EPIDEMIOLOGICAL INVESTIGATION OF SCHISTOSOMOSIS IN DOMESTIC CATTLE IN BANGLADESH

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A Thesis Submitted for the Degree of
Doctor of Philosophy in the Department of Zoology,
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June, 2013

DEDICATED
TO MY
BELOVED PARENTS

DECLARATION

I do hereby declare that the present study was undertaken with my own initiative under the guidance and supervision of Professor Dr. Md. Abdul Mannan, Department of Zoology, University of Rajshahi, Bangladesh and Professor Dr. Md. Abdul Awal, Department of Pharmacology, Bangladesh Agricultural University, Mymensingh, Bangladesh for the Degree of Doctor of Philosophy. The help and sources of information as have been availed during the course of this investigation have been duly acknowledged. The author herself has composed and prepared this thesis and no part of this thesis has been submitted for any degree or diploma anywhere.

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Dated: 25.12.2013

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CERTIFICATE

This is to certify that the thesis **"EPIDEMIOLOGICAL INVESTIGATION OF SCHISTOSOMOSIS IN DOMESTIC CATTLE IN BANGLADESH"** submitted by Nurjahan Begum for the award of **Doctor of Philosophy** in the Department of Zoology, University of Rajshahi is based on the results of her own research work carried out in the Department of Zoology, University of Rajshahi under our supervisions.

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ACKNOWLEDGEMENTS

All praises are due to Almighty Allah who enabled the author to complete this piece of research work peacefully. The author received valuable guidance, constructive suggestion and encouragement from many persons during the course of research work. The author is grateful and indebted to all of them. It would be an act of ungratefulness if the author does not mention their contributions.

The author expresses her heartfelt indebtedness, thanks and sincere appreciation to her reverend supervisor, Professor Dr. Md. Abdul Mannan, Department of Zoology, University of Rajshahi, Rajshahi for his constructive suggestions and encouragement at each stage from starting to completion of the dissertation. The author expresses deep sense of gratitude for her supervisor's time which he had never mind to allocate whenever author desires. The author also expresses her gratitude to her co-supervisor, Professor Dr. Md. Abdul Awal, Department of Pharmacology, Bangladesh Agricultural University,Mymensingh for his valuable suggestions and advice in formulation of research methodology, and preparation of dissertation.

Sincere appreciation is to Professor Dr. Md. Abdul Baki, Department of Pathology, Bangladesh Agricultural University, Mymensingh for performing histopathological studies of the infected tissues.

The author expresses her deep gratitude to Dr.A.K.M.Anisur Rahman, Associate Professor, Department of Medicine, Bangladesh Agricultural University, Mymensingh and Mohammad Kamruzzaman Bhuiya, Associate Professor, Department of Agricultural Statistics, Bangladesh Agricultural University for the statistical analysis of experimental data.

Special thanks are to the teachers of the Department of Zoology, University of Rajshahi, Rajshahi, and teachers of the Department of Parasitology, Bangladesh Agricultural University, Mymensingh for their co-operation, valuable contribution and constructive suggestions at various stages during the tenure of the study.

Thanks are also due to Dr. Md. Mustafizur Rahman, Associate Professor, Department of Agronomy and Agricultural Extension, University of Rajshahi, Rajshahi for his encouragement and instant co-operation during the study period.

The author expresses her deep indebtedness to Upazilla livestock officers of Mymensingh, Netrokona, Kishoregonj, Chittagong and Sirajgonj districts for their kind co-operation and moral support at the time of sample collection in the field level. Special thanks are also due to Md. Jalal Uddin, and Hosneara Begum, Laboratory demonstrator, Bangladesh Agricultural University, Mymensingh for their kind co-operation during the laboratory work.

The author is also grateful to Ministry of Science, Technology and Information Bangladesh for providing financial support to carry out the field work during the study period.

Sincere appreciation is also extended to my family members and other well wishers for their constant encouragement and sacrifice during the research works.

The Author

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ABSTRACT

Bovine schistosomiasis is a disease of great economic importance in cattle production resulted form infection by helminth parasite of the genus *Schistosoma*. An epidemiological study of bovine schistosomiasis was conducted in Mymensigh, Netrokona, Kishorgonj, Chittagong and Sirajgonj areas of Bangladesh. On farm and abattoir survey were done to find out the risk factors associated with epidemiology, abundance of vectors and their relationship with temperature and rainfall during September 2009 to August 2011. Epidemiological data were collected from five different agro-ecological zones and schistosomiasis was found as one of the major bovine parasitic disease. Among the different areas Sirajgonj district, male, adult animal and rainy and summer seasons were identified as significant risk factors for bovine schistosomiasis by egg (egg per gram of feces) count in Bangladesh. Among the three species of mostly common schistosomes in Indian sub-continent two species of were recorded during this period. *Schistosoma indicum* (29.9%) were the most common species than *Schistosoma spindale* (10.6 %).Based on fecal egg examination a significant variation in the prevalence of bovine schistosomiasis were observed according to districts, ages, breeds, sexes, physical conditions, fecal consistency, sanitations, grazing, diets, de-worming, farming systems and seasons. Mesenteric blood vessels examination showed higher prevalence (48.0%) than fecal examination (33.2%).A significant variation in the prevalence of bovine schistosomiasis was observed according to regions and seasons. The highest number of total schistosomes counted from one mesenteric blood vessel was 322 and lowest was 18. In the study of intensity 27.4% of the mesenteric blood vessels showed moderate intensity (21-100 pair) which were statistically significant than low and heavy worm intensity. In order to find out the role of vector host snails were examined and 3.8% were found positive for cercariae of schistosomes of cattle with seasonal variation. Among the three different types of snails *Indoplanorbis exustus* played the dominant role followed by *Lymnaea luteola* and *L. auricularia* for shedding of *Furcocercus* cercariae. The shedding of *Schistosoma* spp. carcariae was significantly higher ($P<0.01$) during rainy season. The miracidial hatchability study clearly showed that miracidia hatching increases with the decreasing age of cattle. This observation clearly indicates that young animal play an important role in the transmission cycle. Though clinical signs were not observed but histopathology of liver, small intestine and large intestine showed slight to moderate parasitic granulomas produced by the *S.indicum* eggs and adult parasites. Two simple methods such as egg detection method and miracidia hatching test was of great help in performing a proper diagnosis and fair assessment of schistosomiasis in the field. It may be concluded that schistosomiasis in cattle could be listed among the infectious diseases with special care because of climate change in the future which will severely affect the disease transmission dynamics. This study indicated that treatment of the infected animal with proper anthelmentic at the end of summer and restrict their movement nearer to water reservoirs could reduce the infection rate to a greater extent.

CHAPTER 1

INTRODUCTION

1.0 BACKGROUND OF THE STUDY

Topographically, Bangladesh lies on the north part of South Asia between 20°34 and 27°38 north latitude and 88°01 to 92°41 east longitudes. Except for the hilly region in the north-east and south-eastern part and high land in the north, the country consists of low and fertile land made up of alluvial soil. The south west region consists of older and moribund delta with large numbers of dead and cut off rivers, often in the form of oxbow lakes (locally called 'Baors'). Within the north-east part of Bangladesh, lie a number of big and depressed basins (locally called 'Haors') which become completely inundated during the monsoon and also remain full of water during the dry winter months. A large number of water collections in the depressed locations (locally called 'Beels') are present in almost every part of Bangladesh.

Wetlands consist of wide variety of types ranging from lakes, rivers, coastal forests to deep water paddy fields and ponds. More than 2/3 of Bangladesh may be classified as wetland according to the definition enunciated in the 'Ramsar' convention, (The Ramsar convention was named after the town of Ramsar, Iran) where wetlands were defined as "areas of marsh, fen, peat land with water that is static or flowing, fresh, brackish or salty including areas of marine water the depth of which at low tide does not exceed 6 meters".About half of the area of Bangladesh can be considered as wetlands. Wetlands in Bangladesh encompass, a wide variety of dynamic ecosystem ranging from mangrove forest (about 577, 100ha), natural lakes, man-made reservoir (Kaptai lake), fresh water marshes (about 400 haors), oxbow lakes (about 54488ha locally known as baors), fresh water depression (about 1000 beels), fish ponds and tanks (about 147000 ha) estuaries and seasonal inundated extensive flood plains (Akonda, 1989, cited in Khan, 1993).

All these wetlands form a unique mosaic of habitats with extremely rich diversity of flora and fauna much of which as yet biologically undiscovered.Wetland of Bangladesh provide one of the major scopes for livelihoods particularly for cultivating food, crops, vegetables, fishing and pasture for cattle which shared most abundance of snails which harbor the infective larval stages of different trematodes including

Schistosoma sp. as intermediaries with other diseases.Accumulation of water due to flood in different parts of the country creates innumerable breeding places for snails which ultimately transmit diseases like schistosomiasis, fascioliasis, paramphistomiasis etc. in man and animal.

Bangladesh has a tropical monsoon climate characterized by heavy seasonal rainfall, moderately warm temperature and high humidity. Regional climatic differences in this flat country are minor. The three main seasons are winter (November to February), summer (March to June) and monsoon (July to October) (Ahmed *et al.,* 1989). January is the coolest month of the year. The average temperature of the country ranges from 7.2c°to12.8°c during winter and 23.9°c to31.1°c during summer (BBS, 2007). The climate of the country is one of the wettest in the world. The annual rainfall ranges from 1432-3903mm (BBS, 2007).Most rainfall (80% of the total) occurs in monsoon (June to September).The average relative humidity for the whole year of the country range from 78.1% at Cox's Bazar to 70.5% at Pabna (Banglapedia). March and April are the least humid months of the country; lowest relative humidity 57% and June to September are the highest humid months, highest relative humidity over 80%. Bangladesh is divided into 30 major agro ecological zones (AEZ) according to physio-graphy, soil type, land type by inundation and agro-climatography of the country (FAO, 1988). The climate makes the country rich in flora and fauna, which grow naturally and abundantly.

Like many other developing countries of the world Bangladesh is also beset with vital health problem of livestock and poultry. Infection and malnutrition constitute a deadly risk factors one predisposing other. Among infectious diseases, i.e. like schistosomiasis, fascioliasis, gastro-intestinal nematodiasis, coccidiosis etc. constitute a big proportion, as the topography, climate makes the country more favorable to their occurrence. The causative agents and their intermediaries find ample opportunity to reproduce and transmit to other hosts.

Most of the diseases in animals of Bangladesh are rooted into ignorance, poverty and apathy of the animals. The body defense mechanism of general animal is low because of scarcity and high cost of animal food and medicine, non-availability of pure drinking water, poor housing, unhygienic disposal of feces and garbage, uncontrolled breeding of snails. All these encourage creation of an environment where not only parasites grow but also vectors find suitable environment for increasing its intensity and disease transmission. In spite of all these discouraging situation, research efforts must be directed to identify the real position of the important parasitic diseases.In recent years, there has been increasing demand of the importance of livestock to the poor with estimates indicating that at least 70% of the rural poor people depend on livestock for part of their livelihoods LID (Livestock in Development,1988). As such, there is an increasing demand for a better understanding of the role of livestock in poverty reduction. With regard to animal health, little is known about the impact of specific diseases on poor households.

The total population of cattle in Bangladesh is about 23.4m (BBS, 2007) of which most of is non-descriptive indigenous Zebu (humped) type multipurpose animal, and among the total population 11.91 million are male and 11.49 million are female. Included among the cattle populations are about 3.53 million are milking cows, 2.61 million dry cows (cows without milk), 2.13 million draught cattle and 4.20 million improved cattle.The national count also reveals that 8 Govrnment cattle /dairy farm are located in different parts of the country and around 20000 private dairy farms are scattered all over the country. Livestock rasing was found to be integral to rural households in Bangladesh representing an important source of income for the rural poor. However, productivity was low due to the lack of suitable shelter, feed and improved breeds combined with poor nutrition and high incidence of diseases. Livestock mainly plays an important role in the national economy, contributing significantly to agriculture and the gross national product. The opportunity for development of large dairy is limited in Bangladesh due to scarcity of land. However, the potential for development of small holder dairy is high. Over the last two decades small-scale dairy farming has increased significantly with the support of credit, feed,

veterinary service and provision of insurance systems.Cattle farming is a way of rearing cattle for profitable production of meat and milk. However, in Bangladesh cattle is mostly reared as a component of traditional crop-based mixed farming or as a source of traction power and manure.In Bangladesh number of cattle per farm and system of cattle management vary with farm sizes. The total populations of dairy are categorized into 3 groups depending on the land size and management system(Saadullah *et al*,1990).Very small to small (0.5 to 2 acre) also known as small scale or semi commercial dairy system mainly practiced semi-zero grazing in which animals are confined partially in stall and are allowed for partial supplementary feed. The common feeding practice is the cut and carry system where by fodder is obtained by cutting from various places and carried back to the housed animals.Supplementation is low, irregular and confined to milking cows.Medium (2.0 to 5.0 acre) which called as traditional farms or subsistence farming systems is sedentary (stationary) herds where farms also practices agricultural activities. Animals are grazed also watered on communal land during the day and housed in open yards or verandah/ corridor at night. Traditional cattle are inadequately provided by supplementation or effective disease control.

Large (above 5 acre) scale or commercial dairy farms have improved or semi-improved management system in which animals are confined full time in stall condition. They mainly practiced zero grazing and animals are given concentrate, chopped grass and mineral supplementation. Since the 1960s, the people of Bangladesh have been rearing three categories of cattle viz.Local breed cattle which are non descriptive indigenous type, Red Chittagong or goyal, Pabna breed, white Munshiganj breed and north Bengal grey cattle.Exotic breed/ pure breed cattle are Haryana, Sindhi, Shahiwal, Jersey, Holstein-Friesian, Australian and Sahiwal-Friesian. A number of exotic pure breeds, their cross breeds and up-graded cattle are found in the government dairy farms, milk pocket area, and in urban and semi-urban areas of Bangladesh. These crossbred cattle are reared by small holder dairy farmers with traditional housing, nutrition and hygienic conditions making them more susceptible to different diseases.Cattle rearing in Bangladesh are considered as a

potential tool in reducing poverty and malnutrition. The Government of Bangladesh is givng much emphasis on both commercial and rural dairy farming. A number of non-Government organizations (NGOs) are also involved in cattle rearing as part of their poverty alleviation programs targeting mostly the destitute women. Because of the patronization from the Government and almost an assured return dairy sector has seen a tremendous development in the recent past. Dairy farming is now considered as a profitable industry in Bangladesh. However, the most likely potential danger is the unawareness and often the ignorance of the producer regarding the possible devasting effect of different infectious diseases and other health problems.

With the increasing interest in dairy productions, evidenced by the proliferation of the cattle farm it is pertinent to continuously evaluate the prevalence and management issues associated with common cattle diseases.Parasites represent an important stressor for many animals, and their effects on host phenotypes can include direct pathologies and host plasticity. For example parasitism can cause liver and intestinal damage, anaemia and increased thermal conductance (Wiger,1977,Holmes and Zohar,1990, Booth *et al.,*1993,Tocque,1993 Meager,1998).Parasitism can also lead to changes in the energetics and performance of animals, including reduced feeding and activity,impaired antipredetor and competitive behavior and altered metabolic rates (Freeland,1981,Rau,1983a, Rau 1983b,Rau and Peter,1984, Symons,1985). Finally parasitized animals may show shifts in organ size and body tissue composition (Tocque, 1993, Kristan and Hammond, 2001, Kristan 2002).Chauhan *et al.*,(1994) attributed 24.5% mortality and 40.0% morbitity to parasitic diseases in various dairy farms in Kangara valley of India. Economic losses are caused by parasites in various ways. Parasitism causes a reduction in food intake and lower weight gain. Milk production can also be affected and mortality can occur in heavily parasitized animals. de Haan and Bekure (1990) estimated in sub- saharan Africa (SSA) endoparasites cause annual mortality and production losses in order of US$2billion. Estimate of losses in Asia have been made difficult by a lack of reliable data (McLeod, 2000) Parasitic disease like schistosomiasis is of particular importance because of their high occurrence in dairy occasioned by tropical and subtropical environmental condition under which the farmers operate (Seifert, 1996).

1.1 General Introduction of the Study

Schistosomiasis also called Bilharziasis after Theodor Bilharz, who first described the disease in 1851, is a snail-borne parasitic disease of large animal and public health importance in endemic countries. Schistosomiasis has for long been recognized as a common parasitic infection in domestic animals in Asia but it is only recent years that the disease had been recognized as a possible veterinary problem. However, information accumulating for experimental studies and outbreak of clinical schistosomiasis occasionally reported leave no doubt that the disease at least under conditions favoring intensive disease transmission, may be of major veterinary importance due to high mortality and morbidity in acute clinical infections and to the less easily recognizable deleterious effects of long standing chronic infections (Dargie,1980). The disease is restricted to subtropical and tropical countries and is estimated to affect the health of around 165 million cattle and 200 million people in countries of Africa, Asia, the Middle East and South America (Rollinson, 1994, DeBont and Vercruysse, 1997, Chitsulo *et al.,* 2000, Agrawal,2011). Schistosomiasis has been a focus of various studies because of its medical, veterinary, social and zoonotic importance (Abdel *et al.,*2000, Ross *et al.,*2002,WHO,2002,Atupele *et al.,*2009,Hirofumi and Hiroshi,2009).In livestock it is a chronic and wasting disease, contributing to considerable economic losses. Schistosomiasis is now recognized as the fifth major helminthosis of domestic animals in the Indian subcontinent (Agrawal, 1999).Veterinary interest also lies in the fact that some animals may act as reservoir of infection for man (David,2000, Julie *et al.,*2007,Gary *et al.,* 2008).Schistosomiasis is considered as one of the most neglected tropical disease , based on the limited resources invested in diagnosis, treatment and control and its strong association with poverty. In general three types of clinical syndromes are seen in animal infection with schistosomiasis, an acute intestinal, a chronic hepatic (Lawrence, 1978b, c) and a nasal granulomatous syndrome (Mulkani, 1933). Intestinal schistosomes such as *S. indicum* and *S. spindale* are considered as most harmful helminthes of adult cattle. In the great majority of cases, bovine schistosomiasis is a chronic disease, characterized in the short term by very undramatic clinical changes, but in the long term, and particularly on a herd basis, by retardation of growth. It should however be

emphasized that in the field other parasites such as gastrointestinal nematodes and liver flukes may also affect productivity, and influence the pathophysiology of schistosomiasis. Infected cattle are anorexic show severe diarrhoea and develop a marked anaemia and hypoalbuminaemia. Because of gut damage and associated protein leak, animals infected with schistosomes fall into a hypercatabolic state with respect to haemoglobin, albumin and globulins. At the sametime they also synthesize much more of these proteins which are needed for host survival. This high turns over associated with the anorexia and reduced digestibility explained the observed substantial loss of body weight from which animals never really appear to recover (Dargie,1980).They cause severe enteritis, anaemia, hypoalbuminaemia, edema, decrease in reproduction, loss of weight, neurological signs may develop, changes in the nitrogen status and digestive efficiency follow the onset of oviposition (Berry *et.al.,*1973).The decline in nitrogen balance correlated with the severity of diarrhoea, and hyper catabolism of albumin.The reduced apparent digestibility coefficients of all dietary constituents might be due to the presence of ova in blood vessels of gastrointestinal tract. Morbid losses are also staggering from sharp fall in body vigor and productivity which also require costly medicine for treatment. The hepatic syndrome is an immunological disease resulting from the host response to schistosome eggs in the liver. This has been studied in mice infected with *S. mansoni* as a model for the pathology seen in human schistosomiasis (Warren and Witt, 1972, Warren and Moore 1977).Chronic hepatic pathology occurs in schistosome infected animals, particularly those repeatedly exposed to heavy infections. Granulomatous lesions and periportal inflammation occur, followed by a progressive portal fibrosis. *S. nasale* causes rhinitis and a mucopurulant discharge manifested clinically by signs of coryza, sneezing, dyspnoea and snoring (snoring disease). Adult parasites cause dilation and thrombosis of the nasal lesions. The nasal mucosa is studded with granulomas and abscess containing eggs. Chronically these are fibrous tissue formation and proliferation of the nasal epithelium. The clinical signs and pathology are less severe in buffaloes than in cattle which suggest that buffaloes are a more suitable definitive host than cattle (Rajamohanan and Peter, 1975).Every form of schistosomiasis is predominantly a rural occupational disease of field workers. It also

affects people coming into contact with water containing cercariae, such as fisherman, persons engaged in building bridges or clearing river banks as well as children bathing in streams and women washing clothes in open waters. The disease saps the health, and therefore, the working capacity of large population groups and for this reason if indeed merits the name 'world scourge'(Agrawal *et al.*,1991).Animal and humans get infected when coming into contact with snail infested waters, the cercarial stage of the parasite is allowed to penetrate the skin (Malek, 1980).

However, it has been established that high rates of prevalence of sub-clinical infections cause significant losses due to long term effects on animal growth and productivity and increased susceptibility to other parasitic or bacterial diseases (Dargie, 1980, Pitchford and Visser, 1982, McCauley *et al.,* 1983, 1984). Despite this schistosomes of animal concern have received little attention compared with species affecting man and most studies have been based on experimental infection with only a small number of field epidemiological reports. This is surprising because infections in cattle are widely distributed and occur commonly throughout Africa and Asia. It has been argued that in the long term, such infection causes significant losses to farms (Dargie, 1980).

S. nasale causes snoring disease. Preliminary accounts on the pathology of infection in cattle were described by Datta (1932) and Rao (1933, 1934). The eggs deposited in the mucosa incite an inflammatory reaction with cellular infiltration and extensive fibrous tissue proliferation leading to large cauliflower- like growths on the nasal mucosa. Compared with visceral schistosomiasis, nasal schistosomiasis is probably a slower developing progressive disease. The histopathology is similar to other schistosome infections in that granuloma formation, owing to the presence of eggs, is a common feature. An epidemic of cercarial dermatitis in paddy field workers caused by cercariae of *S. indicum* and *S.spindale* is noted in different parts of Asia (Preyakullanijaya and Harnwongwaisayawau,1993, Narain *et. al*,1998).The farmers gave a history of itch following immersion in water while planting rice.The epidemiology of schistosomiasis is rapidly changing and new geographical approaches are required to promote prevention and control. Four situations where

schistosomiasis transmission is increasingly linked with infection in animals have now been documented: Urban /periurban, irrigation system, delta areas related to dam construction, and increasing cattle mobility through trading and / or rental increased the possibility of spreading infections sources(Ross *et al.,*2002).

Only a few epidemiological studies have considered on *S. nasale*. This is not just a reflection of the relatively lower economical importance of nasal infections but mainly because it is more difficult to study nasal infections in the definitive host. In practice, *S. nasale* worms or eggs can not be counted in infected cattle and the only measurable parameters at the population level are the number of animals infected and the severity of macroscopic lesions in positive animals. Therefore, apart from prevalence data's (Dutta and Srivastava, 1968, Achuthan and Alwar, 1973, Muraleedharan *et al.,* 1973, 1976b, DeBont *et al.,* 1989a) little is known about the epidemiology of nasal schistosomiasis in cattle. However, the prevalence of *S. nasale* infections and the severity of the lesions are known to increase with the age of the host/ infection (Islam, 1975, 1976, DeBont *et al.,* 1989a).

Now in many developing countries a new threat is emerging the disease in relation to exposure of parasites due to increasing demand of certain agricultural product cultivated by organic farming to avoid health hazard. Bangladesh is not legging behind; such cultivated land where compost is used for cultivation of crops and subsequently the same land is used as pasture for animals will pose a new threat for parasitic infections. Among the ruminants schistosomiasis is much more serious and important infection in cattle than in buffalo and goat (Jeyathilakan *et al.,* 2008) but in contrast to small ruminants, cattle visit regularly and usually get contact with water as source of contamination could explain the high prevalence of *Schistosoma* in cattle. In the most areas cattle show a very high prevalence without clinical signs and even in well managed cattle herds the number of infected animals at slaughter may be over 90% in stock drawn from areas of plentiful natural water (Urquahart, 2001). It has been suggested, however that even in apparent infections there may be a major effect which may be important in the dairy and beef industries. A negative impact of infection on production has been appraised, particularly in cattle (McCauley *et al.,*

1984, Makundi *et al.,* 1988) leading to major economic losses in infected herds. The impact of infection on production is probably still underestimated, but more attention is to be directed towards this disease as cattle is important for small holders farmers is a strategy for reaching the United Nations Millennium Development Goal to end poverty and hunger. (www.un.org./millennium).

In developed countries the data on epidemiology of various helminthiases are published in an efficient manner as an aid to combat infections more effectively. In contrast in developing countries little published information and data exists on the epidemiological aspect of helminth infections particularly on schistosomiasis.While the epidemiology of schistosomiasis in human is well known in most countries but schistosomiasis in animals is still a neglected helminth infection in many countries and under diagnosed and has not received due attention.

The patterns of infection of schistosomiasis are determined by the interactions of environmental factors, the biology of parasites and the production systems in which cattle are expected to perform. Given the essential relationship between parasite schistosomes and water dwelling snails that act as intermediate hosts, it is no surprise the schistosome infection is most common in areas providing a suitable habitat for the intermediate host which may be contaminated with parasite containing cattle feces.The ecology of schistosomes includes tropical lotic (lakes and reservoir) and lentic environments (rivers) and behavior of people and their domestic animals that live near the aquatic environments.Islam and Awal (1991) reported schistosome dermatitis among the farmers working in water lodged paddy and jute field from different regions of Mymensingh district of Bangladesh.

Considering the importance and pathological effects on schistosomiasis in cattle this study will describe the details of schistosomiasis in ruminants and attempt to bridge gap knowledge of these aspects.To understand the unusual biology of schistosomes is further justified by the fact that schistosomiasis remains common in the developing world has been significantly underestimated in its impact on causing animal morbidity in what King *et al.,* (2005) have referred as a "slient pandemic". Recently control of

this disease has become important because of reduced efficacy and undesirable toxic effects of available drugs and drug resistance of schistosomes indicate it may have developed diversity in their genome (Nelson, 1970) that needs to develop alternative control strategy.Lack of information on the epidemiology and pathogenesis of gastro-intestinal parasites is recognised by Lonka *et al.*,(1991) to be a constraint to the development of effective control measures in India. For a rational and sustainable helminthes control program a comprehensive knowledge of the epidemiology of parasites and their interaction with the host in a specific climate and management system is prerequisite (Barger, 1999).

1.2 Diagnosis of Schistosomiasis in Cattle

Diagnosis of infection is essential in epidemiological survey as well as in prevention and control of disease in domestic livestock. Determination of target population for chemotherapy in endemic areas, assesment of morbidity and evaluaton of control strategies all build on the results from diagnostic test (Feldmeier *et al.*, 1993).Ruminant schistosomiasis can not be diagnosed merely by clinical signs, since these are unspecific and often resemble the signs of other diseases such as paratuberculosis, fascioliasis and trypanosomiasis (Kassuku,1985).However light infections and delayed onset of egg output usually prevent detection of eggs in feces (Khalil *et al.*,1993).In the chronic form when the characteristic eggs of the parasite worm are not found in feces, serological tests are of increasing value (Wilcoks and Manson-Bahr1972).Diagnosis of acute schistosomiasis depends on the identification of eggs in feces or in liver or rectal biopsy but rectal biopsy a threat to the host (Pappas,1988).The fecal examination revealed the presence of disease ranging from 7-21% with a mean of 13% both cattle and buffaloes in various group (Anwar and Gill,1990).

Since fecal egg counts in chronic schistosomiasis bovis are usually very low, the sensitivity of the diagnostic method is critical for correct diagnosis. The sensitivity of feces examination may be improved by rectal snip biopsies. However increased number of examined fecal samples makes the two methods equally reliable (Rabello *et*

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al., 1992).The diagnosis in endemic areas depends mainly on microscopic detection of eggs in feces or urine of the infected animal (Saad *et al.,*1994) and miracidia hatching test (WHO,1994).These two parasitological techniques are still the diagnosis of choice in national schistosomiasis control programmes all over the world.

1.3 Distrbution of Schistosomes in Indian Subcontinent and Other Regions of the World

Many domestic and farm animals have their own schistosomes, some of local economic importance in their own right. Because of the close and sometimes intimate association of human and their livestocks, these schistosomes may also confuse human epidemiological studies. The geographical distribution of animal schistosomes differs somewhat from that of human schistosomes.A total of 19 different species of schistosomes are described worldwide in different host. They live in the peri-vesical (*S. haematobium*), nasal (*S. nasale*) or mesenteric and hepatic (remaining species) veins of the host where they feed on blood and produce non-operculated eggs with a characteristic terminal or lateral spine. As many as 10 different species of schistosomes have been reported to naturally infect cattle: *S. spindale*, *S. indicum*, *S. nasale*, and *S. japonicum* in Asia, *S. mattheei, S. bovis* and *S. curassoni* in Africa and other three species are primarily parasites of antelopes (*S. margrebowiei and S. leiperi* in Africa) or pigs. S. *incognitum* and *S. japonicum* causes infection under natural condition both in man and animals known as zoonotic (Cheever and Neafi, 2000).

1.3.1 The distributions of schistosomes are as under

In Asia

i) ***S. indicum*** species was discovered by the British scientist Montgomery (1906a) from equine and infects variety of domestic animals (sheep, goat, waterbuffalo, cattle, camel, donkey and dog) in the Indian sub-continent. The eggs are oval and posses a terminal spine. This parasite resides in portal and mesenteric veins of cattle including other ruminants and the equine in Indo-Pakistan and Bangladesh. Rodents are experimentally susceptible; the geographic range is from southern Europe (sporadically in Portugul, Spain and Cyprus) through the Middle East and Africa

(from the Nile delta to the South) to Indo-Pakistan and Burma. This parasite is found occasionally in dogs in Africa, Indo-Pakistan, Thailand, former Indo-Chinese countries and Indonesia (Dunne, 1978).The blood fluke causes hepato-intestinal schistosomiasis and it was responsible for an outbreak of pulmonary schistosomiasis in 1981 in sheep in Rajasthan leading to considerable mortality. Avariant of *S.indicum* rather than *S.haematobium* was suggested to be responsible for human schistosomiasis in Gimvi village, Ratnagiri district, India.Terminal spined *S.indicum* like eggs have been detected in human stools too.

ii) ***S. spindale*** was discovered by Montgomery (1906b) from the portal and mesenteric veins of small and large intestines of cattle of Muktesar, India and now this parasite is found in Srilanka, Indonesia, Malaysia, Thailand and Vietnam. The parasite is found in ruminants and occasionally horses and dogs in Africa, Indo-Pakistan, Bangladesh, Thailand, the former Indo-Chinese countries and Indonesia (Islam, 1975, Dunne, 1978). This parasite also infects horse and pigs in Asia and Far East (Urquhart *et al.*, 2001). It causes schistosomal dermatitis in man. The cattle may show sub-clinical symptoms but in severe infections anaemia, diarrhoea and edema may develop, occasional mortality has been recorded. Buffaloes are believed to play a major role in transmission because of their close association with rice paddies and irrigation channels harboring their intermediate host *Indoplanorbis*.

iii) ***S. nasale*** described first by Rao (1933) is responsible for nasal schistosomiasis or snoring disease in cattle, sheep and goats. The adult worms are found in the veins of nasal mucosa. The eggs are large and distinctive with a terminal spine. This parasite also infects horses in India and Pakistan (Urquahart *et al.*,2001). In India *S. nasale* has been reported from Andhra pradesh (Rao and Murthy, 1964, Christophen and Rao, 1975, Sreeramula, 1982), Bihar (Varma, 1954, Sahay and Sahai, 1978), Karnataka (Rao and Naik,1957, Naik,1962, Muraleedharan *et al.,* 1976a, b, c). *S. nasale* is relatively more prevalent and cause nasal granuloma in buffaloes and cattle than sheep and goat (Southgate and Agrawal, 1990).

S. incognitum species was described by Chandler (1926) who observed sub-terminal spined eggs in the samples of human stool in Bengal, India and Pakistan but it appears to be a parasite of rodents though it does mature in domestic animals, specially in pigs and dogs but less well in ruminants although sheep and goat infections do occur. The intermediate host in India is lymnaeid, *Lymnaea luteola* but elsewhere is *Radix auricularia rubiginosa*. Natural infections have been found mixed with *S.indicum* and *S.spindale* in India and Thailand and with *S. japonicum* in Sulawesi. The eggs are sub-oval in shape with one side comparatively flattened and the spine is short.

In Africa

v*)* ***S. bovis*** species was discovered by Sonsino (1876) and occurs in genera *Bulinas*, *Planorbis* and *Artiodactyla* in Africa. This is especially true in Sudan where 90% of infection occurs in 18 months age of cattle and is serious veterinary problem in the Mediterranean region, Middle East, Asia and southern Europe. The adult worms were found in the mesenteric veins of the intestine, bladder involvement appears in sheep suffering from a very heavy infection (Hussein *et al.,* 1984 and Urquhart *et al.,* 2001). In a survey study 12% of the cattle and buffaloes were positive with *S. bovis* in Ralwa city and its surroundings in Punjab, Pakistan (Anwar, 1982). The experimentally infected animals develop mucoid followed by diarrhea and become dull and depressed. Packed cell volume and haemoglobin concentrations of the infected animals showed progressive reductions with uninfected controls. The animals were found necropsied and perfused at 12 weeks post-infection (WPI) and tissue egg densities and worm burdens was found to be 245n 215/g in the liver, 1133n 428/g in the small intestine and 758n 516/g in the large intestine. The mean worm burden was 3000 (Aradaib *et al.,* 1995b).

S.mattheei was discovered by Veglia and Le Roux (1929) is transmitted by snails of genus *Bulinus* in southern Africa.Its economic effect is not usually considered significant but serious epidemics have been reported in sheep in South Africa (Lawrence,1978a) and studies in Zambia indicate adverse effects on the growth rate of cattle (De Bont and Vercruysse,1997). *S.mattheei* extends northwards towards the equator, where it overlaps the southern limits of another schistosome of cattle, sheep

and goats, *S bovis*. Hybrid eggs of *S.mattheei* and *S. bovis* have been described from Tanzania and Zambia (Appleton and Stiles, 1976)

S. curassoni a schistosome of domestic ruminants with limited distribution in West Africa (Senegal, Mali, Mauritania and northern Nigeria) is transmitted by *Bulinus* spp. Its distribution overlaps that of *S. bovis* and two may be present in the same animal, sometimes as heterologous pairs. It does not seem to cause serious pathology in sheep (Leptak and Mckerrow, 1997).

S. margrebowiei was discovered by Le Roux in1933 in ruminants and zebra in Zambia and theRepublicofSouthAfrica

S. japonicum was discovered by Katsurada in 1904, in the portal and mesenteric veins of both man and animal in Far East.A wide range of domestic and wild animals have been found to be infected. These include ruminants, equines, pigs, dogs, and rodents.Both man and animals act as maintaince hosts. Four strains of *S.japonicum* occur, the Japanese, Philippine, Chinese and these are recognized both by geographical distribution and by morphological characters.In Taiwan this species only affect animals not humans.

S. haematobium was discovered by Bilharz in 1852 and found in Africa and locus of infection has been reported in India. Man is the only significant maintenance host of this species although the infection has been detected in animals,e.g. baboons and monkeys in EastAfrica, rodents in Kenya and southern Africa,pigs in Nigeria and chimpanzees in West Africa.

S. mansoni was discovered by Sambon in 1907 found in the mesenteric veins of man in Africa, South America and Middle East. However, a variety of animals have been found to be naturally infected with this parasite.These include gerbils and Nile rats in Egypt, rodents in southern Africa and Zaire, various species of rodents, wild mammals and cattle in Brazil and baboons, rodents and dogs in East Africa.

S. leiperi was described from a sitatunga antelope *Tragelaphus spekei* sclater, shot at Mazabuka and further specimens were recovered by Le Roux in 1955 from many other antelopes and also domestic cattle in Zambia.

Schistosomes are dioecious trematode worms of the family Schistosomatidae. The name *Schistosoma* originates from the Greek words for split (schistos) and body (soma), referring to the ventral longitudinal cleft, the gynecophoric canal, of the male worm in which the female worm is carried during copulation and movement. A characteristic feature of schistosomes is that the female worm is longer and more slender than the male worm. The life cycle of schistosomes is indirect, sexual reproduction takes place in the definitive mammalian host and asexual reproduction in the intermediate fresh-water snail host. Adult schistosomes live in pairs in the mesenteric veins of the mammalian host, where the female worms deposit their eggs (Malek, 1980). Pathological effects in schistosomes are mainly due to response to the eggs leading to peri-oval granuloma form and hepatic fibrosis (Taylor, 1987)

1.4 Life-cycle of Schistosome in General

A schematic drawing of the schistosome life cycle is shown in Figure 1. The following account is based on description by Malek (1980) of the schistosome life cycle and on detailed studies by Lengy (1961,1962 b) on all stages of the *Schistosoma* life cycle.

The paired adult worms live in the nasal (*S. nasale*) or mesenteric (other species) veins of the host where they produce eggs which contain embryonic cells (the miracidium takes about 1 week to develop after the eggs have been extruded from the female worm). The female partially or totally leaves the gynecophoric canal of the male to lay her eggs in the finest branches of the venules. The uterus of the female worm contains from 3 to 65 eggs (Lengy, 1962b) and a paired female produces hundreds of eggs per day. Eggs laid in the blood vessel penetrate the wall of the veins and migrate to the intestinal lumen, or the nasal cavity (*S. nasale*) of the host. This passage through the vein wall and tissues of the host is aided by the release of lytic enzymes secreted by the miracidium contained in the egg and by forceful piercing of

the tissues by the pointed end of the eggs (Semuguruka, 1992). Deposited eggs are not developed, but after 6-8 days of maturation the egg contains a fully developed miracidium (Lengy, 1962b) which hatches when the egg has passed out with feces and come into contact with water. Many eggs are carried away in the blood stream and / or trapped in the tissues of various organs mainly in the liver and die. Eggs which reach the lumen are either passed in the feces (intestinal schistosomes) or disseminated while drinking or sneezing (*S. nasale*) and hatch only if they come in contact with fresh water.The miracidium swims actively in search of its intermediate host, a fresh water snail of the family Lymnaediiae, Indoplanorbidae. (*L. auricularia*, *L. luteola*, *I. exustus* and *Planorbis* sp.) (Agrawal and Southgate,2000).

It may penetrate several species of snails but only in the above stated snail asexual reproduction will take place. After entering the snail, the miracidium transforms into a primary sporocyst, which in turn produce large number of fork tailed cercariae which will escape from the snail into freshwater. Under optimal temperature conditions (at about25°c) the time elapsed from the penetration by the miracidium to the liberation of the cercariae is approximately 4 weeks.Infections of cattle by cercariae is usually accomplished by penetration of the skin, although it has been shown that per oral infection while drinking may also be of importance (Fairly and Jasudasan, 1930, Van wyk *et al.,* 1974, Kassaku *et al.,* 1985). After penetrating the skin or mucosa the cercariae shed their tail and transform schistosomula travels by the venous system or the lymphatic to the right heart and lungs and the most probably continues through the systemic circulation to the liver, where it matures into adult worm. Adult male and female worms' forms pair and migrate to the mesenteric veins, where they may live for years. Pairing appears to be of paramount importance to the development and sexual maturation of the female worms (Popiel, 1986). The time from cercariae penetration of the definitive host to the onset of egg laying by female worms, the pre-patent period is around six weeks. The route of migration of the *S. nasale* to the veins of nasal cavity has never been studied.

1.5 Justification of the Epidemiological Studies

Increasingly, epidemiological studies of populations in endemic areas of schistosomiasis are using standard quantitative methods to determine both the intensity of infection and morbidity. The use of these methods describes the understanding of the relationship between intensity of infection and morbidity by allowing comparisons between different geographical areas. It will also provide reliable baseline data for studies on chemotherapy, malacology and contamination patterns in host group and will provide information on the transmission potential of host population, which may be utilized in devising operational and assessment strategies for sustainable schistosomiasis control.Lack of information on the epidemiology and pathogenesis of gastrointestinal parasites is recognized by Lonka *etal.,*(1993) to be a constraint to the development of effective control measures in India.

1.6 Research of Schistosomiasis in Bangladesh

The detailed epidemiology of schistosomiasis of livestock in Bangladesh has not been investigated. As livestock management practices can change the occurrence of parasites in an area, so existing parasite inventries as well as distribution data based on old studies may require updating (Hansen and Perry, 1990).Hence, for the interest of epidemiological studies on schistosomiasis in cattle the species of *Schistosoma* and their distribution data based on study made more than thirty years ago needs to be updating. Only a few isolated surveys have been conducted earlier in domestic ruminants by Bhuyan, 1970 and Islam, 1975, 1976 in Bangladesh. None of the studies attempted to estimate prevalence and associated risk factors of schistosomiasis by using appropriate study design.The age, breed, sex, nutritional status, herdsize,seasons,farm management systems have been identified as risk factors of various parasitic diseases elsewhere in the world (Radostits *et al.,*2000).The dairy farmers in Bangladesh are not always aware of the best practices to control any diseases.Therefore, unhygienic management system,anthelmintic treatment practices without identifying the parasite and costly imported medicine are the main challenges for controlling schistosomiasis in Bangladesh.The aim of this study is to investigate

the epidemiology (prevalence, incidence, intensity, frequency, distribution, transmission and risk factors) of *Schistosoma* spp. in cattle of Bangladesh. Epidemiology is a key step for the control of schistosomiasis (Feldmeier *et al.*, 1993). Determination of target population for control in the endemic areas, assessment of morbidity and the evaluation of control strategies all determine on the basis of epidemiological observation (Martin *et al.,* 2008, Zhou *et al.,* 2008). A greater understanding of their (*Schistosoma*) epidemiology will enable the design of appropriate control measures in different production system in different climatic conditions. So a cross-sectional survey is needed to determine the prevalence and risk factors of schistosomiasis through random sampling from target populations.Random sampling design is used to determine the sample size with desired level of confidence(95%) and error(5%) from the target population where prevalence and risk factors of schistosomiasis in the target population can be determined (Thrushfield,1986).Therefore,a cross-sectional survey is needed for epidemiological investigation of bovine schistosomiasis in Bangladesh.

The work aims to address the following objectives:

1) To determine the prevalence by examination of eggs from feces of live animals through identification of risk factors by schistosome egg count (EPG) and also to observe the relationship of the prevalence with temperature and rainfall.
2) To determine the prevalence by worm count from mesenteric blood vessels examination with their intensity and to observe the relationship of the prevalence with temperature and rainfall.
3) To determine transmission ecology of schistosomiasis by the study of snails and cercariae.
4) To estimate miracidia hatching intensity.
5) To find out pathology produced by schistosome parasite.

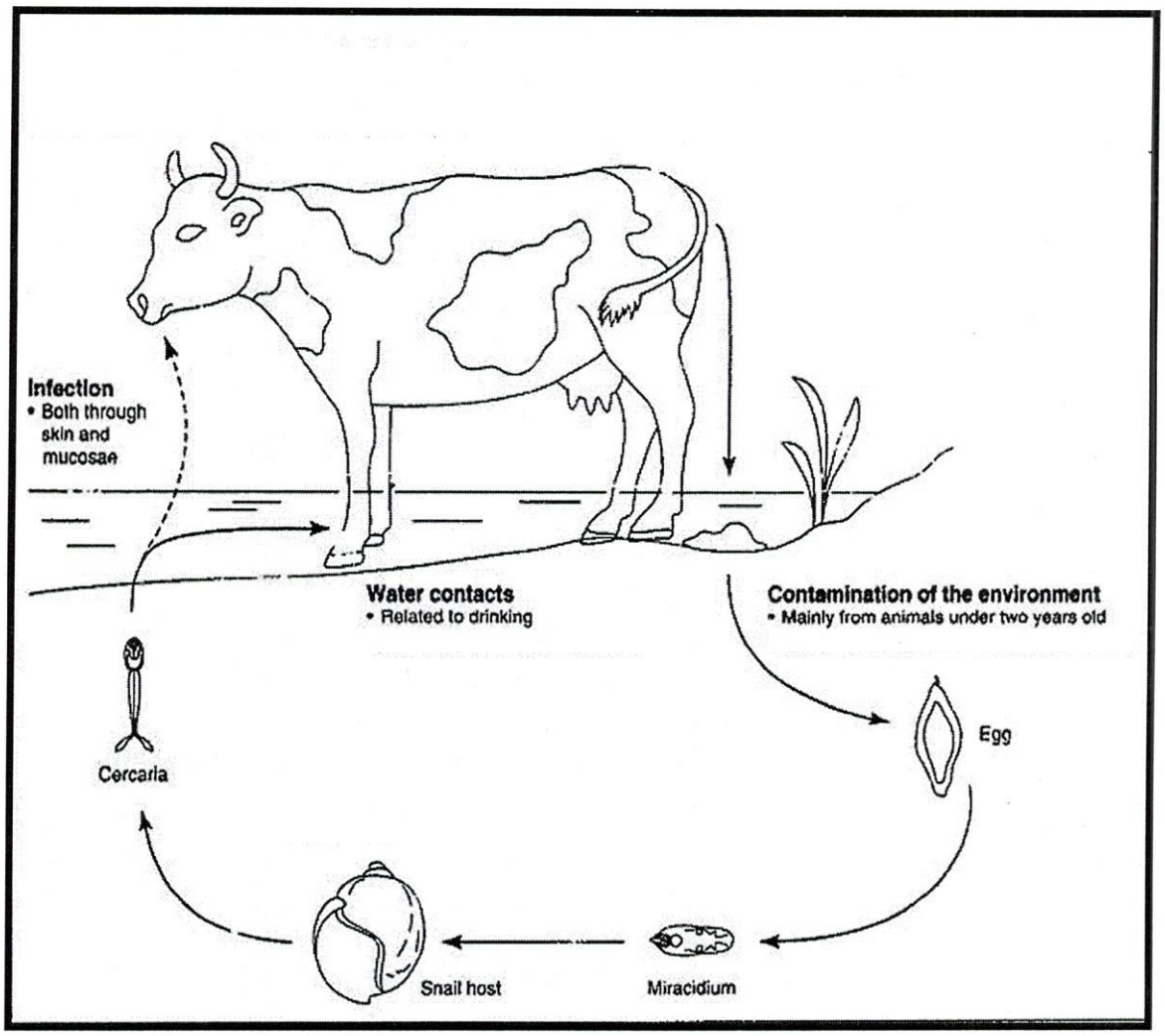

Figure 1. Lifecycle of *Schistosoma* in cattle Lengy (1962)

Plate 1. Collection of feces from rectum of cattle

Plate2.Cattle in schistosome cercariae infected water

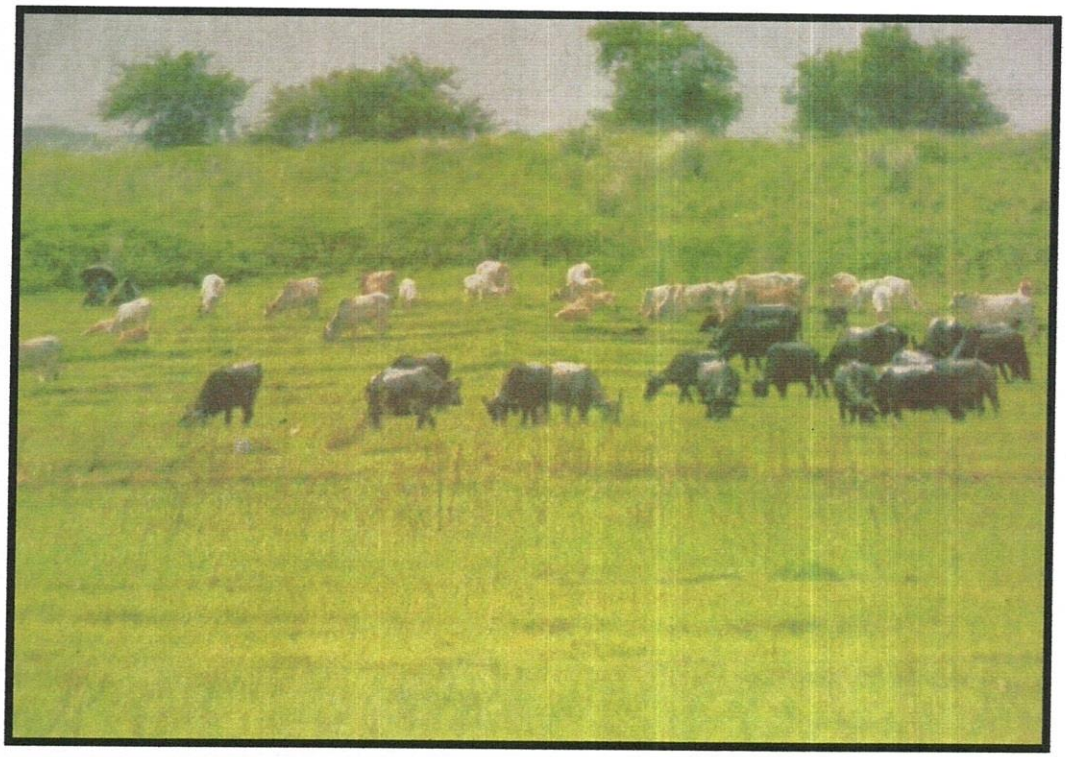

Plate3. Grazed cattle in swampy infected snail contaminated pasture

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The review of literature in any research is necessary as it provides a new dimension for reviewing the stock of knowledge and information relevant to the proposed research. This knowledge gives us a guideline furnishing the future research problem and validation the existing findings. The focus of this chapter is to provide a selective review of the past research works which are related to the present study. In recent years the main purpose of the study of schistosomiasis is to control the disease. A good number of studies carried out on parasitic diseases but a few studies were conducted which concerned the topic of the research. Although schistosomiasis is highly prevalent and pathogenic in Bangladesh, there is little information regarding the research topic. However, a literature review on the available literatures on schistosomiasis and related aspects are presented below. Surveillance for cattle schistosomiasis is generally inadequate and the literature is limited but some idea of the problem can be gained from the past small-scale studies.

Schistosomiasis is known as bilharzia or bilharziosis in many countries, after Theodor Bilharz who first described the cause of urinary schistosomiasis in 1851. The first doctor who described the entire disease cycle was Piraja da Silva in 1908. Schistosome infection in humans is referred to as "Schistosomiasis "according to recommendations by the WHO. For infection in animals the term "Schistosomiasis" is used according to recommendations by the World Association for the Advancement of Veterinary Parasitology (Kassai *et al.,* 1988). Despite major advances in control and substantial decreases in morbidity and mortality, schistosomiasis in human it continues to spread to new geographic areas (Patz *et al.,* 2001). As a result, research has been conducted in a wide range of disciplines aimed at understanding and alleviating schistosomiasis infection (Rollinson and Simpson, 1987, Jordan *et al.,* 1993). The epidemiology of human schistosomiasis in the field has received particular attention. Eggs count in excreta and in recent years, antigen detection assay (Deelder *et al.,* 1994) and ultrasonography (Wiest, 1996) has been the main techniques used to measure intensity and morbidity of infection.

A major factor restricting epidemiological studies of human infection is that it is not possible to count worm ante-mortem in infected humans.Schistosome infections occur in cattle but except for occasional reports of field outbreaks (Kulkarni *et al.,* 1954, Reinecke, 1970, Lawrence and Condy, 1970, Van wyk *et al.*, 1974 and Lawrence, 1978a) there has been little recognition of their veterinary significance. The clinical and pathological aspects of acute schistosomiasis have been examined in detail, together with evolution of the host-parasite relationship and development of immunity in cattle (Lawrence, 1978b, Taylor, 1987). Most studies have been based on experimental infections, with only a small number of field epidemiological reports. The relationship between particular parameters may be studied, or the variation observed in one parameter according the type, level and duration of exposure, or age, sex, breed, nutritional status and physiological condition of the host while the relevance of any findings to human infections is uncertain, until new techniques for the assessment of worm burdens are available for use in human studies, information on the dynamics of schistosome infections in other natural hosts is valuable. Despite this, schistosomes of veterinary concern have received relatively little attention compared to species affecting man, although reviews on particular species of schistosomes infecting cattle or on distinct aspects of the infection have been published (Hussein, 1973, Lawrence 1978a, Christensen *etal.,*1983, Kumar and de Burbure, 1986, Taylor, 1987, Aradaib and Osburn, 1995b, Aradaib *et al.,* 1995a, DeBont and Vercruysse, 1997,Agrawal, 2003).

2.1 Prevalence and Intensity of Infection by Fecal Egg Examination

The prevalence of infection is the percentage of cattle population which is found infected at a given time; where as the intensity of infection is worm burden in infected animals.

The geographical distribution of schistosome species infecting cattle is mainly determined by the distribution of their respective intermediate host snail. There are reports of heterlogous infection of *S. spindale* with other trematodes including schistosomes. In Bangladesh, schistosomiasis is a constant threat to dairy industry. Since the pioneering work (Islam, 1975) no detail studies on schistosomiasis of cattle

or any other hosts have been carried out in Bangladesh. Schistosomiasis in bovines is known to cause severe morbidity in animals. Detection of subclinical schistosomiasis in live animals is difficult, since the affected animals do not show obvious signs of disease and animal act as carriers and continue the chance of transmission. Nevertheless, it is important to detect schistosomiasis in live animals so that they can be treated in order to minimize morbidity and economic losses.

Condy (1960), Dinnik and Dinnik(1965), Islam (1975), Rollinson *et al.,* (1990), DeBont *et al.,* (1991b) and DeBont *et al.,* (1994) in their study reported that in Srilanka,Bangladesh, Zimbabwe, Zambia and West Africa the overall infection rates in cattle populations varied from 31-81%. It is therefore reasonable to speculate that at least 165 million cattle are infected with schistosomes' world wide.While the prevalence rate is high; the intensity of infection is generally low. Majid *et al.,* (1980), Pitchford and Vissar (1982),Kassuku *et al.,* (1986), De Bont *et al.,* (1995a) stated that under field conditions, fecal egg output rarely exceeds 50 eggs/gram although much higher worm and fecal egg counts were recorded during outbreaks of clinical schistosomiasis. Lawrence (1978a), and Dargie, (1980) found fecal egg output during severe outbreaks can vary between 100 and1000 eggs per gram of feces. Majid *et al.,* (1980), Pitchford and Visser (1982) and Kassuku *et al.,* (1986) measured the intensity of infection in some field studies, by fecal egg counts. However, Van wyk *et al.,* (1974), Lawrence(1978a), Dargie (1980),Kassuku *et al.,*(1986),De Bont *et al.,* (1991a, 1995a) stressed that even if fecal egg counts may, in some early infections, provide reasonably accurate indications of the intensity and morbidity of infection, they should not generally be considered as an indirect measure of worm burden when the duration of infection is unknown.However, tissue egg counts (usually of the intestines and liver) are more commonly used as a measure of morbidity, the eggs of schistosomes acting as the primary agent of pathogenesis in the host. The incidence rate is a measure of the level of transmission over a given period of time.

Datta (1932) studied on any morphological or physiological difference between the schistosomiasis infecting cattle or those infecting the buffaloes, in case of nasal schistosomiasis. He also carried out the morphology of the adults, eggs, miracidia and cercariae of *S. nasale* and cross infection of the same. The author found no difference in the morphology of *S. nasale* of cattle and buffalo origin.

MacHattie and Chadwick (1932) opined that *S. bovis* is common in Egypt as well as in Iraq where *Bulinus truncatus* serves as its intermediate host. It is thus not surprising to find this trematode also in Israrel where this snail species is widespread. Datta (1933) studied from pathological viewpoint schistosomiasis in army horses caused by *S.indicum* and concluded that infection of this schistosome to be one of the causes of the so-called nodular cirrhosis of liver in horses and the same is an important factor in the causation of persistent debility of equines in India. Rao (1934) recorded a heterologous schistosome infection incorporating both *S. indicum* and *S. spindale* in a cow and a sheep.

Vaidyanathan (1949) reported a clinical case of *S. spindale* in an 8 year old cross-bred cow at Coimbatore, Tamil Nadu with the history of rapidly running down physical condition and frequent passage of loose feces with blood and mucus. Examination of feces revealed numerous eggs of *S. spindale* suggesting it to be responsible for the clinical condition.

Kulkarni *et al.,* (1954) described two outbreaks of schistosomiasis of erstwhile Bombay state. In these two outbreaks, 121 bullocks, 34 cows and 2 buffaloes died because of *S. spindale* out of a total population of 1248 bovines. Besides the bovines, sheep and goats were also affected. The outbreak was unique in that the flukes were found in almost all the organs of the animals including heart. In some cases, the symptoms were similar to those of rinderpest, coccidiosis or John's disease, but *S. spindale* eggs were present in the feces and urine thereby confirming the diagnosis.
Olafson (1955) stated that until recently, however, knowledge concerning *S. bovis* in Israel was meager and fragmentary and only two records of infection were known in infected sheep in Israel.

Pitchford (1959) reported the highest prevalence upto 90% *S.mattheei* in cattle and more than 90% *S. haematobium* in human in the Transvaal lowveld of South Africa where man and domestic and wild animals often share the same water sources infested with intermediate host.

Dinnik and Dinnik (1965) mentioned that the diagnosis of schistosome infection is most commonly achieved through the demonstration and identification of eggs in feces. In addition counting these eggs is sometimes used as an indicator of the level of infection.

Niphadkar and Rao (1967) examined an unknown number of bandicoots for schistosome infection and were able to recover *S. spindale* worms from a single bandicoot.

Lawrence (1970), Pitchford and Visser, (1975) and Olaechea *et al.*,(1990) described and compared different fecal egg counting techniques based on the examination of between 50mg and 10g of feces. Although time consuming, fecal examination is a valuable diagnostic method for the field veterinarian dealing with groups of animals at risk, particularly severe morbidity in cattle is generally associated with high egg output.

Rao and Devi (1971) in a study observed a mixed infection in five out of twenty cattle in natural infections of *S. nasale* and *S. spindale.* In this study a large number of cattle, 168 out of 300, were infected with both *S. nasale* and *S. spindale.* They also claimed that development of heterologous resistance by *S. spindale* was responsible for protection of buffaloes against clinical nasal schistosomiasis.

Pitchford *et al.,* (1973) calculated the mean daily *S. mattheei* eggs out put from 149 cattle living in three areas near the Kruger National Park in South Africa and found it to vary between 208 and 358 eggs per animal per day.

Hussein, (1973), Southgate and Knowles, (1975) and Pitchford, (1977) recorded *S. bovis* in the Mediterranean region (Spain, Italy, Sicily, Sardinia, Corsica), the Middle East (Iran, Iraq, Israel) and in northern western and eastern Africa (except Egypt) which extended southwards to central Angola and Southern Zaire. Hussein, (1973) and Lawrence, (1978a) identified *S. mattheei* from south-eastern Africa, from the Cape Province in South Africa northwards to Tanzania and Zambia.

Christopher and Rao (1975) surveryed cattle, buffaloes, sheep and goats in Andhra Pradesh for schistosomiasis and found nasal schsitosomosis in 80 percent cattle and 60 percent buffaloes with its absence in sheep and goats. Interestingly, 50 percent of the animals, positive for *S. nasale*, were also positive for the eggs of *S. spindale*. The authors were of the opinion that *S. spindale* was responsible for immunity to cattle against nasal schistosomiasis.

Hiregoudar (1975) examined feces of 5 lions (*Panthera leo*) and found eggs of a number of helminthic parasites including those of *S. spindale*. Islam (1975) observed heterologous schistosome infection incorporating both *S. indicum* and *S. spindale* in a cow and a sheep. In a study in Sadui Arabia (Arafaa, 1976) showed that both urinary and intestinal schistosomiasis in most part of the country, with a patchy distribution and varying infection rates in human.

Pitchford (1976) recorded distribution of *S. leiperi* from Botswana, Namibia, Zambia and Tanzania. Pitchford (1977) recorded *S. margrebowiei* in northern Botswana, Namibia, Zambia, Southern Zaire and Chad. He also recorded *S. spindale* infections from India, Srilanka, Indonesia, Malaysia, Thailand, Laos and and Vietnam.

Agrawal (1978) and Agrawal and Sahasrabudhe (1982b) examined liver tissue and fecal sample of 31 cattle and 34 goats from the Jabalpur slaughter houses. Heterologous infection was seen in 37.5 percent cattle and 12.0 percent goats with *S. spindale* and *S. indicum* and could be detected by liver examination but rectum curettage showed only single infection.

McCauley *et al.,* (1980) estimated mortality in six- to 30-month-old cattle due to presumptive schistosomiasis was 7.1%.This mortality was higher for those herds under sedentary management than for migratory herds (9.4% vs 3.6%).

Agrawal (1981b) and Agrawal and Sahasrabudhe (1982a) reviewed the position of schistosomiasis in domestic animals in and around Jabalpur, Madhya Pradesh. They reported presence of at least three mammalian schistosome species (*S. spindale, S. indicum* and *S. incognitum*) in the area. The pigs were found to harbor only *S. incognitum*, whereas cattle, buffaloes and goats harbored *S. spindale* and *S. indicum* either separately or simultaneously. Schistosomiasis was more common in pigs and buffaloes as compared to cattle and goats. The authors tried to explain this variation on differences of behavior of these domesticated animals. For instance, pigs remain for a longer period in snail inhabited ponds / sewage in search of food as well as for resting. Likewise buffaloes spend more time in water for wallowing purpose. In contrast, cattle and goats visit water sources mainly for drinking hence spend less time.

An epidemiological study of *S. japonicum* in animals by Fernandez *et al.,*(1982) in two municipalities of the Eastern coastal plain of Leyte, Philippines, showed that pigs and dogs have the highest rates of prevalence. Dogs had the highest mean 24-hour egg output, and pigs the proportion of hatchable eggs. However, dogs serve an important role in maintaining the transmission of the parasite as indicated by a high transmission potential and the close habitual contact of the animal to human. Although necropsy of 49 dogs and 35 pigs which were coprologically and serologically negative revealed 10 dogs and 6 pigs with adult schistosomes in the mesenteric and portal veins.

Mohanty *et al.,* (1984) reported an out break of *S. spindale* in 11 bulls from Keonjhar district of Orissa. All the animals were of less than two years of age and were reported passing blood and mucus in their feces and suffering from colic pain. The out break was confirmed to be due to *S. spindale* by observing the characteristic eggs in the feces.

Malek and Ongon (1984) found *S.margrebowiei* from cattle and buffalo of Uganda.Saharee *et al.,*(1984) recorded *S. nasale* from cattle of Malaysia. Ramachandran *et al.,* (1986) recorded a cutaneous granuloma containing *S. spindale* worm from the left vulval labia of a 12 year old cow which appeared to be an unusual site from where the worm was recovered. Kumar and deBurbure, (1986) found that the distribution of *S. indicum* is apparently confined to the Indian subcontinent.

Rollinson and Southgate (1987) identified *S.margrebowiei* from cattle of Sudan.Rollinson and Simpson (1987) and Jordan *et al.,*(1993) described that except for *S.nasale* all species are parasitic in the mesenteric and rectal veins and are responsible for intestinal schistosomasis.In endemic areas, the occurrence of cattle schistosomiasis is known to be highly focal because of the underlying aggregated distribution of the intermediate snail hosts and somewhat restricted stock movement from one farm to another.

Rollinson and Simpson (1987) in their study observed and FAO (1993) reported about 530 million head of cattle live in areas known to be endemic for cattle schistosomiasis in Africa and Asia.Cattle generally move as a herd and therefore, individuals from the same herd are likely to be exposed to similar ranges of cercarial challenges.

Banerjee (1988) and Banerjee and Agrawal (1992) surveyed 310 buffaloes and 200 cattle respectively for hepato-intestinal schistosomiasis at Jabalpur by applying both hatching and sieving tests. Banerjee (1988) failed to identity by *Schistosoma* spp. in hatching positive cases and could report them only from egg positive cases which were 26 (8.39 percent) in buffaloes and 34 (17 percent) in cattle. Among the positive cattle 14 (41.2 percent) cases were positive for *S. spindale*, 14 (41.2 percent) for *S. indicum* and 6 (17.6 percent) for mixed infections of two. He also recorded that buffaloes, 10 (38.5 percent) were positive for *S. spindale*, 7 (26.9 percent) for *S indicum*, 1 (3.8 percent) for *Ornithobilharzia dattai* and 8 (30.8 percent) were positive for mixed infections of *S. spindale* and *S. indicum*. On analysis, infections of *S. spindale* was found more common in cattle (41.2 percent) than in buffaloes (26.9

percent) indicated that *S. spindale* was well adapted in cattle in this area. Agrawal and Shah (1989) reported *S. incognitum* from India, Thailand and Indonesia.

Rajkhowa (1990) examined cattle, buffaloes and goats and saw heterologous infection only in cattle, one with *S. spindale* and *S. indicum* and one with *S. spindale* and *S. nasale* and three with *S. indicum* and *S. nasale*.

Rollinson, *et al.,*(1990a) recorded *S. curassoni* from ruminants in Senegal, The Gambia, Mauritania, Mali, Niger and Nigeria.Lam *et al.,* (1991) reported one case of *S.japonicum* and 0.0% infection with other human *Schistosoma* spp. in Singapore.

Rajkhowa *et al.,* (1992) detected 2.9 percent *S. spindale* in cattle, 2.7 percent in goat and 16 percent in buffaloes from three districts of Assam. Jagannath and Setty (1992) reported a case of 6 year old cross-bred cow with non-febrile persistent diarrhea, reduced appetite and emaciations, which did not respond to sulphar drugs or antibiotics. The fecal examination revealed large number of *S. spindale* eggs. Agrawal and Alwar (1992) in their study found *S. nasale* from India, Srilanka, Pakistan, Bangladesh and Myanmar.

Chen (1993) found that *S.japonicum* is endemic in mainland China, Taiwan, the Philippines and central Sulawesi in Indonesia.

Vercruysse *et al.,(*1994) recorded *S.mansoni*, *S.bovis*, *S.haematobium* and *S.curassoni* from the Senegal river basin.They also carried a survey in an attempt to confirm the recent independent reports of the presence of *S. intercalatum*. Data based on egg morphology and zielnelson staining of egg shells suggested the possible occurrence of *S .haematobium* x *S.intercalatum* hybrids.

Imadeldin *et al.,*(1995) in their experimental infection with 30,000 cercariae of *S. bovis* found that *S. bovis* eggs first appeared in feces of the infected animals by week 5 post infection and all animals were shedding eggs by week 6 post infections.The animals were necropsied and perfused at week 12 post infection and tissue egg densities and worm burdens were found high.

De Bont and Vercruysse (1997) demonstrated that as for the identification of the parasite, the morphological characteristics of the eggs may be sufficient for differentiating species in some parts of the world such as the Indian sub-continent, but may of limited value in those places where several closely related species coexist and may interact.

Singh *et al.,* (1997) studied on cercarial dermatitis in paddy growing areas in Peninsular Malaysia. The authors observed that dermatitis in paddy planters occurred in paddy fields which were cultivated using animals such as buffaloes or fields where domestic animals were allowed to graze during the off planting season as these animals harbored the parasite. The authors observed that the causative agent of cercarial dermatitis was *S. spindale.*

Sing *et al.,* (2000) investigated the cattle and buffalo mortality with particular reference to hepatic / intestinal schistosomiasis. The authors found twenty five of 199 cattle and five of 105 Murrah buffaloes of either sex died within 15-20 days after showing symptoms of intestinal schistosomiasis.

Izhar *et al.,* (2002) reported 0.0% human schistosomiasis in Indonesia.Leping (2002) studied on the epidemiological variables for five years to find out the main infection sources of schistosomasis in Jiangsu marshland, the infection status of farm cattle and the contamination of marshland in four villages along the Yangtze river and two islands. The results showed that the prevalence rate of farm cattle ranged from 2.68%-15.25% withan average rate of 8.23%.The cattle fecal contaminant rate was85.15% in marshland and positive rate of schistosome egg in cattle feces was 3.05% with geometric mean of EPG 2.9.

Agrawal *et al.,*(2003) determined the prevalence of schistosomiasis along with other parasitic infections in cattle, sheep, pigs and goats in two districts of Orissa, India. The authors observed that the *Schistosoma* infection in cattle was 67% with *S. spindale* and *S. indicum* causing the 45% of the infection. They also observed comparison of the hatching method and formal ether methods revealed that hatching method was more sensitive in detecting schistosome infection but was not able to identify the species.

Agrawal *et al.*,(2004) conducted a survey work for helminth parasites of buffaloes of different age groups in the Malwa region of Madhya Pradesh. One of the helminthes reported by these workers was *S. spindale*, but no other details have been provided by the authors.

Krishnasamy *et al.,* (2004) conducted a one year survey in an area at Peninsular Malaysia showed that *S. spindale* had a high zoonotic potential in this area. Among the rodents, birds, bats and domestic animals examined for *S. spindale* infection, only the buffalo and the field rat were found positive.

Sumanth *et al.,* (2004) studied the incidence of nasal and visceral schistosomiasis in 300 male cattle that were slaughtered in 2001 at the Karnataka Meat and Poultry Marketing Corporation Limited abattoir in Bangalore, South India. The authors observed eggs of *S. nasale* and *S. spindale* in 197 and 151 samples and worms in 218 and 204 carcasses respectively. A mild infection of *S. nasale* was observed in 129 animals and mild infection of *S. spindale* in 124 animals a moderate infection of *S. spindale* in six animals. Twelve animals were heavily infected with *S. nasale* and 14 cattle were heavily infected with *S. spindale.* Mixed infections of both species occurred in 163 animals. The level of infection was determined by the eggs per gram methods and was found to be in the range of 1 to 310 eggs per gram for *S. nasale* and 1 to 201 eggs per gram for *S. spindale*, this was a higher result than the eggs per cubic centimeter methods, which indicated lower egg counts. Wiwanitkit (2005) stated that human schistosomiasis is sporadic in South Asian countries.

Liang *et al.,*(2006) investigated on the prevalence of infection among cattle in various province of China via hatch test,which ranged from 0 to 22.3%, with the highest prevalence in Luojiang.

Pfukenyi *et al.,* (2006) investigated an epidemiological study of *S. mattheei* infections in cattle in Zimbabwe. A total of 16264 (5418 calves, 5461weaners and 5385 adults) fecal samples were examined and 734 (4.5%) were found positive for *S.mattheei* eggs. Significantly higher prevalence was recorded in calves compared to adult and in wet season compared to dry season.

Steven *et al.,* (2006) estimated the association between the intensity of animal infection with *S. japonicum* and human infection in Samar province, the Philippines. The crude parasitological findings for animals indicated that 2.9% cats, 19.2% dogs, 2.1% pigs, 30.0% rats and 3.2% water buffaloes were infected and human infection was 17.7%. The authors found strong associations between the intensity of infection in cats and dogs and that in humans.

Zhuojian *et al.,*(2006) studied on the epidemiology and controlling techniques of *S. japonicum* of mountaing livestock in Sichuan province with a view to prevent and cure schistosomiasis.Epidemiology position and functions of every kind of domestic animals were revealed and controlling techniques were researched. Fcees on pasture, the majority of which was defecated by bovine were very important transmission factor. Epidemic situations of schistosomiasis among human being and animals had a marked and positive correlation (r 0.01%, P< 0.01).

Agbolade *et al.,* (2007) reported negligible prevalence 0.6% of human schistosomiasis from Southeast Nigeria.

Modena *et al.,*(2008) described natural infection with *S. mansoni* in wild vertebrates and domesticated animals in Brazil. Some species of wild rodents showed high infection rates under natural conditions.Despite showing low natural infection rates cattle were found very susceptible to infection under experimental condition.

Gholamreza *et al.,* (2008) reported 0.0% infection of human schistosomiasis in Iran. Yabe *et al.,* (2008) investigated an interaction among *Fasciola gigantica*, *Schistosoma* spp. and *Amphistomum* spp.and concurrent natural infections in Zambian cattle, based on egg and worm counts. The authors revealed (42.2%) of the abattoir examined cattle harbored one, two or all three trematodes of 133 cattle, 50 were randomly selected for worm and egg counts. The mean numbers (SD) of Amphistomum, *Schistosoma* and *Fasciola* were 62.1 (>97.9), 33.7 (>7.4) and 19.5 (>4.6) respectively. A total of 32% harbored all the three treamatodes, 66% had *F. gigantica* and *Amphistomum* spp. infections, 52% had *Schistosoma* spp. and *Amphistomum* spp. infections while 32% had *F. gigantica* and *Schistosoma* spp. infections.

Cherian and D'Souza (2009) screened fecal sample from 650 sheep belonging to fifteen districts of Karnataka for schistosome egg or miracidia following different laborotary techniques.The authors observed 44 (6.76%) samples were positive. Majority of the cases had *S. indicum* infection (95.1%) and very few were positive for *S. spindale*. Among the different techniques miracidial hatching test was found to be superior detecting natural ovine schistosomiasis.

Habtamu and Wolde (2010) conducted a study from April 2008 to February 2009 at Bahir Dar Woreda adjoining upper Blue Nile Abay river basin, northwest Ethiopia from benefit of repeated examination of fecal sample in diagnosis of bovine schistosomiasis and determining the prevalence of the disease in naturally infected cattle. Out of 687 fecal samples examined, 256 samples were found positive for *S. bovis* giving an overall prevalence of 37.3%.

Hai *et al.,* (2010) reported that water buffaloes are a major reservoir for transmission of *S. japonicum* to humans in China. However, in the Philippines, reports based on microscopic examination of buffalo feces identified a low prevalence of *S. japonicum*, and mathematical models using these data concluded that water buffaloes are not a major reservoir for transmission of *S. japonicum* to humans. They collected feces from 81 buffaloes in Macanip, Leyte, the Philippines, and assayed for *S. japonicum* infection by the Danish Bilharziasis Laboratory technique, the Kato-Katz technique, miracidia hatching, and a highly validated real-time polymerase chain reaction. The prevalence defined by each assay was 3.7%, 3.7%, 0%, and 51.5% respectively. Their results demonstrated that microscopic-based techniques dramatically underestimate the prevalence of *S. japonicum* infection in water buffaloes in the Philippines and warrant reexamination of the role of bovines in transmission of *S. japonicum* to humans in the Philippines.

Niaz *et al.,* (2010a) made an epidemiological investigation of human schistosomiasis in four districts of Pakistan showed that out of 2400 human fecal sample the out come of prevalence was noted zero.Three hundred human blood samples from the same study areas were also found negative for the prevalence of specific ImmunoglobulinG (IgG) antischistosoma antibiodies.

Niaz *et al.,* (2010b) conducted a prevalence study in cows and buffaloes at different sites of Punjab, Pakistan and overall prevalence was found 15.6% in cows and 15.1% in buffaloes.They also reported positive significant relationship with ecological variables.

2.2 Prevalence of Schistosome by Worm Count

Schistosomiasis in cattle is not diagnosed frequently by fecal sample examination (Agrawal, 1999). Many times, the sensitivity of the fecal sample examination as a diagnostic tool, which is the most widely used technique for a field veterinarian is questioned (De Bont *et al.,* 1991a). Hence it is essential to assess the prevalence of intestinal schistosomiasis by worm counts from mesenteric blood vessels. However, little information is available on the average worm burden in cattle. Only a limited number of prevalence studies have been conducted at a regional and national level, of which few included worm detection in animals from as many different herds as possible and were carried out in abattoirs.

Rao (1934) surveyed 112 bovines, 170 sheep and unknown number of goats for hepatointestinal schistosomiasis at Bellary and Anantapur districts of erstwhile Madras Presidency.The author was able to collect more than 500 schistosomes from portal veins from some of these animals. This collection contained a higher male population thus giving a sex ratio of 16:1.

Bhat (1942) reported *S.spindale* infection in two dogs causing illness while one dog died, the recovered after its treatment with sodium antimony tartate.

Salam and Sarwar (1953) reported *S.indicum*, *S.spindale* and *S. nasale* from various localities of Punjab province of Pakistan.

In Somalia *S.bovis* was recorded by Sobrero (1960) in134 out of 355 cattle. He examined a total of 336 cattle from west Tanzania which were slaughtered at Liebig's factory near Nairobi in 1949-61 at the Mwanza and Musoma abattoirs in the Lake Province of Tanzania and this showed that 66% of the local cattle were infected with schistosomiasis.In northern and central Tanzania 52% of cattle slaughered at Arusha

and 31% of those at Dodoma abattoir had schistosomiasis. In the southern highlands the disease was also common against cattle, possibly the incidence exceeds 50% but the small number of cattle examined (only ten animals out of which eight contained schistosomes) was insufficient to workout a more exact figure.

A survey carried by Dinnik and Dinnik (1965) at various abattoir showed that schistosomiasis in cattle was very common in East Africa. Forty nine percent of cattle from Karamoja in north east of Uganda which was slaughtered at Kampala abattoir were found to be infected with schistosomiasis in the Kenya highlands. In Riftvalley 663 out of 2365 beef cattle from European farms contained schistosomes in their mesenteric veins. The rate of infection varied from 100% (with heavy infection in some cases) on the farms close to Lake Bolossat with their swampy pastures to none from the dry pastures on ranches where the cattle were watered from trough. Twenty five percent of cattle in Wkambani and 23% of the Masai cattle in Kenya harbored schistosomes, while 16% of those from the Northern Province slaughtered at Isiolo were found to be infected.

Malek (1969) recovered schistosomiasis bovis from mesenteric blood vessels of cattle, sheep, goats, equines, the camel and the pig in the White Nile reservoir, along the White Nile South to Malakal in Singa, Gedaref, the Gezira and the Ingasane area in the east, in Darfur and Kordofan provinces in the west, in Bahr-el-Ghazal province in the south and near Khartoum.

Van wyk *et al.,*(1974) in their study showed that perfusions of untreated and healthy adult cattle form fams infected with *S.mattheei* in South Africa showed average counts of 2660 and 3634 worms respectively.They also recovered an average of 57721(range 41654-71083) adult *S. matheei* worms from five severely affected animals selected during an outbreak on a farm in South Africa.

In Bangladesh Islam (1975) examined the visceral organs of 224 cattle for the presence of *Schistosoma* species. The overall prevalence was 62% with 43% *S .indicum*,33% *S.spindale* and 13%mixed infection of both; animals older than 4 years had a higher prevalence of infection (95%) and had an average heavier worm burdens (147) than younger cattle (23) worms. One animal had a burden of 1665 *S. spindale* worms.

Lawrence (1977) counted 40679 schistosomes from one clinically affected animal from Zimbabwe. Dargie (1980) estimated that the overwhelming majority of cattle infected with *S. bovis* in Sudan had less than 500 worms.

McCauley *et al.,*(1984) conducted a study in the White Nile Province to characterise the history and signs of naturally occurring *S. bovis* infection in cattle (Gorag). Necropsy and laboratory examinations were performed on 10 animals, six to 30 months of age which were in poor condition. They were selected because of a history suggestive of schistosomiasis. All the animals showed some degree of *S. bovis* infection; eight had a moderate or high degree of infection.Signs of haemorrhagic diarrhoea, severely sunken eyed appearance and only moderate inappetance were common in animals with acute schistosomiasis.

Vercruysse *et al.,* (1984) conducted a survey of 5722 sheep and 1752 goats at the abattoir in Dakar, Senegal and showed that the overall prevalence of schisosomosis was 2.1%. Of the 112 animals where identification of the schistosome species was possible, all were infected with *S.curassoni* and 20.7%had mixed infection with *S.bovis*.

Kassuku *et al.,*(1986) showed from an abattoir survey of two farms that overall 30.8% cattle and 3.8% goats were infected with *S.bovis* in the area of Tanzania and field studies of two farms found that both providing good opportunities for schistosome transmission ecology of *S. bovis* in relation to different types of grazing and water supply.

Ndifon *et al.,* (1988) recovered adult schistosomes *S. bovis* and *S. curassoni* from the intraheptic branches of the portal vein and from the mesenteric venules of cattle in northern Nigeria. Rectal scrapings of 90 cows slaughtered at the Kano abaittoir, Nigeria during March and April 1986 revealed prevalence of 7.8% *S. bovis* and 2.2% *S. curassoni*. Further examination of the mesenteric and rectal veins of 502 cows showed that the overall prevalence of schistosomiasis was 31.1%.

In northern Thailand, Upatoom *et al.,* (1988) reported a prevalence of *S. spindale* of 60-80% sometimes associated with severe clinical symptoms and mortality, particularly in calves.

Anwar and Gill (1990) examined a total of 20,000 animals from different localities of Punjab province of Pakistan and 13% of cattle and buffaloes were found infected with *S.indicum* and *S.bovis*.The rate of infection was related to feed source (grazing, stall feeding and mixed) and water source.The highest rate of infection occured in grazing animals which drank stagnant water from pond and ditches.

Of the 112 animals where identification of the schistosome species was possible, all were infected with *S. curassoni* and 2.7% had mixed infection with *S. bovis*. By combining field and experimental investigation Vercruysse (1990) was able to study several new aspects of fundamental problems concerning animal and human schistosomiasis in Senegal. Epidemiological studies proved that in Senegal,*Bulinus guernei* is the main vector of *S.bovis,B.senegalensis* of *S. haematobium* (northern Senegal) and *B. umbilicatus* of S. *curassoni* and *S.haematobium* (southern Senegal.).

De Bont *et al.,* (1990a) surveyed an abattior and found that over 92% of the animals infected with *S. spindale* in Srilanka. De Bont *et al.*,(1991a) examined in abattoirs and found that vast majority of infected animals (>90%) had <100 worm pairs in the mesenteric veins. De Bont *et al.,* (1991b) examined the mesenteric veins of 901 cattle for the presence of *S. spindale* at a slaughterhouse in Srilanka revealed an overall of infection of 31.2%.

De Bont *et al.,* (1994) examined a total of 358 cattle for schistosome infection in Zambian slaughter houses. A total of 542 worms collected from 104 infected individual was examined for glucose-6-phosphate dehydrogenase and phosphoglucomutase using isoelectric focusing. The overall prevalence of infection was 51%. Ninety three percent of the infected animals had less than 100 worm pairs in the mesenteric veins. *S. mattheei* was the predominant species (75%), *S. leiperi* (12%) and *S. margrebowiei* (2%) were also identified.

Natural infections of *S. bovis* were observed in cattle slaughtered at Praia abattior on Santiago Island, Cape Verde by Rossa and Simoes (1999). *S. bovis* were collected from mesenteric veins and prevalence of infection was 3.5% and schistosomes were present in cattle of Monte Negro and Vale Cachopo. The main pathological finding was haemorrhagic enteritis. Various aspects of *S.bovis* were studied over a period in Iringa region, Tanzania.

An investigation to a primary school was conducted by Fenwick (2004) where the prevalence of intestinal schistosomiasis (*S. mansoni*) in children was in excess of 90% was unusally emaciated. To test the hypothesis that there may have been anthropozoonotic focus of *S. mansoni* within the local cattle herd a young female heifer of 9 months old was slaughtered from schistosome worms were later recommended by dissection. Microscopic examination of tubercles on the tegument of the male worms at high magnification (x400) showed that these worms were without spines, a characteristic of *S.bovis.*

Stothard *et al.,* (2004) investigated a routine parasitological surveillance and monitoring activities within a National Control Programme for control of human schistosomiasis in Uganda.The authors noted that cattle grazing in a watermeadow immediately adjacent *S. haematobium* is being endemic in the lake region of Tanganyika, East Africa and bovine bilharziosis occur 50% of cattle examined over a period of six months at the Mawanza abattoir were infected. All the eight cattle examined on three occasions at Nzega, and all the six cattle examined on three occasions at Bariadi were infected. Both *S. bovis*, a more northerly, and *S.mattheei*, a more southerly species, occur in this region.

Sumanth *et al.*,(2004) conducted a survey and found that overall infection rate of 68% based on the detection of *S. spindale* in the mesenteric veins of 300 animals, indicating that the disease was much more prevalent in an around Bangalore,where there seems to be a strong focus of infection.

Based on two post mortem techniques for diagnosis of visceral schistosomiasis from goat and sheep Vhora and Agrawal (2007) recorded 68 (34%) of the 200 goat mesenteries examined for *S. spindale*, *S. indicum* and *S. incognitum* flukes with the recovery varying from 1-28 flukes per mesentery. From 50 sheep mesenteries examined 22 (44%) were positive for *S.spindale* and / or *S. indicum* flukes with a range of 1-20 flukes per mesentery with higher concentration in heterologous infection. The other method was the examination of intestinal scrapings, which detected *Schistosoma* eggs in 113(66.5%) goats and 38 (76%) sheep. The authors showed lower eggs concentration in the small intestine, whereas the ileocecal junction and caecum had the highest egg concentration.

Ravindran *et al.,* (2007) reported the visceral schistosomiasis by worm counts from the mesentery of domestic ruminants of hilly district of Wayanad, located in Kerala, South India. They found 57.3% prevalence of visceral schistosomiasis in cattle, with the majority (58.1%) suffering from moderate infection. A considerable number of animals (41.8%) harbored a high intensity of infection. The highest number of worm pairs was 430 in a cow. In the case of buffaloes, the majority suffered from mild infection (66.7%) while the rest of the animals showed moderate infection (33.3%). Even though the percentage of positivity in goats was only 4.7%, among the positive cases, 42.8% harbored a high intensity of infection. Their findings showed that the prevalence of *S. spindale* infection was very high in Wayanad in comparison to previous reports from this and neighbouring countries.

2.3 Prevalence of Snails and Cercariae

The gastropod intermediate host is regarded as the most vulnerable link in the chain of transmission. A study of ecology, bionomics and population dynamics of the snails is accordingly essential for the understanding of the dynamics of the transmission for a critical assessment of control measures. Cercariae emerge daily from the snail host in a circadian rhythm, dependent on ambient temperature and light. Young cercariae are highly mobile, alternating between vigorous upward movements and sinking to maintain their position in the water. Cercarial activity is particulary stimulated by water turbulence, by shadows and by chemicals found on host skin (Wikipedia-Schistosomiais).

Lengy (1962b) collected over 350 *Bulinas trancatus* snails from a small swampy area of Regavin Israel upon examination 9% of these snails were found to shed cercariae subsequently identified as those of *S. bovis.*

In a study of lakeregion of Tangangiaka, Kinoti (1964) suggested that *Biomphylaria africanus ovoideus* was entirely responsible for the transmission of *S. bovis* in this region. He observed that streams were the main habitats of *B. a. ovoideus* and it would therefore be expected that the transmission of *S. bovis* mostly took place there. The author proved that in pools snail's populations build up rapidly in the dry season and with increased cattle watering cercarial infection rates went up. In the rainy season floods may greatly reduce snail populations.The transmission of *S. haematobium* by this snail followed the same pattern as that of *S. bovis*.

Soulsby (1965) in his study found that the optimum temperature ranged for development of the snail was 15-20^{0}c, when rapid production of snail egg masses occurs. These eggs hatched within two weeks and the resulting snail matured a month later. Thus one snail can produce several thousands descendents within a period of 10-12 weeks. No development and no reproductive activity took place at temperature below 10^{0}c but snail may survive adverse conditions for months buried at the mud.

Shaikh and Rahman (1968) identified at least nine species of snails in Bangladesh of which at least four species namely *Indoplanorbis exustus, Lymnaea* spp., *Planorbis* sp. and *Thiara tuberculata* were found as the possible intermediate host of *Schistosoma* spp.

Patnaik and Ray (1968), Muraleedharan *et al.,* (1975), Muraleedharan *et al.,* (1977) and Islam (1977) described that seasonal factors influence snail populations, their density, dispersal and survival rates, and transmission patterns in endemic areas. The age-distribution of the snail population in a locality is essential for an understanding of the transmission of the parasite.

Malek and Cheng (1974),Sing and Agrawal(1981) and Jairajpuri(1991) found several species of fresh water mollusks in Indian sub-continent including *Indoplanorbis exustus, Lymnaea luteola, L. auricularia, Planorbis planorbis, Paludomus obesa, Ferressia tenuis* and *Melanorides tuberculate* and acted as intermediate host of *S.incognitum* and *S. nasale* in the north part of India.

Rahman (1977) studied on the prevalence of cercariae from fresh water snails in and around the then East Pakistan Agricultural University campus. The author observed 14.9% infections by various cercariae in snail belong to six different species.

Ali *et al.,* (1978) reported abundance of biomass of the fresh-water snails of three fish ponds of Dhaka city. Rahman and Gillman (1978) observed prevalence of fresh-water snails in Bangladesh with their behavior and nature of habitat.They mentioned that mainly lymnaeid and planorbid groups of snails are economically important because they serve as intermediate hosts of parasite flukes of domestic animals. Very little is known about the ecology of these snails in the tropical countries and particularly in Bangladesh where a long summer rainfall and short dry winter weathers prevail.

Qadir (1980) observed that lymnaeids and planorbids snails were abundant from January to August ie during winter, summer and monsoon and from March to August ie summer and monsoon time respectively. Lymnaeiid and Planorbid snails live in deeper water and are close to bringing true aquatic snails in their behavior. They can however, adapt to an amphibious existence in adverse conditions.

Majid *et al.,* (1980), Chandiwana *et al.,* (1987b) and De Bont *et al.,*(1995b) found that the effects of rainfall and temperature were the most important factors, resulting in clear seasonal patterns of transmission in most habitats. For example in South Africa the prepatent period of *S. mattheei* in *Bulinus* snails can very from a mean of 38 days in the summer to 126 days in the winter (Pitchford and Visser, 1969). In Zimbabwe, *S. mattheei* is most intensive during the hot, dry season (September/November), moderate and variable during the rainy (December/February) and warm, post rainy (March/May) seasons, and markedly reduced during the cold, dry season (June/August).

Chandiwana *et al.,* (1987b) and DeBont *et al.,* (1995 b) observed that transmission occurred throughout the year on a farm with average worm recoveries ranging from 71 worms during the cold dry season to 736 worms at the end of warmer rainy season. There was a strong correlation between worm burdens at a particular time and the infected snail densities recorded at the main transmission site.

Madsen (1992) studied on the ecology of intermediate snail hosts and detected that infected snails were found almost only in well defined host water contact sites. The author recorded infection rates with schistosomes were often high upto 27% in *B. pfeifferi.*

Vercruysse *et al.*,(1994) recorded four species of schistosomes in the Senegal river basin as well as the following intermediate hosts namely *B.pfeifferi, B.globusus, B. umbilacatus, B.senegalensis* and *B.forskalii.* Recent ecological changes caused by the construction of dams at Diama and Mananatali on the Senegal river such as reduction of salanity, more stable water flow, creation of irrigation canals and development of rice culture have contributed towards the occurrence of new outbreaks of both intestinal and urinary schistosomiasis in the Senegal river basin with their intermediate host.

Rahman *et al.,* (1997) studied on the distribution and association for the development of *Furcocercous* and other cercariae in snails and their release with ecological and temporal variables for identification of risk factors. Of the 3512 snail examined during one year period *L. auricularia* var *rufescens* (26.0%), *L. luteola* (33.7%), *P. planorbis* (2.4%) and *I.exustus* (17.2%) were identified. The cercariae identified were *Furcocercus* (3.3%), *Gymnocephalus* (0.8%), *Echinostome* (1.8%) and *Amphistome* (2.4%).

De Bont and Vercruysse (1997) observed that contamination of transmission sites with feces containing schistosome eggs is essential for the transmission of the parasite. The dynamics of transmission intricate and exhibit variations dependent on behavior and habits of the bovines and the ecology of the molluscan intermediate hosts.Transmission patterns would undoubtedly influence the incidence, intensity and pathology of the infection in the bovine.

Narin *et al.,* (1998) observed that cercarial dermatitis of paddy field workers in Assam was very common. However it was not known which species of animal schistosomes is its causative agent in Assam. Studies in two dermatitis affected villages of Dihrugarh district revealed that cercariae of *S. spindale* was involved in its etiology.*I.exustus* the only snail species found infected with apharyngeate brevifurcate schistosome cercariae.An epidemic of cercarial dermatitis caused by *S.spindale* cercariae occurred in November1989 in district of Chaiya,Surajthani province in southern Thailand. Forty eight Thai farmers gave a history of itch following immersion in water while planting rice. The area of rice field was flooded for 10 days before farmers started working.The duration of the disease ranging from 2 days to 1 month.

Zhang *et al.,* (2000) and Zhou *et al.,* (2008) reported that the increase in population of snails ultimately increased the prevalence of schistosomiasis.

Yakubu *et al.,* (2003) collected 4119 snails from temporary flowing habitat and found that 204 (5%) of these were shedding human schistosome cercariae and schistosome infection rate was 0.6% in *Bulinus* and 7.8% in *Biomphylaria.*

Arijo *et al.,*(2005)described that snail fauna of Pakistan is quite rich and many species are involved in transmission of parasitic infection. They opined that it was interesting to found a good number of *Biomphalaria* spp. snail sheded *Fasciola hepatica* cercariae alone, with some snails were found to shed both *Fasciola* and *Schistosoma* cercariae.

A toal of 2802snails of different species from two fish ponds and one swamp in Czech Republic were examined, 951 (33.9%) were found to be infected with larval trematodes of 28 species (Faltynkova, 2005). In Quena, Egypt 2070 snails were examined about the infections of *S.mansoni* and a total of 0.48% infection was recorded.

A total of 2936 snail *Bulinus globusus* was examined by Pfukenyi *et al.,* (2006) in Zimbabwe and found that overall 2.5% *B. globusus* were shedding the cercariae in the highveld (range 0-18.8%) compared to 1.5% in the lowveld (range 0-4.5%). The shedding of schistosome cercariae was significantly higher ($P<0.01$) during the hot-dry season (September to November), post-rainy (March to May) and rainy season (December to February).

Issac (2009) studied on 648 snails comprising 392(60.5%) *B.globusus* and 256 (39.5%) *B. pfeifferi* obtained from stream and dam of Nigeria for collection of cercariae.Two schistosome cercariae were observed during the study namely *S.mansoni* and *S haematobium* with the former being more prevelant (20.3%) than the later.

2.4 Pathology of Schistosomiasis

Preliminary accounts on the pathology of *S. nasale* infections in cattle were described by Datta (1932) and Rao (1933,1934).The eggs deposited in the mucosa incite an inflammatory reaction with cellular infiltration and extensive fibrous tissue proliferation, leading to large cauli-flower like growths on the nasal mucosa.Rao (1934) briefly described the pathological changes observed in two calves slaughtered 86 and 118 days after exposure to 127000 cercariae of *S. spindale*, and Kalapesi and Puhorit (1954) reported the lesions found in the tissue of one naturally infected bull.

Sadun *et al.,* (1970) and Von Lichentenbery *et al.,*(1971) reported that severe liver fibrosis is much more common in chimpanzees with *S. japonicum* and *S. mansoni* and occasional oesophageal varices was found but there is no portal hypertension occured in this primate.

Warren and De Witt, (1972) and Warren and Moore, (1977) reported that mice infected with *S. mansoni* and *S. japonicum* developed hepatomegaly, spleenomegaly and portal hypertension. Bloch *et al.,*(1972) studied and revealed that microcirculation of eggs did not alone significantly obstruct liver blood flow, the large avascular granulomas which developed around them and the subsequent fibrosis were responsible for the pathophysiological consequences of this infection.

Warren, (1973) investigated an experimental murine model and unequivocally showed that the parasitic factor responsible for there changes is the egg.

Hussein *et al.,* (1976) experimentally infected five desert sheep with 5000 or 10000 *S. bovis* cercariae each and the parasitological, clinical and pathological parameters were recorded. The prepatent period was approximately 7 weeks and from that time onwards, the sheep become progressively ill and emaciated. Following necropsy at the 12th week, a percentage schistosome recovery ranging from 36-63% was found, and there were high egg densities in the intestinal tract and liver. These organs revealed severe pathological lesions.

Histopathology of twenty six Friesian steers infected with between 5000 and 45000 cercariae of *S. mattheei* were studied by Lawrence (1978b). The animals were slaughtered at intervals between 7 and 107 weeks after infection. Lesions developed in the liver, lung and intestines around dead parasites lodging in blood vessels, and many progressed to form lymphoid nodules. The large vein of the intestine becomes involved in an inflammatory reaction which may be an immunological response to antigen released from dead worms in the veins. The medium-sized intrahepatic portal veins become involved in a reaction which could represent a response to antigen released from dead and dying worms in the mesenteric veins. In heavy infections liver and lungs became grey with pigment regurgitated by the parasites.

McCauley *et al.,* (1982) worked on mortality in Sudanese cattle and found that 82% of the total mortality in 6 to 30 months age cattle is due to schistosomiasis.

Saharee *et al.,* (1984) reported a case of bovine nasal schistosomiasis in Malaysia. Clinically the animal had loud respiration, tachypnoea, bilateral serous nasal discharge and numerous raised nodules in the anterior nasal mucosa. Histopathological section showed an infiltration of eosinophils, lymphocytes, plasma cells, macrophages, fibroblasts and a few Langarhan's giant cell in the lamina propia of the nasal mucosa. A structure resembling *Schistosoma* eggs were also seen amidst these cells. Numerous boomerang shaped eggs were seen in the digested tissue biopsy sample.

Vercruysse *et al.,* (1985) studied the gross and histopathological lesions caused by natural and experimental *S. curassoni* infection in sheep.The data obtained showed that *S. curassoni* in sheep causes only slighty clinico-pathological manifestations with preferential involvement of the liver, the lower intestine and the urinary bladder.Variable spectrum of host reaction to the eggs within an individual animal was observed, reflecting the duration of presence of eggs in the organs. In the liver, egg granulomas were most numerous in the perilobular regions, while in the intestine, lesion were most pronounced in the mucosa of the rectum. The presence of eggs in 10% of the urinary bladders examined indicated some bladder involvement.

Obowolo and Rogers,(1988) made an inspection on bovine female genetalia at a major abattoir in north-eastern Zimbabwe and showed schistosome induced granulomas in uterine walls. Lesions were most severe in the myometrium and consisted of rings of multinucleated giant cells and macrophages occurring around eggs and masses of eosinophils on the outside. The parasite was presumed to be *S. mattheei*.They also detected lesions caused by eggs in the intestine, abomasum, forestomach, pancreas, lymphnodes, liver, lung, bladder and omentum. They reported haemorrhagic lesions throughout the large intestine and were responsible for acute diarrhoea during the early weeks after patency and were thought to result for immunological hyper-reactivity to migrating eggs.

Wang *et al.,* (1988) described an outbreak of bovine schistosomiasis japonica in Taiwan, in a herd of 75 beef cattle where 15 cattle were found dead after showing severe diarrhoea. The most striking gross lesions were multifocal parasite egg granulomas, found mainly in liver, lungs and intestines. Histologically, the circumoval granuloma consisted of *S. japonicum* eggs, surrounded by epithelioid cells and fibrous stoma.

A detailed description of the pathological features in infected animals was given by De Bont *et al.,* (1989a).They found that the lesions tend to get more severe in older animals.Compared with visceral schistosomiasis; nasal schistosomiasis is probably a slower developing progressive disease. The histopathology was similar to other schistosome infections are that granuloma formation, owing to the presence of eggs, is a common feature. The granulomas, which are often associated with Hoeppli reactions, began by accumulation of mononuclear cells and eosinophils. The numerous, striking Hoeppli phenomena observed in cattle infected with *S. nasale* may be correlated with a strong hypersensitivity reaction of the respiratory mucosa.

Fransen *et al.,* (1990) studied the pathology of natural *S. spindale* infections in cattle in Srilanka.The authors observed that hepatic lesions were moderate with periportal epitheloid cell granulomas with perilobular zones. Submucosal and mucosal granulomas accompained by cellular changes were present in the small and large

intestine.Two unusual observations included the migration of an adult worm from the mesenteric veins to the mucosa of the small intestines in one bull and the presence of epitheloid cell granulomas containing slender living eggs in the urinary bladder of one animal.They also found that these natural infections were of much lower intensity than those obtained experimentally in studies on *S. mattheei* and *S. bovis.* The authors observed egg granulomas mainly in the intestines and liver, and to a much lesser extent in the lungs. Hepatic lesions were moderate, with peri-portal cell infiltration and peri-portal epitheloid cell granulomas within perilobular zones.Submucosal and mucosal granulomas accompanied by cellular changes were present in the small and large intestines.

Chen (1993) considered that *S. japonicum* to be more severe than other schistosome infection because *S.japonicum* female worms have a much greater egg output than female of other species and because eggs are deposited in large aggregate leading to intense focal intestinal reactions Both the schistosome isolate and the host species are important determinants of the pathology induced by *S. japonicum*. However, little information was available on the pathology of natural *S. japonicum* infection in cattle. Johansen *et al.,* (1996a) and Saad *et al.,*(1984) observed the gross pathological and histopathological changes with *S. bovis* infection in goat and sheep. The gross pathological changes seen were mild to moderate. The liver appeared discolured and moderately enlarged. The intestinal lesions were most prominent in the small intestines, which showed catarrahal inflammation with numerous tiny corpuscles beneath luminal surface. The mesenteric lymphnodes were slightly to moderately enlarged. In contrast, on macroscopical examination, the infected animals invariably showed pronounced liver changes and marked enlargement of the lymphnodes, whereas the lesions in the intestines were comparatively slight. Histological lesions related to dead worms were seen in the livers of animals. These lesions characterized by pronunced inflammatory cellular infiltrates, thrombophlebitis, necrosis and periportal fibrosis.

Lindberg *et al.,*(1997) demonastrated a histopathological study of goats experimentally infected with *S. bovis.* They compared the characteristics of the inflammatory response in the small intestine and liver related to tissue egg counts and fecal egg output between goats at different time periods of primary infection followed by challenge. At early patency coinciding with increasing egg output, the intestinal lamina propia showed numerous intact schistosome eggs devoid of any inflammatory reaction. Later in primary infection and after challenge, intestinal changes were marked by a granulomatous anti egg response with only a minor component of egg lacking inflammatory change.Hepatocellular necrosis with eosinophil infiltration was pronunced only during the early patent stage. The results indicated that the early cascade of fecal egg output in caprine *Schistosomiasis bovis* is aided by a low degree of tissue reactivity to eggs in the intestine.

Ferreras-Estrada *et al.,* (1998) evaluated the pathological response of sheep to two levels (400 or 1000 cercariae) of *S. bovis* after 24 weeks of infection. The results confirmed that a single low or high dose causes lesions in the liver and intestines, and that the lungs, lymphnodes, pancreas and abomasum were affected in sheep given a single high dose. In addition, the study showed that pathological changes (mainly a granulomatous inflammatory reaction) were induced not only by eggs but also by adult worms, and their severity was in general related to the dose of *S. bovis.* Hoeppli reaction product, observed on the surface of adult schistosomes in some parasitic gramulomas.

Maria *et al.,* (2000) investigated the tissue responses of pigs exposed to either 100 or 2,000 *S. japonicum* cercariae at 4, 11, 17 and 24 weeks post infection to explore the pig as an animal model for pathologic aspects of human *Schistosomiasis japonica.* Egg granulomas were present in the liver intestine and occasionally in the lungs from 11 weeks post infection. There were also many free eggs and early exudative reactions to eggs in the intestine were found.At 11 weeks post infection pigs in the higher dose group showed marked periportal and septal fibrosis with minimal parenchymal destruction.There after, lesions regressed spontanaeously as the pigs underwent a self-cure. The lower dose group showed only mild lesions throughout the study. The

degree of hepatic fibrosis was correlated with the density of eggs and granulomas in liver tissue. The result indicated that the pig would be particulary useful for studies of the development and resolution of schistosomal hepatic fibrosis, and also for investigations of the mechanisms behind the self-cure phenomenon.

Chiti *et al*.,(2000) made a post mortem examination of an adult male Jack Russell dog from Zambia and revealed that it was heavily infected with schistosomes.At necropsy the liver was found to be enlarged with multiple pinpoint yellowish white foci scattered diffusely through out the organ. Multiple pinpoint recent and old haemorrage were seemed on the mucosal surface of the gastrointestinal tracts particularly in the stomach and duodenum. Large number of schistosome worm pairs and eggs were found in all mesenteric lymphnodes, liver,spleen, pancreas,stomach and lungs revealed numerous strongly fibrotic encapsulated epitheloid giant cell granuloma containing dead, degenarating and viable eggs.DNA analysis of female worms indicated that the schistosomes were either *S.haematobium* or a hybrid of *S.mattheei* and *S.haematobium*.

Soren *et al.,* (2009) observed in goat after treatment in experimental *S. bovis* infections that pathological effects in schistosome were mainly due to immune responses to the eggs of the parasite leading to intestinal and hepatic granulomatous disease and hepatic fibrosis.

2.5 METHODOLOGY

The study was conducted through 5 investigations under the following headings:

- Investigation1. Prevalence study by fecal egg examination and to identify associated risk factors of schistosomes by egg count
- Investigation 2.Prevalence study by mesenteric blood vessels examination with intensity of parasites
- Investigation 3. Study of snails for the development of *Furcocercus* cercariae in snails and their release
- Investigation 4. Miracidial hatchability
- Investigation 5. Pathology of naturally infected schistosomiasis of cattle by *S.indicum*.

The data related to herd and animal level were collected by interviewing farmers and clinical examination of the animals.Data from each animal and herds were recorded by means of a designed questionnaire. The animal-level co-variates were age, breed, gender, physical condition and consistency of feces.The herds were examined for the selected variables.The herd-level co-variates were production type, grazing, feeding of cut-grass, sanitation, diet and de worming.The age of the animal were determined from the official record book or by asking the farmers or by examining the teeth (Sharma, 1981). Physical condition of the animal was determined by the methods used by Samad, (2001). The other co-variates were recorded either by distance inspection or by physical examination or by interviewing the owners.

For the convenience of the study naturally infected animals were categorized into three age groups describing the method of Samad (2001).These are-

6 months to <1.5 years as calves

1.5 years to 3 years as yearlings

> 3 years as adult animals.

Other co-variates will also be categorized into different groups, such as :

Breed: Indigenous or Cross

Gender: Male or Female

Physical condition: Moderate to Very thin, Slight thin or Normal.

Consistency of Feces: Normal or Liquid

Diet: Good or Poor

De worming: Yes or No

Production type: Subsistence or Commercial

Grazing: Yes or No

Sanitation: Poor or Good

At the time of collection of sample meteorological data from each area was collected from local sources.

CHAPTER 3
INVESTIGATION 1

PREVALENCE STUDY BY FECAL EGG EXAMINATION AND TO IDENTIFY ASSOCIATED RISK FACTORS OF SCHISTOSOMIASIS BY EGG COUNT

3.1 INVESTIGATION 1

PREVALENCE STUDY BY FECAL EGG EXAMINATION AND TO IDENTIFY ASSOCIATED RISK FACTORS OF SCHISTOSOMIASIS BY EGG COUNT

3.1.1 INTRODUCTION

Inadequate feed, widespread diseases and inefficient extension services are considered among the most binding constraints of livestock development in Bangladesh (FAO STAT, 2003). At least two- thirds of the total livestock populations (23 million) are infected sub- clinically with parasitic helminth infections (Garrels, 1975). Sub-clinical diseases are often ignored resulting of heavy economic losses. The economic losses due to sub-clinical problems in populations are significantly higher than clinical problems in individual animal (Martin *et al.,* 1994). A prerequisite for disease control/prevention is to identify factors responsible for that disease. Knowledge of the risk factors facilitates identification of categories of animals that are at particular risk of developing an infection (Thrushfield, 1986). Risk factors for animal health and production problems include specific etiologic agents, host factors and environmental factors (Dohoo *et al.,* 1996).

Epidemiology is the study of a disease in populations and of factors that influence the occurrence of a particular disease. Therefore, epidemiology of any disease of cattle is the indespensible basic science of cattle population health that provides the logical framework for the facts that enable the scientists to identify health problems and to delineate their dimensions.Epidemiological methods are used to these health problems of dairy industry; to classify, identify and elucidate their causes; and to plan and evaluate rational control measures. As a result, it needs the case control study, a systematic extension of routine history taking of cattle and therefore, a cohort study that identifies cattle in a defined population who are exposed to varying levels of known or suspected risk and the population is observed over a certain period.Epidemiology deliberately deals with the disease triad- the agent, the host

cattle and the environment.Agent factors include dose, environmental hardiness, virulence, infectivity, toxicity. Host factors include innate resistance, previous exposure, age, gender, behaviour, production status, vaccination status and response, reproductive status, genetics etc. Environmental factors include animal stocking density, animal movements between groups, housing, environmental conditions and nutrition etc.

Knowledge of the associated factors of a disease is an aid in differential diagnosis also. About 530 million heads of cattle live in areas of endemic for cattle schistosomiasis in Africa and Asia which at least 165 million cattle are infected with schistosome's world wide (De Bont and Vercruysse, 1997). Although little or no overt clinical signs may be seen over a short period, frequent chronic schistosome infection in the long-term cause significant losses of the herd (Ravindran *et al.,* 2007). Detailed studies on the epidemiology of *S. bovis* and *S. mattheei* in cattle in parts of Africa have been reported (Lawrence, 1978a, Kassuku *et al.,* 1986) but little information exists on the epidemiology of bovine schistosomiasis in Asia.

Hence, this study was undertaken with a view to fulfill the following objectives:

(i) To asses the prevalence of intestinal schistosomiasis by fecal examination from live animals.

(ii) To determine the risk factors of the infection by fecal egg count.

(iii) To findout the relationship of prevalence with temperature and rainfall.

3.1.2 MATERIALS and METHODS

The purpose of this Chapter is to describe the methods and procedures followed in collecting data for achieving the objectives of the study. This Chapter is divided into three sections. The first section deals with selection of the study areas and samples. The instruments and techniques of collecting data are described in the second section. The last section describes the statistical techniques used for analysis of the data.A pre-survey was carried out in five different study areas, namely Mymensingh, Netrokona,Kishoregonj, Chittagong and Sirajgonj districts of Bangladesh before developing the forms for recording data.The pre-survey activities included examination of the topography of the region, socio-economic condition of the farmers, types of herds and management system of the farms.Appendix 1 shows the forms for the examination of farms and animals that were validated by pre-survey and interaction with the farmers.The use of ecological terms is in accordance with the definitions of Margolis *et al.*,(1982).

3.1.2.1 Study design, selection of farms and sampling

The study was a cross-sectional observational type and conducted in five different districts of Bangladesh. A multi-stage stratified random sampling method was used to select animals from different villages, management systems, farms, age groups and sex groups. Farms in each areas/ villages were categorized into subsistence and commercial. A total of 330 subsistence cattle farms were included in this study.Among them the study was carried out on 51, 45, 54, 41 and 39 subsistence farms of Mymensingh, Netrokona, Kishoreganj, Chittagong and Sirajgong districts respectively. A total of 60 (12 farms from each district) commercial farms were identified and included in this study. All the five districts were selected on the basis of endemicity report of schistosomiasis in those areas prepared by DLS (2001) (Directeriote of Livestock Service), Bangladesh. The preliminary field observations and descrete water contact points where animals consistently move for grazing and drinking water were considered. For this study a farm is defined as any livestock establishment with a minimum of one animal. During this study some farm is continued with its normal management practices including anthelmintic treatment.

3.1.2.2 Study period

The areas were prospectively studied for two years from September 2009 to August2011. The climate of the study areas was tropical with summer and monsoon rains. In Bangladesh three main seasons can be distinguished, namely the cool-dry winter from November to February, the hot dry summer from March to June and the hot-wet rainy season from July to October (Ahmed *et al.,* 1989). The average monthly temperature of the study areas of Bangladesh varied from 20^0c to 37^0c. Rainfall occurring mostly during monsoon (April to September average 2234.0 mm).Relative humidity at 8.0 am varies from a monthly average of 50% to 95%.

3.1.2.3 Locale of the study

The study was conducted in five selected districts of Bangladesh. The study areas were selected by considering its geographical position, topography of the land and primary report of diseases investigated by Directorate of Livestock Services, Bangladesh.The areas are:Plain areas of Mymensingh district, low lying areas of Netrokona district, marshy areas of Kishoregonj district,hilly areas of Chittagong district and char areas (eroded river island)of Sirajgonj district.

For collection of data from Mymensingh district two upazilas were selected. One union from each of the upazilas was the locale selected of the study.A total of six villages from each of the two unions were selected randomly and included in this study.Similar methods were followed for other districts (Netrokona, Kishoregonj, Chittagong and Sirajgonj).

Flow diagrams of the selection procedure of the study areas have been shown in Figure 2,3,4,5 and 6. Map of the Bangladesh showing study areas have been presented in Figure7. Geographical location and agro-ecological data of the study areas have been presented in Table 1.

STUDY AREAS

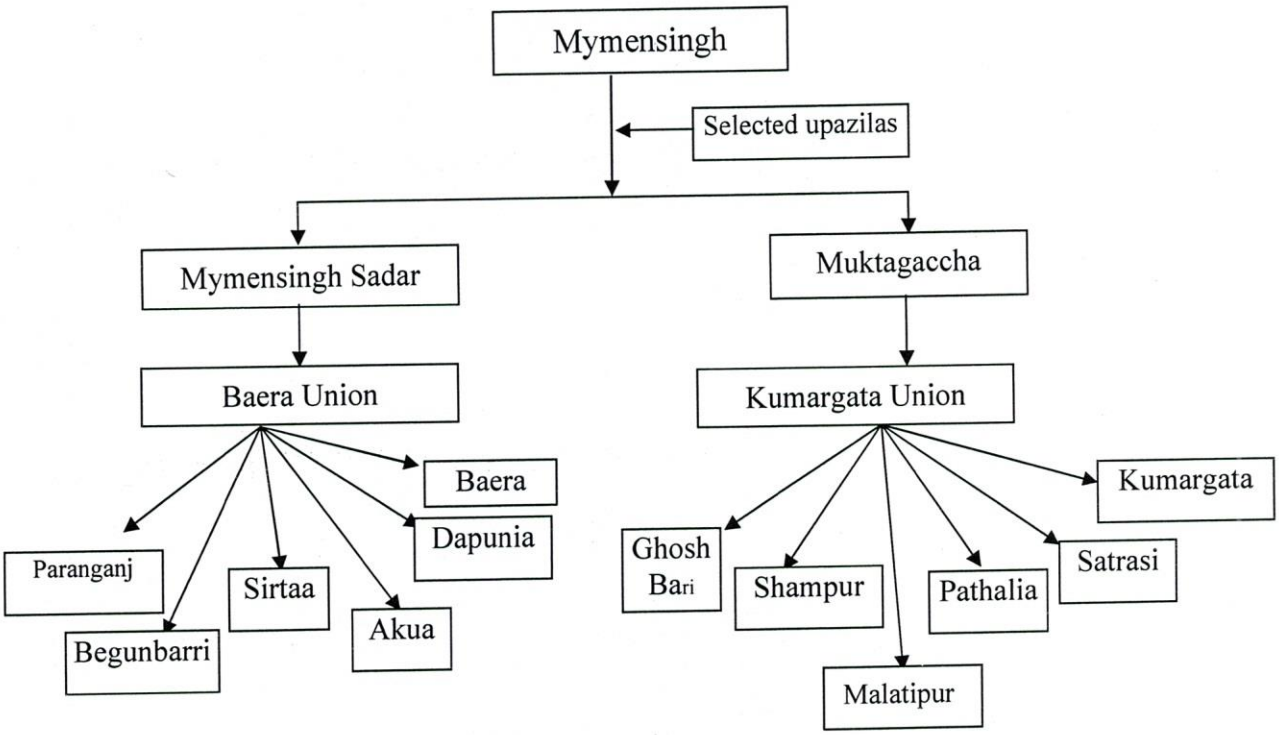

Figure 2: Diagram showing the selection procedure of Mymensingh district

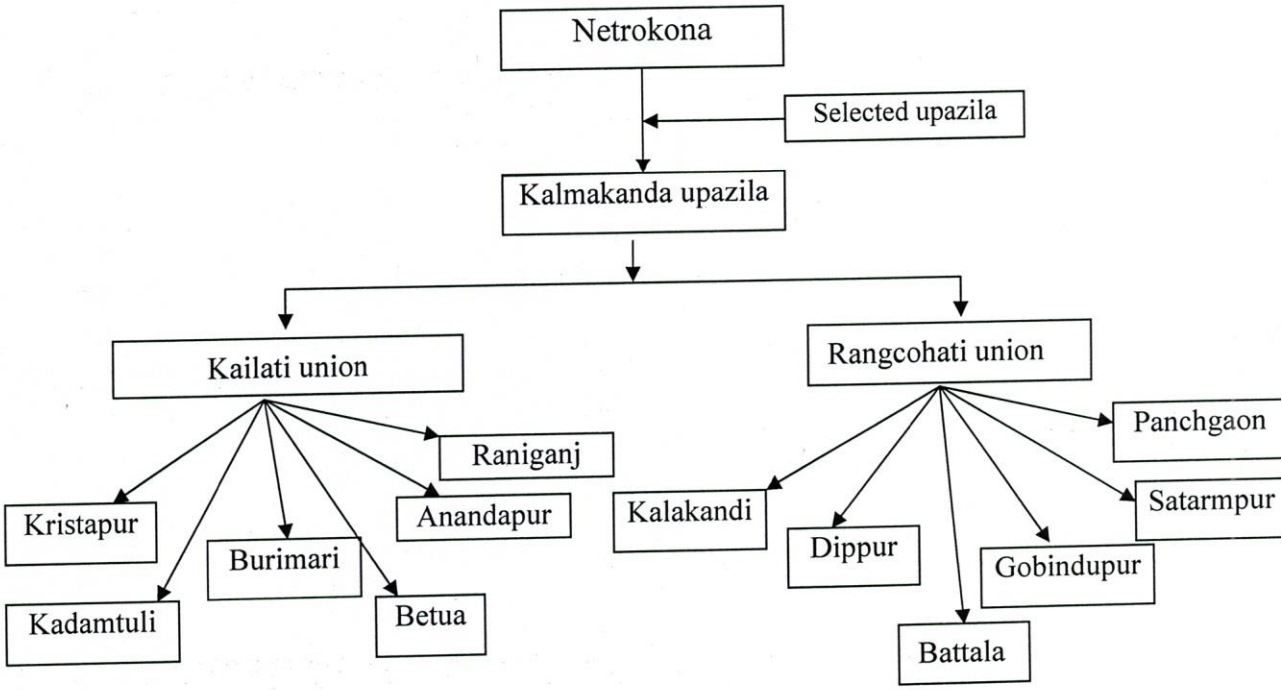

Figure3. Diagram showing the selection procedure of Netrokona district

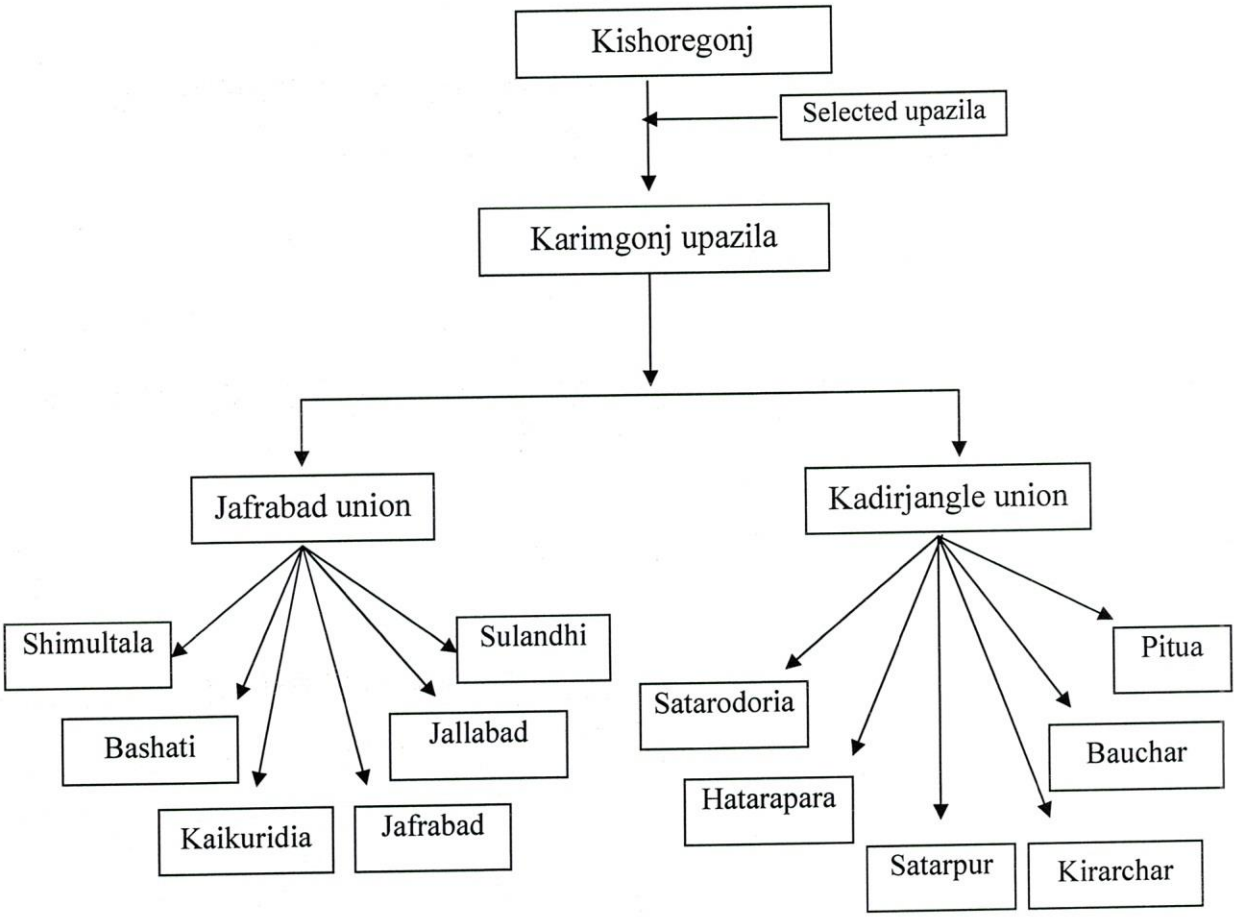

Figure4. Diagram showing the selection procedure of Kishoregonj district

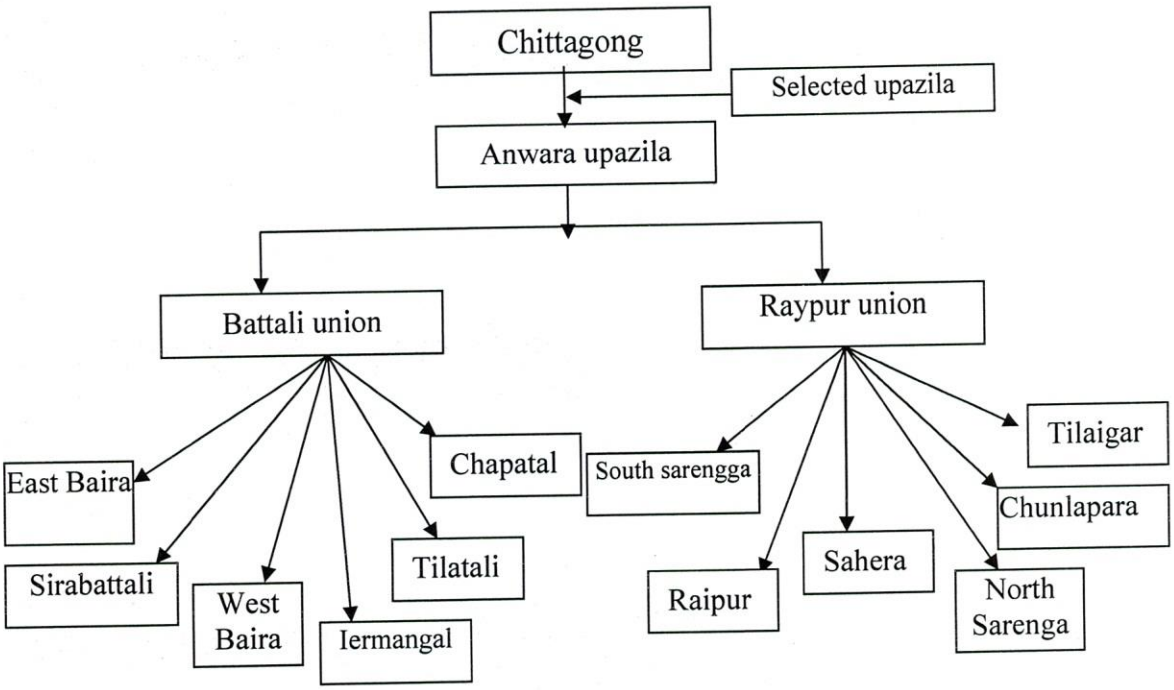

Figure 5. Diagram showing the selection procedure of Chittagong district

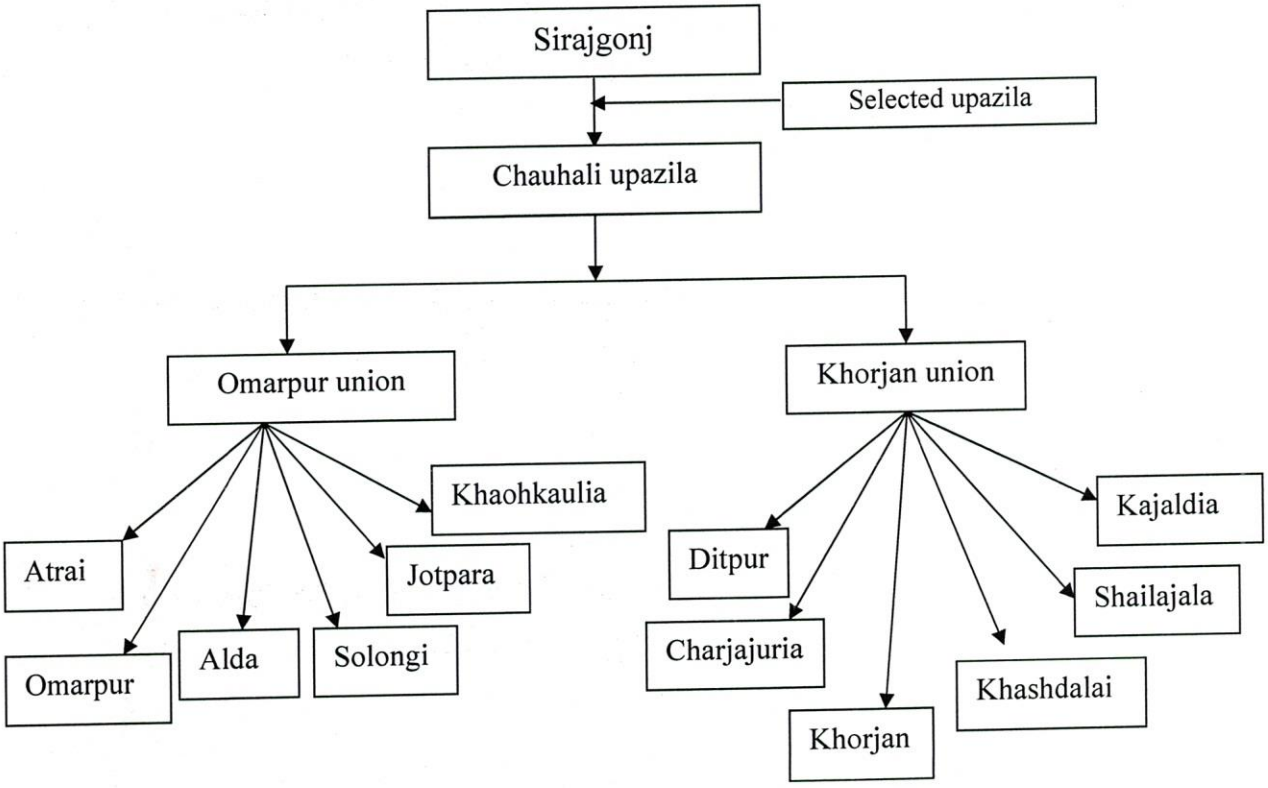

Figure6. Diagram showing the selection procedure of Sirajgonj district

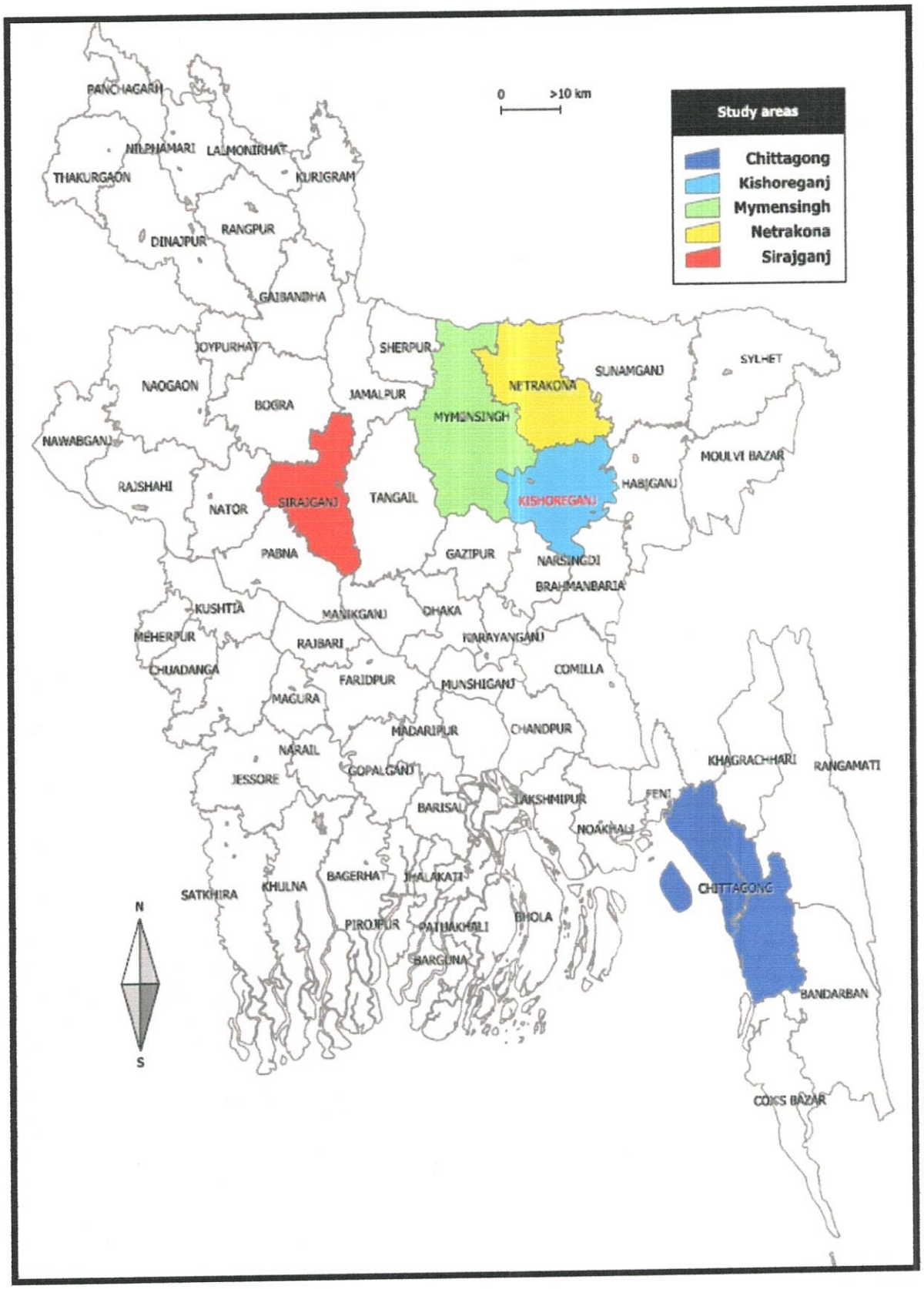

Figure7. Map of Bangladesh showing study areas

Table1. Agro ecological data and geographical locations of the study areas

Location	Agro ecological zones	Description		
		Soil	Mean annul temperature (^{0}c)	Annual mean rainfall (mm)
1.Mymensingh district is at24.7500°N latitude and 90.4167°E longitude.	9 old Brahmaputra flood plain	Soil type: Dark grey flood plain and grey piedmont, hill brown and terrace. Soil texture: Medium to very strongly acidic. Land type: Medium high, Medium low and high land.	25.3-26.5^{0}c	2000 mm in the west and 4000 mm in the north west.
2. Kishoregonj is locatedat24.433°N latitude and 90.7833°E longitudes.	9 old Brahmaputra flood plain	Soil type: Alluvial sand.	33.3^{0}c	2174 mm
3. Netrokona is at the position of 24.88°N latitude and 90.73 °E longitude.	9 old Brahmaputra flood plain	Soil type: Alluvial sand. Land type: Medium low, Medium high and high land.	33.3^{0}c	2174 mm
4. Sirajgonj is located at North latitude of 24.1708° and East longitude of 89.5875°.	7 active Brahmaputra-Jamuna flood plains.	Soil type: Dry grey, heavy acidic clay soils. Soil texture: Non-Cal careous grey flood plain. Land type: Medium low land to medium high land.	25.8-25.5^{0}c	1500 mm in south east and 1800 mm in north east.
5. Chittagong is located at the position of North latitude of 22.4833° and East longitude of 91.9667°.	18 Young Meghna estuarine flood plain	Soil type: Relatively uniform brown hill soils. Soil texture: Loamy and loamy skeletal. Land type: Mostly flat, alluvial plain, hilly in southeast	33.3^{0}c	2687mm

Source: Report-2. Agro ecological regions of Bangladesh; Report prepared for the Government of People of Bangladesh by FAO, Rome-1988(Modified).

3.1.2.4 Fecal sample collection and examination

Fecal sample were collected for examination of parasitic egg with its count. A total of 2514 fecal samples of the naturally infected cattle of the study areas of the 330 subsistence farms and sixty commercial farms were collected weekly.Fecal samples were collected directly from the rectum (Plate1) and also from recently voided feces using gloved fingers. The samples were labeled and few drops of 10% formalin were added to fix the eggs of schistosomes. The samples were then transported to the laboratory of Parasitology Department of Bangladesh Agricultural University, Mymensingh and refrigerated at 4^0c and examined.

3.1.2.5 Examination of fecal sample

The fecal samples were examined to findout the eggs of schistosomes by direct smear method as described by Soulsby (1982) and Rahman *et al.*, (1996).

3.1.2.6 Direct smear method

The technique was as follows:

A drop of water was placed on the center of a clean glass slide.A small amount of feces is detached from the given sample with the help of tooth pick and spread out to form a thin smear(thick smears would have prevent passing of light through it). This can be done gently drawing the coarse particles towards a side on the glass slide.A suitable coverslip preferably was put over the smear and slide was then placed under the low power objective of a microscope for examination. In total three slides from different parts of the fecal samples from each animal were examined.

3.1.2.7 Fecal egg count

Individual schistosome fecal egg counts were carried out by using the concentration technique of Lawrence (1970) modified as follows:

Two gm of feces were thoroughly mixed with 10% formal saline, passed through a coarse sieve and washed successively through Endecott sieves of 212 and 38 μm aperture. The material on the last sieve was centrifuged in a graduated test tube and residue topped up with a 2% methyl cellulose solution added to the 4 ml mark. The

viscosity of the later solution allows the formation of a uniform suspension of the fecal material and fluke eggs. On two separate occasions, schistosome eggs were counted in 0.1 ml of the thoroughly mixed suspension of each egg counted representatively 10 eggs per gram feces (EPG). The individual animal was categorized infected by positive egg.The prevalence of the disease was calculated as described by Thursfield (1995).

The schistosome eggs were identified on the basis of their characteristics morphological features as described by Soulsby (1982).

1. Spindle-shaped and 200µm x 70-90µm with a terminal spine .....*Schistosoma spindale.*
2. Oval-shaped and 57-140µm x 18-72µm with a lateral spine ..... *Schistosoma indicum.*
3. Boomerang shaped and 350-380µm x 50-80µm .....*Schistosoma nasale.*

3.2 RESULTS

3.2.1 Data exploration

In this study 2,514 fecal samples from naturally infected cattle of the study areas (330 subsistence farms and 60 commercial farms) were examined through the year ofSeptember 2009 to August2011. The total population of male cattle is 1121 and female cattle is1393. Mean age of the animal is 5.54±0.08. In subsistence farming system most of the cattle was indigenous breed and in commercial farms most of the cattle population was Frisian cross and Holstein cross. A total of 1,097 mesenteric blood vessels from the local slaughter houses of the study areas were sampled. Mean age of the slaughtered animals are 9.3 ±0.09 and most of the slaughtered animals are male and indigenous breed with few female and cross bred animals. During this study 5,272 snails were examined which are collected from four different inhabitants during January 2010 to December 2011. Temperature and rainfall data from five regions (Mymensingh, Netrokona and Kishoregonj districts recorded data from same meteorological station) were also analyzed for the same period. To study the histopathology of naturally infected animals by schistosome parasites 150 visceral samples (50 liver, 50 small intestine and 50 large intestine) were collected from different local markets of the study areas.Sampling scheme to study the prevalence and risk factors of schistosomiasis by fecal examination in the selected study areas is presented in Table2.

3.2.1.1 Statistical analysis

The data obtained from the questionnaire and laboratory tests were entered in the application. The collected data were compiled, coded, tabulated and analyzed in accordance with the objectives of the study.Descriptive statistics were performed to describe the presented data.

The data consists of qualitative egg examination from feces and quantative egg count by egg per gram (EPG) method from fecal samples of live animals, adult schistosomes counted from mesenteric blood vessels, miracidia counted from snails and weather data from secondary sources. Some animal level variables and herd level variables

were also collected during sample collection using a pre-tested questionnaire. All data were entered in Microsoft Excels 2003 and transferred to R 2.14.2 (The R Foundation for Statistical Computing, Vienna, Austria) for both descriptive statistics and regression analysis. Descriptive statistics were obtained from data mining package of the said software. For the identification of risk factors of bovine schistosomiasis from egg count, poisson regression is traditionally used.

A pre-requisite for association study is that the explanatory variables should be statistically independent. One of the problems that complicate association study is multicollinearity. Multicollinearity occurs when the predictor variables are related to each other. Multcollinearity results in unstable estimates of regression coefficients, incorrect variance estimates for the coefficients of those parameters in regression models leading to inflated standard error and loss of statistical power. Pairwise correlation (Spearman's) analysis was used to determine if any pairs of predictor variables are highly correlated and therefore likely to result in multicollinearity. If any such pair is found, one of the predictor variable is selected for inclusion in the final analysis. (Dohoo *et al.,* 1996). A multiple poisson regression model was built using backward elimination method (Dohoo *et al.,* 2003). The goodness of fit of the poisson model was evaluated by poisgof (poisson goodness of fit) test. In case of poisson regression of egg counted from fecal samples of live animal, the poisgof was significantly different from zero (χ^2=81407.0, df=2503, p<0) which indicates there is over dispersion in the data. So accordingly a negative binomial regression model with backward elimination method was fitted (Gurmu and Trivedi, 1996). Then negative binomial regression model was compared with poisson regression model using vuong test (Vuong, 1989). A significant vuong test with a large negative test statistic (-19.94, p<0.000) indicates that the second model (i.e. negative binomial) is superior than poisson model.

Table2. Sampling scheme to study the prevalence and risk factors of schistosomiasis by fecal examination in the selected study areas

Criteria	Mymensingh	Netrokona	Kishoregonj	Chittagong	Sirajgonj	Total
Selected herds	63	57	67	56	87	330
Number of cattle	517	436	414	481	660	2514

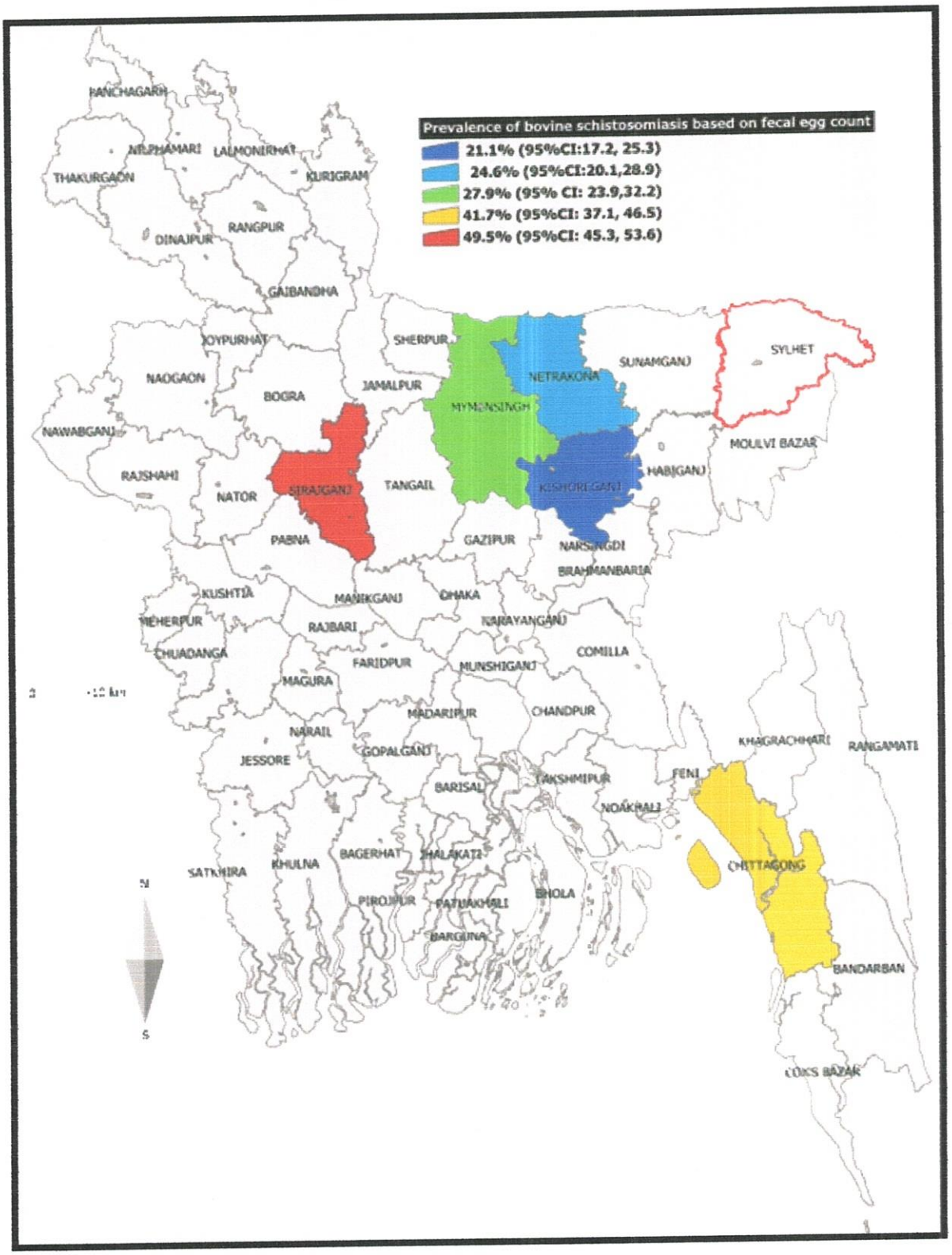

Figure8. Map of Bangladesh showing distribution of prevalence of bovine schistosomiasis based on fecal egg examination

3.2.1.1 Prevalence based on fecal egg examination

An overview of data describing overall prevalence and distribution of bovine schistosomiasis based on fecal egg examination are presented in Table 3 and Figure 8.Collected eggs of schistosomes were identified as *S.indicum* and *S.spindale* are presented in Plate 4a and 4b respectively. Eggs of *S.indicum* were characterized by oval shaped with a lateral spine and eggs of *S.spindale* were characterized by spindle shaped with a terminal spine (Soulsby,1965). About 29.9% (751/2514) and 10.6% (267/2514) cattle were found to be *Schistosoma indicum* and *Schistosoma spindale* egg positive respectively which are statistically different.The overall prevalence of bovine schistosomiasis in Bangladesh is 33.2%. Based on Chi-Square test results, significant variation in the prevalence of bovine schistosomiasis are observed according to districts, ages, breeds, sexes, physical conditions, fecal consistency, sanitations, grazing, diets, de-worming, farming systems and seasons.

Table-3.Prevalence and distribution of bovine schistosomiasis based on fecal egg examination in Bangladesh (n=2514)

Variables	Tested	Positive	Prevalence (%)	95% CI
Schistosoma indicum: (n=751),Prevalence 29.9%				
Schistosoma spindale: (n=267),Prevalence 10.6%				
District***				
Chittagong	484	199	41.7	37.1, 46.5[b]
Kishoregonj	437	92	21.1	17.4, 25.3[a]
Mymensingh	517	130	27.9	23.9, 32.2[a]
Netrokona	451	113	24.6	20.1, 28.9[a]
Sirajgonj	625	301	49.5	45.3, 53.6[b]
Age* **				
Calf (≤1 yr)	353	96	27.2	22.6,32.2[a]
Young (>1-3 yrs)	824	263	31.9	28.7,35.2

Contd.....

Variables	Tested	Positive	Prevalence (%)	95% CI
Schistosoma indicum: (n=751),Prevalence 29.9%				
Schistosoma spindale: (n=267),Prevalence 10.6%				
Adult (>3 yrs)	1337	476	35.6	33.0,38.2[b]
Breed* **				
Indigenous	1821	700	38.4	36.2, 40.7
Friesian Cross	693	135	19.5	16.6, 22.6
Sex* **				
Female	1220	316	25.9	23.5, 28.5
Male	1294	519	40.1	37.4, 42.8
Physical condition* **				
Normal	860	186	21.6	18.9, 24.5[a]
Slightly thin	403	154	38.2	33.4, 43.2[b]
Very thin	1251	495	39.6	36.8, 42.3[b]
Fecal consistency* **				
Normal	1464	118	7.9	6.6, 9.4
Liquid	1050	717	67.0	64.0, 69.8
Sanitation* **				
Poor	1252	500	39.9	37.2, 42.7
Good	1262	335	26.5	24.1, 29.1
Grazing* **				
No	973	242	24.9	22.2, 27.7
Yes	1541	593	38.5	36.0, 40.9
Diet* **				
Poor	1479	570	38.5	36.1, 41.1

Contd.....

Variables	Tested	Positive	Prevalence (%)	95% CI
Schistosoma indicum: (n=751),Prevalence 29.9%				
Schistosoma spindale: (n=267),Prevalence 10.6%				
Good	1035	265	25.6	22.9, 28.4
Deworming* **				
No	1021	385	37.7	34.7, 40.8
Yes	1493	450	30.1	27.8, 32.5
Farming system* **				
Subsistence	1598	611	38.2	35.8,40.7
Commercial	916	224	24.4	21.7,27.4
Season* **				
Winter	855	183	21.4	18.9, 24.3[a]
Summer	618	187	30.3	26.7, 34.0[b]
Rainy	1041	465	44.7	41.6, 47.7[c]
Overall	2514	835	**33.2**	31.4, 35.1[b]

*Siginificant at p<0.001
Values with different letters within a column differ significantly (p<0.01)

3.2.1.3 Area wise prevalence

Area wise prevalence in cattle of five districts of Bangladesh the data indicate that the infection is highest in Sirajgonj (49.5%,CI.45.3,53.6) followed by Chittagong (41.7%,CI.37.1,46.5),Mymensingh(27.9%,CI.23.9,32.2)andNetrokona(24.6%,CI.1,28.9) and the lowest in Kishoregonj (21.1%,CI.17.4,25.3)Table 3.Statistical analysis showed significant differences (p<0.001) among the five districts.

3.2.1.4 Age, breed and sex related prevalence

It is evident from the data presented in Table3 that overall prevalence in adult cattle were significantly (p<0.001) higher (35.6%,CI.33.0,38.2) than young (31.9%,CI.28.7, 35.2) and calves (27.2%,CI. 22.6,32.2).Overall breed wise prevalence showed that indigenous cattle had high level (38.4%,CI.36.2,40.7) of prevalence than cross breed cattle (19.5%,CI.16.6,22.6) and significant (p<0.001) difference was also found between two breeds (Table3).Sex wise prevalence showed male animal (40.1%,CI.37.4,42.8) was more susceptible than females (25.9%,CI.23.5,28.5) and statistical difference(p<0.001) was observed between male and female cattle (Table3).

3.2.1.5 Prevalence on the basis of physical condition of cattle

It is observed from Table3 that overall prevalence (39.6%,CI.36.8,42.3) was statistically significantly (P<0.001) higher in very thin animal than normal healthy animal(21.6%,CI.18.9,24.5) and slightly thin animal (38.2%,CI.33.4,43.2).

3.2.1.6 Prevalence on the basis of fecal consistency of host

The data from Table 3 show that animals defecated diarrheic feces had significantly (P<0.001) higher prevalence of schistosomiasis (67.0%,CI.64.0,69.8) than normal defecated animals (7.9%,CI.6.6,9.4).

3.2.1.7 Prevalence on the basis of sanitation

The informaton in the Table3 indicates that poor sanitary condition of the cattle shed showed significantly (P<0.001) higher (39.9%,CI.37.2,42.7) prevalence than good sanitary condition (26.5%,CI.24.1,29.1).

3.2.1.8 Prevalence on the basis of grazing system

From Table 3 it shows that animal grazed on the pastures showed significantly (P<0.001) higher prevalence (38.5%,CI.36.0,40.9) than stall feded animals (24.9%,CI.22.2,22.7).

3.2.1.9 Prevalence on the basis of diet

It is reported from Table 3 that animals which were fed with poor diet showed significantly ($p<0.001$) higher prevalence of schistosome infection (38.5%,CI.36.1,41.1) than animals having balanced diet (25.6%,CI.22.9,28.4).

3.2.1.10 Prevalence on the basis of de-worming

The data in the Table3 depicted that de-wormed animals had significantly ($P<0.001$) lower (30.1%,CI.27.8,32.5) prevalence than that of animals which were not de-wormed (37.7%,CI.34.7,40.8).

3.2.1.11 Prevalence on the basis of rearing system

It is depicted in Table3 that animals which were reared in subsistence farming system had significantly ($P<0.001$) higher (38.2%,CI.35.8,40.7) prevalence than the others reared in commercial farms (24.4%,CI.21.7,27.4).

3.2.1.12 Season wise prevalence

It is evident from data in Table3 that significantly higher ($P<0.001$) prevalence in all the studied areas of Bangladesh showed in rainy season (44.7%,CI.41.6,47.7) followed by summer (30.3%,CI.26.7,34.0) and winter (21.4%,CI.18.9,24.3).

3.3 Overall Monthwise Mean Prevalence and Mean Egg Count by Fecal Egg Examination

Table4. Overall month wise mean prevalence and mean egg count

Month	Mean Prevalence	95% CI	Mean Egg count (range)
January[a]	17.5	12.8, 23.0	96 (10-160)
February[b]	22.1	16.9, 27.9	97 (10-200)
March[c]	24.9	18.9, 31.7	81 (10-220)
April[d]	31.7[a]	25.5, 38.5	102(10-210)
May[e]	39.0[abc]	32.2, 46.1	100(10-260)
June[f]	39.2[abc]	32.5, 46.1	110(10-280)
July[g]	44.1[abck]	37.5, 50.9	102(10-280)
August[h]	45.2[abckl]	38.3, 52.2	91(10-180)
September[i]	59.0[abcdefgjkl]	52.0, 65.7	104(10-240)
October[j]	33.2[a]	26.6, 40.2	94(10-260)
November[k]	27.7	21.3, 34.8	100(10-270)
December[l]	17.9	12.9, 23.8	78(10-180)

Letters[a-l] corresponds to months from January to December.Month wise prevalence when significantly higher ($p<0.05$) than other month(s) are shown by that (those) month specific letters in superscript

To find out the year's specific period associated with prevalence rate, overall month wise data analyzed and presented in Table4.The data showed an overall highest prevalence (59%,CI.52.0,65.7) were in September,which gradually decreased in December (17.9%,CI.12.9,23.8) and January(17.5%,CI.12.8,23.0).Statistical analysis showed that insignificant difference($P<0.05$) among the prevalence of November, December, January,February and March. Months of July, August and September was noted significantly highest ($P<0.05$) prevalence as compared to December and January.Months of February, March,April,May,June and October showed significantly higher($P<0.05$) difference than December and January. The highest recorded monthly mean egg count during the study period was 110 with the range of 10-280 in the month of June.Otherwise fecal egg count was persistent during all months of the study period.

3.4 Herd Prevalence

The herd size, herd level prevalence, within herd prevalence of bovine schistosomiasis is shown in Table 5. The median and range of herd size are 6 and 1-27 respectively. About 76.7% herds have less than 10 animals. Prevalence of bovine schistosomiasis increases with the increase of herd size. Significantly higher prevalence (P<0.002)of bovine schistosomiasis were observed in herds containing 5-9 and 10-15 animals(P<0.006) than those herds having less than 5 animals. Within herd prevalence of bovine schistosomiasis varied from 3.7 to 100%. The range and median of within herd prevalence's decrease gradually as herd size increases. The median of within herd prevalence's varied from 23.5% to 50% (Table5).

Table5. Herd level and within herd prevalence of bovine schistosomiasis based on fecal egg examination (n=2514)

Herd size	Frequency	*Schistosoma* positive	Herd prevalence (%)	Range and Median within herd prevalence	Odds Ratio	95% CI	p-value
1-4	70	53	75.5	25-100%, 50%	1	-	-
5-9	183	166	90.7	12.5-100%, 42.9%	3.1	1.5, 6.6	0.002
10-15	54	52	96.3	7.7-75%, 27.3%	8.3	1.8, 37.9	0.006
16-27	23	23	100	3.7-45.5%, 23.5%	$1.4x10^7$	0.0, Infinity	0.98
Total	330	294	89.1	3.7-100%, 36.9%			
Herd size: Median=6, Range=1-27							

3.5 Risk Factors of Schistosomiasis

3.5.1 Reducing the number of explanatory variables

The risk factors of prevalence of schistosomiasis in cattle have been identified by fecal egg count. Thirteen explanatory/independent variables like district, age, breed, sex, physical condition, fecal consistency, sanitation, grazing, diet, de-worming, farming system and season were analyzed initially for risk factor identification. Pair wise Spearman's correlation among explanatory variables is shown in Table 6. Twenty two pairs of explanatory variables were significantly associated with each other. One of the pairs of significantly associated variables was selected for inclusion in the final analysis and the other was ignored (Dohoo *et al.*, 1996).

Table6. Significant association among explanatory variables

Variable pairs	Correlation coefficients	p-value
Physical condition + Deworming	-0.60	0.000
Breed + Deworming	-0.35	0.000
Sanitation + Deworming	-0.41	0.000
Grazing + Deworming	-0.62	0.000
Diet + Deworming	-0.61	0.000
Farming + Deworming	-0.63	0.000
Physical condition + Breed	0.48	0.000
Physical condition + Sanitation	0.62	0.000
Physical condition + Grazing	0.80	0.000
Physical condition + Diet	0.76	0.000
Physical condition + Farming	0.79	0.000
Sanitation + Breed	0.40	0.000
Grazing + Breed	0.55	0.000
Diet + Breed	0.49	0.000
Farming + Breed	0.51	0.000
Grazing + Sanitation	0.71	0.000
Diet + Sanitation	0.72	0.000
Grazing + Physical condition	0.80	0.000
Grazing + Diet	0.91	0.000
Grazing + Farming	0.95	0.000
Diet + Farming	0.91	0.000
Sanitation + Farming	0.72	0.000

A negative binomial regression model study was done to find out the risk factors associated with schistosome infection in cattle. Risk factors of schistosomiasis by egg count in cattle of Bangladesh are presented in Table 7. Districts, ages, sexes, grazing, fecal consistency and seasons were identified as significant risk factors for bovine schistosomiasis by egg count in Bangladesh. Significantly higher ($P<0.003$) egg count of bovine schistosomes were found in calves and youngs ($P<0.01$) than those in adults. The egg count of bovine schistosomes was significantly higher ($P<0.001$) in males than females. Grazing cattle had significantly higher egg count ($P<0.005$) those which did not graze. Liquid fecal consistency was found to be significantly associated with bovine schistosomes than normal feces defecated by the animals. The distribution of eggs was significantly different ($P<0.001$) in the rainy season (mean 64,range10-280) and summer season(mean60,range10-60) and showed higher egg counts compared with winter season(mean59,range10-270).Regional variations were also noted for bovine schistosomiasis by egg count. Significantly higher ($p<0.001$) egg count was observed in Sirajgonj and Chittagong districts followed by Mymensingh and Netrokona districts and lowest in Kishoregonj district (Table7).Age wise analysis showed that calves had mean egg count of131 (20-280),young had 130 (10-270) and adult had 90 (10-170).Male animal had mean egg of 122 (10-260) and female had 132 (10-280)(not shown in Table).The mean egg count for *S. indicum* and *S. spindale* were 51.5 and 49.9 with a range of 10-200 and 10-170 respectively (Table7).

Table7. Significant risk factors of bovine schistosomiasis by egg count in Bangladesh identified by negative binomial multiple regression models (n=2514)

EPG *Schistosoma indicum*: Mean ±SD=51.5±36.5, Range=10-200				
EPG *Schistosoma spindale*: Mean ±SD=49.9±26.9, Range=10-170				
Factors	**Estimate**	**Std. Error**	**z value**	**p value**
District				
Kishoregonj	-	-	-	-
Chittagong	2.04	0.21	9.56	0.000
Mymensingh	0.64	0.20	3.14	0.002
Netrokona	0.06	0.20	0.34	0.73
Sirajgonj	1.94	0.19	9.87	0.000
Age category				
Calf (≤1 yr)	0.55	0.18	2.97	0.003
Young (>1-3 yrs)	0.31	0.19	1.64	0.10
Adult (>3 yrs)	-	-	-	-
Sex				
Female	-	-	-	-
Male	0.53	0.12	4.44	0.000
Grazing				
No	-	-	-	-
Yes	0.35	0.13	2.79	0.005
Fecal consistency				
Normal	-	-	-	-
Liquid	3.48	0.14	25.00	0.000
Season				
Winter	-	-	-	-
Summer	0.56	0.17	3.42	0.000
Rainy	1.12	0.14	7.85	0.000

3.6 Relationship of Mean Monthly Prevalence of Bovine Schistosomiasis by Fecal Examination with Temperature and Rainfall (mean) at Five Study Areas

Table8.Monthwise mean prevalence of bovine schistosomiasis by fecal examination and its relationship with temperature and rainfall (mean) in Mymensingh

Months	Temperature (°C)	Rainfall (mm)	Meanprevalence (%)	95% CI
January[a]	18.5	0	7.8	2.2, 18.9
February[b]	21.7	2.0	6.9	1.5, 19.1
March[c]	25.6	17.2	12.5	4.2, 26.8
April[d]	28.1	86.3	26.3	13.4, 43.1
May[e]	28.3	373.4	26.1	14.3, 41.1
June[f]	28.8	247.5	25.0	12.7, 41.2
July[g]	29.4	443.0	38.9	23.1, 56.5
August[h]	29.3	358.0	47.1[abckl]	29.8, 64.9
September[i]	28.9	218.3	63.2[abcdefjkl]	50.7, 74.6
October[j]	27.8	25.9	23.7	11.4, 40.2
November[k]	23.8	2.0	13.9	4.7, 29.5
December[l]	19.5	0.65	6.4	1.3, 17.5
Correlation	r=0.77**	r=0.69*		

* Significant at $p<0.01$, ** Significant at $p<0.003$, letters [a-l] correspond to months from January to December. Month wise prevalence when significantly higher ($p<0.05$) than other month(s) are shown by that (those) month specific letters in superscript

In Mymensingh district significantly (P<0.05) highest mean prevalence in cattle was 63.2% in September followed by 47.1% in August when mean temperature ranged from 28.9°C ° to29.3C and rainfall varied from 218.3mm to358.0mm.Lowest mean prevalence was recorded in December 6.4% when mean temperature was 19.5°C and mean rainfall was 0.65mm.August had significantly highest (P<0.05) mean prevalence than the months of January, February, March, November and December. In September mean prevalence was significantly highest (P<0.05) as compared to January, February, March, April, May, June, October, Novmber and December (Table8).In order to to find out the correlation of prevalence of schistosomiasis with temperature and rainfall mean values were considered. It was noted from the correlation study that prevalence of schistosomiasis had significant positive (P<0.003), (P>0.01) relationship with temperature and rainfall respectively (Table8).

Table9.Monthwise mean prevalence of bovine schistosomiasis by fecal egg examination and its relationship with temperature and rainfall (mean) in Netrokona

Months	Temperature (°C)	Rainfall (mm)	Mean prevalence (%)	95% CI
January[a]	18.5	0	6.2	1.3, 17.2
February[b]	21.7	2	9.8	2.7, 23.1
March[c]	25.6	17.2	13.5	4.5, 28.8
April[d]	28.1	86.3	21.1	9.6, 37.3
May[e]	28.3	373.4	27.8	14.2, 45.2
June[f]	28.8	247.5	25.0	12.1, 42.2
July[g]	29.4	443	29.3	16.1, 45.5
August[h]	29.3	358	37.1[a]	21.5, 55.1
September[i]	28.9	218.3	56.4[abcdk]	39.9, 72.2
October[j]	27.8	25.9	27.5	14.6, 43.9
November[k]	23.8	2	22.2	10.1, 39.2
December[l]	19.5	0.65	25.0	9.8, 46.7
Correlation	r=0.64*	r=0.55		

*Significant at p<0.03, letters [a-l] correspond to months from January to December. Month wise prevalence when significantly higher (p<0.05) than other month(s) are shown by that (those) month specific letters in superscript

In the district of Netrokona the mean prevalence was significantly higher (P<0.05) in September and August and lowest in January. In September the mean prevalence was significantly higher (P<0.05) than the month of January,February,March,April and November and insignificant difference with the month of May ,June, July,October and December. In August the mean prevalence was significantly higher (P<0.05) as compared to January.It was observed from the correlation study that mean temperature had significant (P<0.03) positive relationship and rainfall had insignificant positive relationship with the mean prevalence (Table9).

Table10.Monthwise mean prevalence of bovine schistosomiasis by fecal egg examination and it's relatioship with temperature and rainfall (mean) in Kishoregonj

Months	Temperature (°C)	Rainfall (mm)	Mean prevalence (%)	95% CI
January[a]	18.5	0	2.9	0.07, 15.3
February[b]	21.7	2	7.7	1.6, 20.9
March[c]	25.6	17.2	8.3	1.8, 22.5
April[d]	28.1	86.3	16.2	6.2, 32.0
May[e]	28.3	373.4	17.6	6.8, 34.5
June[f]	28.8	247.5	22.2	10.1, 39.2
July[g]	29.4	443	38.2[ab]	22.2, 56.4
August[h]	29.3	358	41.0[abc]	25.6, 57.9
September[i]	28.9	218.3	48.6[abc]	31.3, 66.0
October[j]	27.8	25.9	25.6	13.0, 42.1
November[k]	23.8	2	8.0	2.2, 19.2
December[l]	19.5	0.65	4.2	0.1, 21.1
Correlation	r=0.79**	r=0.72**		

**Significant at p<0.002; letters [a-l] correspond to months from January to December. Month wise prevalence when significantly higher (p<0.05) than other month(s) are shown by that (those) month specific letters in superscript

The relationship of mean monthly prevalence of schistosomiasis in cattle with mean temperature and mean rainfall of Kishoregonj district is shown in Table 10. Both the mean temperature and rainfall had shown significant (P<0.002) positive relationship with prevalence.The study revealed significant higher (P>0.05) prevalence in the month ofAugust and September than the month of January, February and March.The prevalence in the month of July showed significantly higher (P<0.05) as compared to January and February.

Table11. Monthwise mean prevalence of bovine schistosomiasis by fecal examination and its relationship with temperature and rainfall (mean) in Chittagong

Months	Temperature (°C)	Rainfall (mm)	Mean prevalence (%)	95% CI
January[a]	19.8	5.3	20.5	9.8, 35.3
February[b]	22.1	7.1	28.2	15.0, 44.9
March[c]	25.3	25.6	36.1	20.8, 53.8
April[d]	27.6	115.9	40.0	25.7, 55.7
May[e]	28.5	401.3	47.4	30.9, 64.2
June[f]	28.4	437.8	46.7	31.7, 62.1
July[g]	28	493.1	48.9	34.1, 63.9
August[h]	28.1	386.5	54.1[a]	36.9, 70.5
September[i]	28.4	289.3	63.2[b]	45.9, 78.2
October[j]	27.8	89.9	39.0	24.2 55.5
November[k]	25.2	53.3	36.8	21.8, 54.0
December[l]	20.8	6.0	30.6	16.3, 48.1
Correlation	r=0.86**	r=0.78**		

** Significant at p<0.003, letters [a-l] correspond to months from January to December. Month wise prevalence when significantly higher (p<0.05) than other month(s) are shown by that (those) month specific letters in superscript

Monthwise prevalence showed peak in September (63.2%) and followed by August (54.1%) which are significantly different (P<0.05) and lowest in January (20.5%).Both the temperature and rainfall revealed significant positive (P<0.003) correlation with the mean prevalence (Table11) in Chittagong district.

Table12.Monthwise mean prevalence of bovine schistosomiasis by fecal examination and its relationship with temperature and rainfall (mean) in Sirajgonj

Months	Temperature (°C)	Rainfall (mm)	Mean prevalence (%)	95% CI
January[a]	17.5	22.6	34.8	23.5, 47.6
February[b]	19.8	45.5	41.1	29.7, 53.2
March[c]	25	10.4	47.9	33.3, 62.8
April[d]	29.3	45.7	48.0	33.7, 62.6
May[e]	28.9	168.1	51.1	35.8, 66.3
June[f]	29.5	340.3	52.7	38.8, 66.3
July[g]	29	145.5	54.7	41.7, 67.2
August[h]	29.1	268.3	64.3[a]	50.4, 76.6
September[i]	28.8	149.3	64.1	47.2, 78.8
October[j]	27.4	137.7	44.2	29.1, 60.1
November[k]	23.4	7.6	48.8	32.9, 64.9
December[l]	19.5	0.0	35.6	21.8, 51.2
Correlation	r=0.81**	r=0.65*		

*Significant at p<0.02 ** Significant at p<0.002, letters [a-l] correspond to months from January to December. Month wise prevalence when significantly higher (p<0.05) than other month(s) are shown by that (those) month specific letters in superscript

When monthwise data analysed in Sirajgonj district it showed significantly (P<0.05) highest prevalence (64.3%) in August which gradually decreased and lowest in January (34.8%).There was no significant variation was recorded among the different months of the year with the prevalence of schistosomiasis. A positive significant (P<0.002) correlation between the prevalence with temperature and rainfall (P<0.02) was observed (Table12).

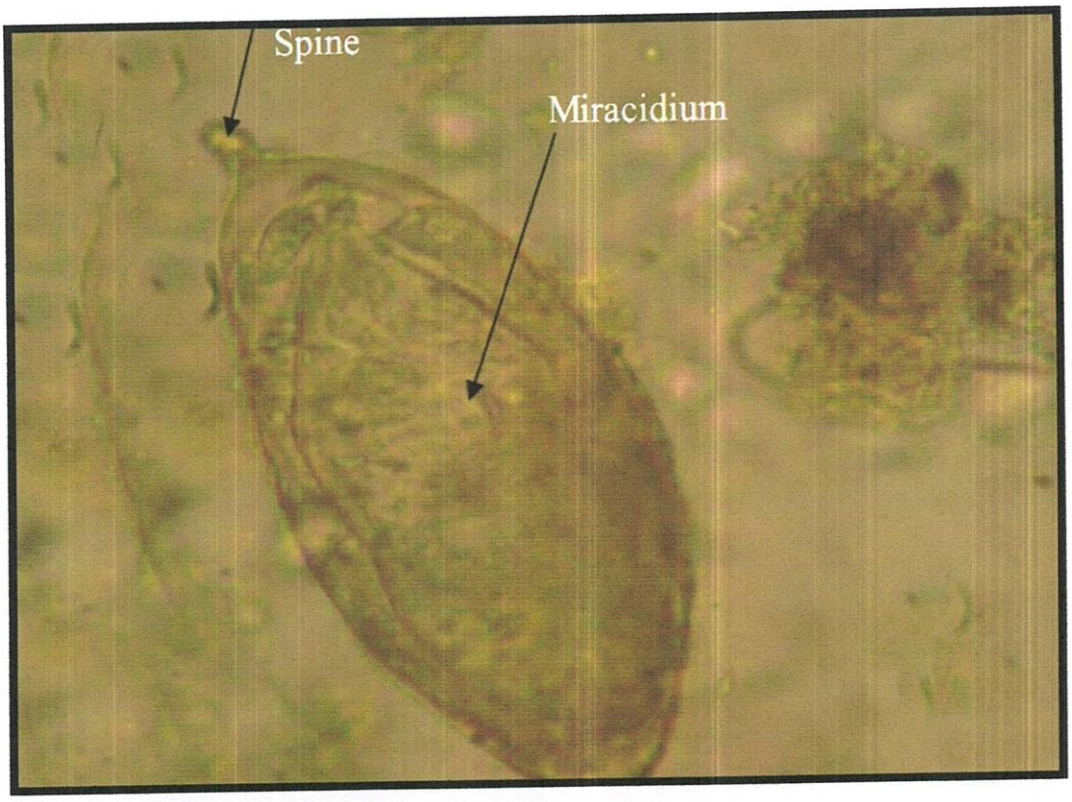

Plate4a: Egg of *Schistosoma indicum* (lateral spine) collected from fecal sample (x 40)

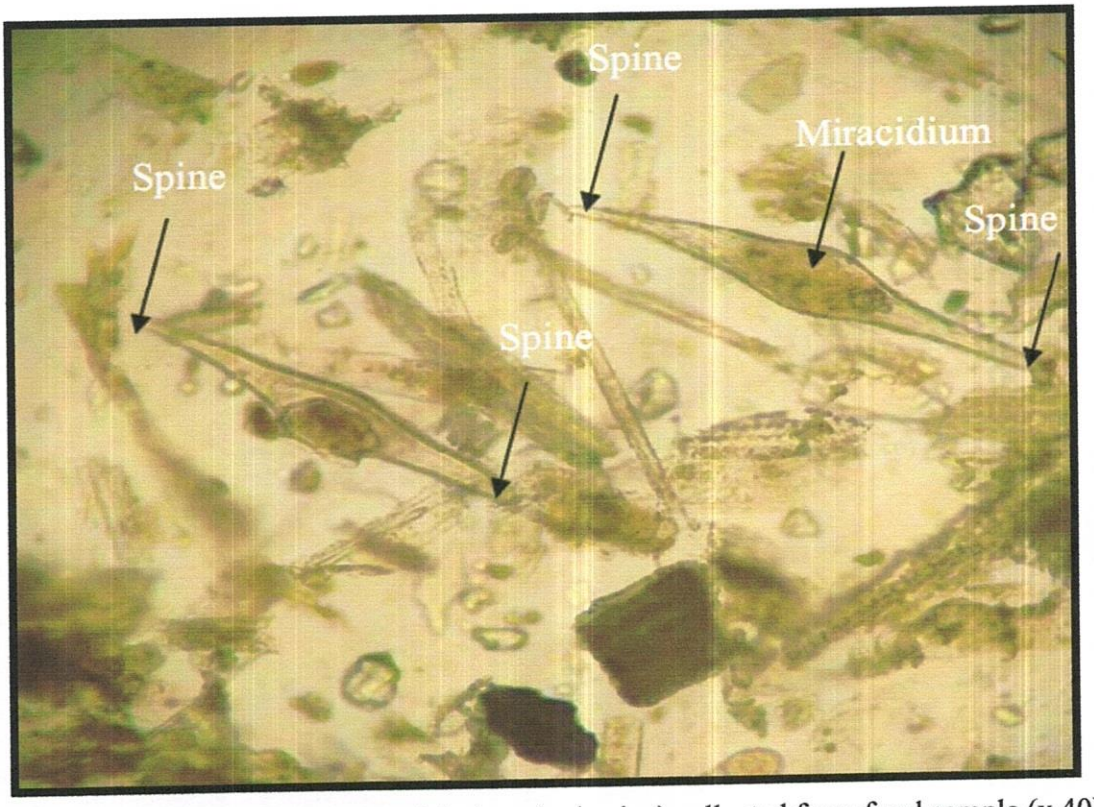

Plate4b: Egg of *Schistosoma spindale* (terminal spine) collected from fecal sample (x 40)

3.7 DISCUSSION

3.7.1 Overall prevalence by fecal egg examination

The prevalence of schistosomiasis is considerably influenced by climatic conditions and as far as possible the evidence for the distribution and prevalence of the disease is presented by geographical areas with corresponding climatic conditions.The present study determined the prevalence of schistosomiasis and attempted to identify the risk factors associated with it.The farmers having at least one cattle were approached without any prior thinking. The herd size represents the real picture of traditional and commercial management system. Those who agreed to provide their animals for sampling were included. Although time-consuming, fecal examination is a valuable diagnostic method for the field veterinarian dealing with group of animals at risk, particularly because severe morbidity in cattle is generally associated with high egg output. From epidemiological work, the major disadvantage of fecal egg counting methods in their decreasing sensitivity in ageing animals, which is clearly associated with immune related reduction of the fecundity of the female worms.In contrast to various experimental infection studies only a few studies have been carried out on the epidemiology of schistosomiasis in naturally infected animals.

The occurrence of schistosomiasis in an area is influenced by a multifactorial system, which composes hosts, parasitic agents, transmission process and environmental effects. In natural foci of schistosomiasis the parasites and their intermediate host and final host form an association of potential epidemiological risk and it is important that the existence and localization of such an association should be recognized beforehand so that it can be brought under control.The detection of ova in feces (Katz *et al.,* 1972) is the most widely used diagnostic method of schistosome infections. Though in this processes the specificity is high but the sensitivity is low due to large day to day variations in egg output (Ruiz-Tibu *et al.,* 1979, Utzinger *et al.,* 2001, Ye *et al.,* 1998) and uneven distribution of eggs in feces (Engels *et al.,* 1997, Ye *et al.,* 1998). The sensitivity of fecal examination may be improved by taking rectal snip biopsies however increased number of examined fecal samples makes the two methods equally reliable (Rabello *et al.*, 1992).

The present work recorded higher (33.2%) prevalence but the result does not agree with that of Anwar and Gill (1990) who reported 7-21% prevalence with a mean of 13% infecting both cattle and buffaloes though the number of cases of schistosomiasis is increasing world wide.According to Makundi *et al.*, (1998) bovine schistosomiasis is an endemic disease in Tanzania and other East African countries.Recent study in Jimma area byLiromsa *et al.*, (2011) suggested that the prevalence was 7.07% at coproscopy. This finding was lower when compared with the reported prevalence rate of 22% reported from Kafu and Zambezi river basins of Zambia by Yabe *et al.,* (2008) and 33.9% from endemic areas of Tanzania (Makundi *et al.,* 1998). This difference could be attributed to the difference in the ecology of the study sites, the age of the animals or worm control practice in the area. The cattle studied by Yabe *et al.,* (2008) were from areas where worm control was not routinely practicised. An increase marked in the prevalence of infection frequently occurs in association with different water resources development schemes. The creation of man made lakes and the introduction of new irrigation schemes or extension of existing ones in endemic areas or in areas adjacent to existing transmission foci are significant factors in the spread of the infection.

The overall prevalence of *Schistosoma* in cattle (33.2%) reported in this study varies with that reported by various authors in Bangladesh.Howlader *et al.,*(1990) reported 8.0% *Schistosoma* spp. from Dhaka district of Bangladesh.Bari *et al.,*(1991)reported lower prevalence than that of present study.They recorded 2.0% prevalence of schistosome infection from organized dairy farm of Bangladesh Agricultural University, Mymensingh. However, it is lower than that the reported from abattoir surveys (Condy, 1960, DeBont *et al.,* 1994). The low prevalence noted by fecal examination than slaughter house survey in this study could possible be ascribed to limitations of the egg finding techniques (DeBont *et al.,* 1996a, DeBont and Vercruysse, 1997, 1998) coupled with a low egg production due to an immunological responses by the host to recurrent infection (Lawrence, 1973a, 1977a). From the animal health and epidemiological points of view, many adult aminals with fecundity suppressed worms are missed by fecal examination (DeBont and Vercruysse, 1997).

However, higher prevalence has been recorded in various parts of the world. In Bubaneshwar 67% cattle was found positive for *S. spindale* and *S. indicum* causing 45% of the infection (Agrawal *et al.*,2003). In Sudan high prevalence 37.2% of bovine schistosomiasis was found by Malek (1969) in the White Nile reservoir. The schistosomes recovered from cattle, sheep, goat, equines, camel and the pig were all *S.bovis* as indicated by the shape and measurements of the eggs.

An epidemiological study done by Habtamu and Wolde (2011) has revealed relatively high prevalence of *S. bovis* (37.3%) in cattle than value reported by previous attempts (12.3-29%) (Amero,1993, Hailu 1999, Yalelet, 2004, Almaz, 2007). This wide gap may be due to the fact that the previous studies were undertaken by means of single fecal sample examination which is used traditionally in the diagnosis of parasite egg. This has resulted in lower probability of detecting *Schistosoma* eggs in feces. It is believed that trematodes are intermittent egg layers so that the chance of detecting eggs during single fecal sample examination may be minimal (Bushara *et al.,* 1980). The prevalence and severity of infection presumably varies according to the distribution of the intermediate snail hosts and the local conditions affecting their infection with the larval stages of schistosomes from the grazing animals. In Kenya highlands and Rift Valley the rate of schistosome infection varied from 100% (with heavy infestation in some cases) on the farms close to Lake Naivasha and Lake Bolossat with their swampy pastures to none from the dry pastures on ranches where the cattle were watered from trough (Dinnik and Dinnik, 1965).Higher prevalence (29.0%) of *S. nasale* ova were identified by Krishnasamy *et al.,* (1998) from naturally infected buffaloes from Labu, Negeri Sembilan region of Malaysia. In another study Krishnasamy *et al.,* (2004) reported *S.spindale* from other ruminants in the same region of Malaysia. The rate of *S. spindale* in cattle, buffaloes, sheep and goats were 23.9%, 24.9%, 25.5% and 24.2% respectively and it is higher than the present study (10.6%) found in cattle. Ram *et al.,* (2004) reported higher prevalence (36.8%) of nasal schistosomiasis by the screening of nasal scraping from cattle where the prevalence of infection was lower in buffaloes (8.3%).

In India, there have been reports of *S. indicum* schistosomiasis in sheep from some states including Rajasthan (Vashista *et al.,* 1981), Haryana (Singh *et al.,* 1984), Karnataka (Chandra *et al.,* 2003) and Madhya Pradesh (Agrawal *et al.,* 2004). The disease seems to be highly prevalent but is underdiagnosed and has not received due attention. The routine method such as direct smear test showed only 30% positive cases, which the other methods go, undetected (Agrawal, 1999). But Miracidial Hatching Test was found to be superior as it detected 72.7% positive cases. A previous report by some of the authors based on examination of fecal sample by sieving and sedimentation techniques reported an incidence rate of *S. spindale* infection of 4.27% in cattle aged over eight years in the Mysore and Mandya districts of Karnataka (D' Souza *et al.,* 1988) and this result was not coroborate the present finding (29.9%). The average schistosome infection rate showed higher infection rate in buffaloes (56.5%) as compared to cattle, sheep and goats and could be due to habit of buffaloes to wade in the water for a longer period (Bedarkar *et al.*, 2000). Chaudhri *et al.,* (2007) also noted that buffaloes have more infection than cattle whereas Banerjee and Agrawal (1992) showed that the cattle have more infection than buffaloes.

Overall prevalence of schistosomiasis in cows was noted 15.6% in Punjab province by Niaz *et al.,* (2010b). Before that no similar published data was available on schistosomiasis from Punjab, Pakistan except Anwar and Gill (1990). They reported 13.0% *S.bovis* in Raiwind area of Punjab. Wang *et al.,* (1998) reported 30.8% and 21.3% infection of schistosomiasis in cattle and buffaloes respectively in Anhvi province of China. Yang *et al.,* (1999) investigated 18.5% infection of *Schistosoma* of cattle in China. Solomon, (1985), Aemro,(1993),Hailu, (1999),Yalet,(2004), Almaz, (2007) reported12.3-29% prevalence of *S. bovis* in Bahir Dar of Ethiopia by coproscopic examination. Lower prevalence (4.5%) also recorded from an Afican country (Zimbabwe) by Pfukenyi *et al.,* (2006). This difference may be due to varied environmental conditions and different geographical regions which influences the prevalence of the schistosomes in cattle.

Results of the present study showed variations in the prevalence of schistosomiasis in different districts.The highest prevalence was recorded at Sirajgonj (49.5%) followed by Chittagong (41.7%) and moderate in Mymensingh (27.9%) and Netrokona (24.6%) and are equally prone to schistosome infection and while the lowest (21.1%) in Kishoregonj. Infection was varied in different geographic areas and influenced by various factors, may be due to the fact that a high level of infection in Sirajgonj and Chittagong was thought to be associated with the extension of canal system providing additional areas of swamp and marsh where the cattle were exposed to infective cercariae as was also noted by Misra *et al.,* (1997) and Pfukenyi *et al.,* (2005b). The lower prevalence of schistosomiasis in Kishoregonj areas might be related to the scarcity of the snail vector *Lymneae* and *Indoplanorbis* which was attributed to the unfavourable condition such as scouring of the river bed by the silt-laden water prevented the establishment of aquatic weeds which harbor the snail hosts. The fast rate of flow of the rivers in this area may also prevent the establishment of snails, as they require still / stagnant waters to breed. Moreover, the herd size is bigger in Sirajgonj and Chittagong districts compared to the herd size in Mymensingh, Netrokona and Kishoregonj districts resulting in dirtier floor with poor sanitation and hygiene.However, the prevalence of a disease in a specific area depends on various factors like geo-climatic condition, biological barriers, immunization status of the host, social awerness and mostly on the health status of the animals.

There are many other reports showing various prevalence in different geographic areas such as the highest prevalence of schistosomiasis of cattle was noted at Lahore (19.0%) followed by Sheikhupura (17.3%), Gujranwal (14.8%) and the lowest (11.6%) in Sialkot by Niaz *et al.*, (2010b).It is happened that prevalence of schistosomiasis was influcenced by local climatic conditions, presence or absence of water reservoirs, lakes, rivers and availability of suitable intermediate hosts of parasites (Maqbool *et. al.,* 2002, 2003, Khan 2008, Zhou *et al.*,2008). There is a large body of literature indicating that global warming could potentially alter the spatio temporal distribution of disease vectors and thus change the frequency and transmission dynamics of vector borne diseases like fasciolosis, schistosomiasis, filariasis and malaria etc. (Reiter,

2001, Hunter, 2003, Southerst, 2004). The general agreement is that transmission dynamics is most sensitive to climate change around the boundaries of endemic areas (Southerest, 2004).The variation among different areas might be related to the environmental conditions which facilitate the presence and propagation of the intermediate host (Al-Gaabary and Nasr, 1997), climate and geographical parameters which affect the hatchability of schistosome eggs (Hirani *et al.*,1999).

By age related data analysis showed that adults (35.6%) and young animals (31.9%) were more susceptible than calves (27.2%) to schistosome infection.It is found that in endemic areas, virtually all animals from herds in contact with cercarial infested water become infected very early in life, depending upon how soon new born calves are allowed to join the herd for grazing. Fecal egg output has been observed as early as during the second month after birth (Majid *et al.,* 1980a) but begins more commonly between the fourth and eighth month of life (DeBont *et al.,* 1995). Maqbool *et al.,* (2002), Qureshi *et al.,* (2005), Qureshi (2008) and Niaz *et al.,* (2010b) recorded the prevalence of fascioliasis and schistosomiasis in respect to the physiological status of cows revealed significantly higher prevalence in adult animals than young ones which support the present finding. This may be due to that calvs and young animals are also kept under more care and also may be due to longtime exposure of adult animals to infective larvae of flukes as compare to young ones.However opposite finding have been reported by Samanta and Santrap (2007) and Khan (2008) with high infection of trematode flukes in young ones than that of adult cattle.Clinical schistosomiasis due to *S. spindale* characterized by blood and mucus in their feces reported high in bulls from Keojhar district of Orissa which was of less than 2 years of age (Mohanty *et al.,* 1984). It is found by Islam *et al.,*(2011) that the prevalence of *Schistosoma* infection had an increased trend with the age in cattle of various districts of Bangladesh.There are many similar reports of higher fluke prevalence in adults than calves (Cringoli *et al.,* 2002, Molina *et al.,* 2005, Pfukenyi *et al.,* 2005b, 2006) which are in conformity with the present findings. It is expected because age is surrogate measure of amount of exposure time.

Epizootiological studies on nasal schistosomiasis in bovines in the Bangalore and Mandys districts of Karnataka state indicated that old and female animals were more affected (Muraleedharn *et al.,* 1976a). Banerjee and Agrawal (1992) have observed a high incidence of schistosomiasis in older animals (44%) and the study by Sumanth *et al.,* (2004) also revealed the incidence among older animals was higher (69.16%).

The highest prevalence of (41.2%) *Schistosoma* in cattle was recorded in the age group of > 36 months by Sarder *et al.,* (2006). Similar trend of prevalence (39.6%) with older age group has also been reported by Rahman and Mondal (1983).The present findings are also in agreement with Sarder *et al.*,(2006) and Rahman and Mondal (1983).Islam (1975) recorded that animal older than 4 years had a higher prevalence of infection (95%) and had an average heavier worm burdens (147worms/animal) than younger cattle (23worms/animal). One animal had a burden of 1665 *S. spindale* worms. A study in Srilanka by Bedarkar *et al.,* (2000) also showed the prevalence increased significantly (χ^2 = 50.8, 2DF, P<0.01) with age, between animals aged 2 years or less (21.3%), 3-4 years (31.5%) and more than 5 years (47.9%). Age wise analysis of other fluke infection mainly fascioliasis by Bedarkar *et al.,* (2000) indicated that peak infection attained during maturity of age. On the contrary high prevalence of the schistosomiasis was observed by Bont (1995) in young and adult cattle groups rather than in older ones. This could be attributed to the fact that calves are traditionally wined at about 1.5-2 years and than allowed to graze with adult cattle. In addition to this adult group of cattle are left to graze on the field where cercarial infection was high, furthermore in this group of animals acquired immunity is not established hence egg shed in these animals was high.While in older group of cattle due to development of acquired resistance there is decrease in egg release.In the present study the older age group of animals were found to be infected more with *Schistosoma* spp. may be due to the high susceptibility and low resistance in older animals. 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 moderate level of parasitism reported in young animals are attributable to the delay in development of immunity and high level of infection in adult animal is due to the

development of significant immunity which is initially low but increases with the intensity and duration of exposure of infection. According to Soulsby (1982) previous infection and age of the host afford some protection against reinfection and hence acute disease is usually seen in young animal.

The higher prevalence in adult than in young animal concurs with other studies of Silangwa (1973) and Holland *et al.,*(2000). The high prevalence in adult might be due to long exposure time as well as management practices, whereby adults were trekked long distance to graze in ditches, flood plains and swampy areas.Negesse (1994) and Pfukenyi *et al.,* (2005b) reported similar observation that adult animal had significantly higher prevalences of fluke infections than young cattle. In Zambia, adult cattle acquire such infections because they move long distances in search of scarce pastures and water, thereby increasing their chances of infecting as well as becoming infected at overcrowded watering holes. Phiri *et al.,* (2007) reported that the prevalence of the trematodes was higher in adults than young ones which contrast the other parasites and are due to prolonged exposure of adult animals to eggs of trematodes and development of limited immunity in the host. However, a longitudinal study perfomed by De Bont *et al.,* (1995a) at an endemic area revealed a significant decline in infection prevalence in cattle of more than 3 years of age. But all of the cattle included in this study were >3 years of age which could potentially affect the actual prevalence in the study area.

It was detected from the study (Table3) that prevalence of schistosomiasis was found higher ($p<0.001$) in male cattle (40.1%, CI.37.4,42.8) than in female (25.9%,CI.,23.5,28.5). There are similar reports made by Armour and Urquhart, (1974) that male cattle have increased susceptibility to parasitic infections. This phenomenon is related to androgen. The male animal in this country are here used for draught purpose and thereby more exposed to the pasture and also more stressed. Male animals are also fed relatively poor diet. Poor diet increases the susceptibility to parasitic infection (Haudijk and Athanosiadou, 2003).So all these factors may increase the risk of being infected by schistosome of male cattle.

Niaz *et al.,* (2010b) showed that males (17.3%) were more susceptible than females (13.0%) by schistosome infection at different sites of Punjub district of Pakistant but the difference was (P>0.05) insignificant.Their results are in close agreement with the present obsevations. The reason seems to be related to social practice of keeping female under better management and feeding conditions may be related to their better care for milk production and breeding and male are generally let lose to graze freely in pastures. Similar results have been reported by Maqbool *et al.,* (2002), Qureshi *et al.,* (2005) and Khan *et al.,* (2006) they also observed high infection rate of different fluke in male (12-14%) than females (6-10%).The exact cause of higher prevalence of schistosome infection in male cattle could not be explained but it could be hypothesized that some hormonal influences might be associated with this phenomenon. Although the elevated plasma-T (testosterone) levels were generally associated with increased parasitism (Roberts *et al.,* 2004), the causal mechanisms underlying this pattern were not well understood. Two primary, nonexclusive hypotheses have been proposed to link T level with parasite load (Klukowski and Nelson, 2001). One possibility was that T increased the probability of encountering parasites by stimulating movement and extending daily activity period. Alternatively, T might increase susceptibility to infection via suppression of the immune system (Klein, 2004). This mechanism has received considerable attention in light of the immuno competence handicap hypothesis (Folstad and Karter, 1992). On the otherhand female hormone estrogen has been found to be responsible for the resistance of female hosts to various parasites.

Sex wise analysis of fluke infections especially fascioliasis and schistosomiasis by Bedarkar *et al.,* (2000) indicated that females of all ruminant species had higher incidence than males.The influence of sex on the susceptibility of animals to infections could be attributed to genetic predisposition and differential susceptibility owing to hormonal control. Further experimental studies are needed to confirm the assumption. Differences in susceptibility to infection between sexes have been observed by various workers. The observed disparity may not soley be due to difference in susceptibility but may also depend on a sex related variation in

behaviour that results in differences in exposure (Magona and Musisi, 2002). Males (Ram) were found to be more infected (72.5%) as compared to females (63.07%) with *Haemonchus contortus* (Barger, 1993). These results are also in agreement with a study from Poland where in males were found more infected with the nematode species (Courteny *et al.,* 1985). However, many studies have reported female more infected than male. Higher infection of intestinal helminthiasis was also found in female goats than males (Hasan *et al.,* 2005). Although sex plays a significant role in the preponderance of the infection but environmental and climatic conditions have a greater role to paly in the onset of parasitism in goats despite the gender differences reported by several authors. Other findings, such as the incidence of 60.3% in nondescript bullocks and 68.87% in Hallikar bullocks of *S. nasale* infection showed that males were more susceptible than female based on the detection of eggs.Ottalwar *et al.,* (2004) recorded similar finding in case of *S. nasale* that male cattle had higher schistosomiasis prevalence (36.59%) compared to female (28.84%) and purebred Sahiwal cattle were unaffected by the disease.

Results of Phiri *et al.,* (2007) confirmed that female cattle had higher prevalence rates than the male cattle in fluke infections. Female animals were observed to have high prevalence and intensity of trematodes than males which are similar to what is reported in other studies and could be due to effect of female hormones (Raza *et al.,*2007). Hormonal changes during the late pregnancy and lactation lower the resistance of host to helminth and consequently result in the establishemt of higher worm burdens.Similarly Kumari and Hafeez (2005) and Khan *et al.,* (2006) recorded the prevalence of other fluke disease such as paramphistomiasis was generally higher in females than males.In another study Bachal *et al.,*(2002) also reported a slightly higher prevalence (48.30%)of helminthes in female than male(45.12%) buffalo calves.But in case of human infection by *S.mansoni* males had a higher prevalence of infection than females and were found 1.6 times more likely to be infected than females,41.0% of the males had *S.mansoni* ova in their stool compared with 31.4% of the females (Abdel-Wahab *et al.*,2000).The authors concluded that man may get more chance to become infected than female as they standing in the shallow waters of dams ,rivers,paddyfields etc.during fishing, planting and building bridges.

The difference in the level of parasitism ($p<0.001$) between two breeds of cattle observed in the present study are in agreement with the observations of other workers in various parts of the world where, some breeds were found to be more susceptible to helminth infection than others.Some of this variation is due to genetic factors in the host, but there is the possibility that the observed difference in resistance may be due to heterosis if the animals are not pure bred (Stear and Murray, 1994).It is likely that farmers pay less attention to local breed of animal than crossbred animal that may have resulted in differences.On the otherhand high value in prevelance was also recorded in crossbreed cattle as compared to local breeds (Habtamu and Wolde,2011).The likely explanation might be local breeds acquired a high degree of immunity as a result of repeated natural exposure. The main manifestation of immunity was suppression of worm fecundity (Bushara *et al.,* 1980). It was also reported that local cattle that are naturally acquired infections are capable of reducing egg production. Furthermore, difference in natural or innate immunity between indigenous and cross breed of cattle was observed by Fekade *et al.,*(1989).

Prevalence of schistosomiasis was found higher ($p<0.001$) in cattle reared in subsistence farming system than those reared in intensive farming system (Table3). The prevalence was generally associated with the type of management of especially the grazing habit. The prevalence was highest in the traditional system, lowest in the intensive dairy system. In all management systems, adult had the highest prevalence (35.6%), weaners / yearlings were intermediate (31.9%) and calves had the lowest prevalence (27.2%).In subsistence production system there are more indigenous animals and mainly used for ploughing land. They are also provided with poor diet. They may also be less dewormed than those for commercial production. These may the reasons for relatively higher prevalence of schistosome infection in subsistence production type. Indigenous cattle are used for ploughing land and are more exposed to snail habitat. The only drug available against *Schistosoma* infection in Bangladesh is antimony compound (Anthiomaline ®) in injectable form, expensive and not readily available. This imported drug is usally used for severe from of nasal schistosomiasis (snoring disease). These may be the reasons for higher prevalence of *Schistosoma* in indigenous cattle.

Most cattle population in Bangladesh comes from primitive and low productive breeds. Most animals are reared in house under the age old traditional husbandry practices. Many cattle are overworked and most of them under fed or half fed during most of the time of the years. They are not supplied with adequate balanced ration. As a result the general nutritional status of most of the cattle are in sub-normal level, which greatly increases susceptibility to parasitic diseases (Bradford,1996).The prevalence of higher proportion of egg positive animals on subsistence farms in the present study compared to other management system concurs with the studies on traditional migratory cattle in Mali (Tembely *et al.,* 1988).The high prevalence might be due to heavy contamination of snail habitats and infection by larval stage *Furcocercus* cercariae as a result of high stocking density and local overcrowding around watering points.Traditional farms those were investigated during this study were stationary herds where traditional farmers also practiced agricultural activities. Animals were grazed and practiced agricultural activities during the day and housed around in open areas and cattle were denied any supplementation or effective disease control.

Management systems for the animals have a strong influence on the epidemiology of transmision of any disease.High stocking density increases the contamination of the environment with trematode eggs or larvae and thus makes the infective stages to be more accessible to susceptible animals.The intensive dairy farms had improved or semi improved management systems. The animals were given concentrates, chopped grass and mineral supplementation and animals were permanently housed. They get better sanitation. As a result the present study reported lower prevalence of schistosomiasis in cattle reared at intensive farms in Bangladesh. Sumanth *et al.,* (2004) reported no cases of schistosomiasis in cattle reared at intensive farms at Bangalore and also highlighted the fact that proper management practice alone can minimize the schistosome prevalence.

During the present study it is recorded that poorly fed animals had significantly higher ($p<0.001$) prevalence than balanced fed animals.Poor nutrition lowers the resistance of the animals thus enhancing the establishment of worm burden and increasing the pathogenecity of the parasites. Consequently worm burdens tend to be higher in

poorly fed than in well fed animals. Malnutrition during the dry season has been found to lower the resistance of animals to *Haemonchus contortus* in Nigeria and Kenya (Cheijna,1986) resulting in heavy mortalities. While restricted feeding due to tethering during the rainy season associated with high worm burdens and mortality of animals.Intercurrent infections and other stress factors also enhance the establishment of higher worm burdens.Tethering has been found to be associated with low weight gains and predisposition of animals to heavy helminth burdens.

Based on a nation wide survey on parasites epidemiology in dairy animals in seven different agroclimatic zones, Sanyal and Singh (1995) indicated an increased, parasite prevalence in the host and on pasture during the rainy seasons, the months for which vary in different agroclimates which are in close agreement with the present findings. Jithendran and Bhat (1999) also indicated the same trend, but the months differed owing to different climatic conditions and timing of the onset of monsoon in the region. There was a seasonal trend in the proportion of animals passing *Schistosoma* eggs were found in the present investigation. The proportion of egg positive animals decreased towards the cold dry season, then increased gradually through the hot and summer season and peaked towards the beginning of the rainy season.The highest prevalence in rainy season may be due to high humidity, heavy rainfall and mostly due to abundance of vectors because annual rainfall appeared to be the most important predictive variable affecting both the probability of presence and incidence of the disease. Rainfall may affect the vector hosts by affecting the vegetation, temperature and relative humidity.Moreover, the higher prevalence of schistosomiasis during wet period might be due to stress on cows resulting from an extreme temperature and humidity in the study areas.The key features of interest are a fall in the prevalence of patent infections during the cold season and a rapid rise to a peak early in the hot season. This pattern results from the accumulation of pre-patent infections which fail to develop at low temperature but nature enmasse, together with new infections, after a few weeks at higher temparature.Seasonal and year to year variations in temperature may therefore have significant epidemiological effects.

From Jammu the tropical part of Jammu and Kashmir state maximum fluke infections was reported in summer (Anish *et al.,* 2006). From Kathua region of the Jammu and Kashmir state Stear and Murray, (1994) also reported highest fluke infection (70.07% to 94.25%) in summer. The higher prevalence rate (47.1%) recorded during monsoon and post-monsoon by Khajuria and Kapoor (2003), Tariq *et al.,* (2008)(31.4%) in different districts of Punjab and other adjoining areas in ruminants is also in conformity with the present observations.

The maximum infections of flukes (*Schistosoma, Paramphistomum*) in summer rainy season in the goat were recoreded from different parts of Jammu and Kashmir State, India and other parts of the world with similar or different climatic conditions. The highest infection rate was observed in summer rainy season could be due to several factors; abundance of snails (viz., *Lymnaea, Indoplanorbis*) owing to rapid multiplication and dispersion and furthermore, dispersion of fecal matter due to rain splashes. These factors enhance hatching of miracidia, infection of snails and cercarial and metacarcarial contamination to adjacent areas through water.

The lowest prevalence in winter is also well documented by (Patel *et al.,* 2001).The low prevalence in winter could be attributed to harsh winter conditions during which whole area remains dry and there are no chances of fresh infection due to non availability of intermediate snail hosts. Thus the agroecological conditions play a significant role in the preponderance of infection with *Schistosoma* in hosts. Nevertheless, in other latitudes, some differences are observed. For instance in Australia, the prevalence of *Calicophoron caliphorum*, decreased in spring and summer and increased in autumn and winter (Rangel-Ruiz *et al.*, 2003).Similarly Lima *et al.,* (2001) reported that the highest level of pasture contamination occur during the dry season. Thus climatic conditions have a great role to play in the onset of helminth infections in ruminants.In many other studies by (Mahalu.1970,Makundi,2001, Keyyu *et al.,* 2005,) have clearly indicated a seasonal pattern in the prevalence of fluke (*Fasciola*) infections in cattle.

In various parts of India *S. indicum, S. spindale, Ornithobilharzia* sp. and *Paramphistomum* showed marked seasonal influence which was highest during the monsoon season and lowest in summer (Mohanty *etal.,* 1984 and Phiri *etal.,* 2007). Fluke infections mainly *F. gigantica, S.spindale, Amphistomum* were also showed higher prevalence in cattle, buffaloes, sheep and goats during monsoon season, moderate during the winter season and lowest during the summer season.Possible reasons for this apparent higher prevalence may be the snail intermediate host (*I. exustus, L. luteola* and *L. auricularia*) which are present in ponds, ditches, beels and rice paddy fields was influcenced by season reaching to its peak in the beginning of October with lowest population in May and June. On the otherhand, however, the capability of the definitive host to contaminate the environment with viable eggs may also affect the level of transmission.In most cases cattle would have free access to water holes while grazing or would be led one to three times a day to an irrigation canal or river (Majid *et al.,* 1980a, Kassuku *et al.,* 1986). Data on the behaviour of cattle in relation to water is scarce, but the results of Pitchford, (1963) and Pitchford *et al.,*(1973) found that they often enter and wade and defecate into water when drinking. Higher prevalence of schistosomiasis also observed in monsoon season (33%) and lowest in summer season (21.3%) among ruminants by Bedarkar *et al.,* (2000) and supports the present finding.

In the present investigation the monthwise data shows overall highest prevalence in September (59.0%) in cattle and lowest in December and January (17.5-17.9%)(Table4). All the study areas individually followed the same pattern of prevalence. In Bangladesh with the onset of rainy season (June to August) there was a pronounced changes in the environmental temperature and rainfall, that favors the emergence of cercariae of schistosome from snail, and it may show their existence in June and July and are infected by cattle therby causing schistosome infection in cattle.That is the reason for highest prevalence (44.1-59.0%) of animal schistosomiasis was recorded in the month of July to September. This results could get favor from the results of Markovics *et al.,*(1993) who recorded highest prevalence of schistosomiasis (29 to33%) for the month of September and October.

3.7.2 Herd size

The cattle of large herds were more susceptible to schistosomiasis than that of small herds. Similarly, several studies have shown that large herds increase the risk of schistosomiasis like any other diseases such as coccidiosis, mastitis in the bovine (Soulsby,1982, Radostis *et al.,*2000).It is likely that increased exposure of cattle to infective stage from moist and contaminated floor in large herd might be a contributing factor.

3.7.3 Reducing the number of independent variable

3.7.3.1 Association among explanatory variable

To solve the problem of multicollinearity by $2\times2\chi^2$ test or pairwise coorelation has some limitations.The choice of which independent varible is to be removed arbitraryly.If pair wise coorelation is used then selecting the level of co-relation co-efficient that represent a problem is also arbitrary.Knowledge of the production system may help to make an appropriate decision (Dohoo *et al.*,1996).

3.7.3.2 Risk factors study by egg count

In the negative binomial multiple regression model analysis by fecal egg count several variables such as topographic areas, ages of animals, sexes of animals, grazing habits of animals, fecal consistency and seasons significantly influenced the prevalence of schistosome infection and identified as risk factors for schistosome infection in cattle. In the present study the egg count of schistosome infection showed has an increased trend with the lower age in cattle than the higher age group.There are many reports which corroborates the present finding. In endemic areas, the fecal egg count (range 70-310 egg /gram of feces) normally peaks in animals of about nine months of age (range 6-15 months) and decrease markedly by the age of 18 months (Majid *et al.,* 1980a, DeBont *et al.,* 1995a, Lawrence, 1977a). DeBont *et al.*, (1995a), Majid *et al.,* (1980a) and Kasuku *et al.*,(1986) found egg counts remain low (< 20 eggs /gram of feces) in older animals and are not affected by variation in levels of transmission.The decline in egg counts observed in ageing, naturally infected cattle is not associated with reduced water contact. This decline could be due to age-dependent resistance or the development of immunity. However, since cattle reach sexual maturity around the

age of 12-15 months it is difficult to relate the decline in fecal egg count to either of these two factors. The existence of pure age-dependent resistance to schistosome infection appears to unlikely in cattle. Outbreaks in susceptible herds appear to affect all age groups of animals equally (Kulkarni *etal.,* 1954, Lawrence and Condy 1970). A prevalence study by Makundi *et al.,* (1988) at Lulanzi dairy farms revealed a peak egg output in 1-3 years old animal which was follwed by a decline within the age group of 3-9 year old animal.This finding is in close agreement with the present finding.

By a longitudinal survey on *S. mattheei* in cattle DeBont *et al.,* (1995a) showed a significant decline in the intensity of infection with age as measured by fecal egg counts. With the present observation this confirms the findings of Pitchford and Visser (1982) who observed that in cattle living in a highly endemic area of South Africa, high *S. mattheei* egg counts (> 76 epg) were found almost exclusively in young animals' upto 3 years old. Although a decline in fecal egg counts with increasing age may be expected in growing cattle as a result of greater egg dilution in increasing fecal masses that is when fluke loads and egg production remain constant, it has been demonstrated experimentally (McCully and Kruger, 1969, Lawrence, 1973a, 1977c) that the time related decline in *S. mattheei* fecal egg output was mainly due to an immune mediated suppression of fluke fecundity.It has been suggested that acquired cell mediated immunity was associated that leads to develop protection against fecal egg output due to schistosomiasis in adult cattle.The finding of significantly higher egg positive cattle among the young in the present study agrees with Lima *et al.,* (2001) who demonstrated that infection in calves can be recorded from as early as 2 months of age.

Fecal egg counts may be influenced by the amount of feces produced per day, time of day of fecal sampling, low sensitivity of coproscopic techniques and other factors. Moreover, acquisition of immunity by cattle in chronic infections may decrease egg out put (Bitakaramire, 1973).

In caprine schistosomiasis bovis, fecal egg output commences 4-8 weeks post infection, reaches its maximum by week 8-10 post infection and declines to minimum values 16-24 weeks post infection (Saad *et al* ., 1989, Kassuku, 1985, Johansen *etal.* , ,1997). A decrease in the egg output with time is seen in both cattle and goats and has been suggested to be an effect of worm death, egg retention in the tissues and reduced worm fecundity (Saad *et al.,* 1984a). However, evidence indicates that the decline in egg output results from lowered worm fecundity rather than from attrition of worm populations (Bushara *et al.,* 1983a, 1993, 1994, Johansen *et al.*, 1997). This antifecundity effect is considered to be an immune regulation phenomenon.The resistance is expressed as a reduction of the egg output with time and has been shown to remain after challenge exposure (Bushara *et al.,*1983b,Johansen *et al.*,1996b Johansen *et al.,*1997).The regulating mechanism presumably immunogically mediated has proved to be an anti-fecundity effect with reduced egg production and output, but with a persistent worm population.

Animals used in the present study were most likely to be continuously exposed to reinfection, and the low monthly mean egg count (10-280eggs/animal) encountered in this study agrees with findings from other parts of the world (Pitchford and Visser, 1982, Kassuku *et al.,* 1986).It has been well known that under field conditions fecal egg output rarely exceeds 50 eggs per gram (Majid *et al.*, 1980a, Pitchford and visser, 1982, Kassuku *et al*, 1986, De Bont *et al.*, 1995a) although much higher worm and fecal egg counts were recorded during outbreaks of clinical schistosomiasis. Fecal egg output during severe outbreaks can vary between 100 and 1000 eggs per gram of feces (Vanwyk *et al.,* 1974, Lawrence, 1978a, Dargie 1980, Markovics *et al.,* 1993, Lawrence, 1978b).

In the present study and studies by Pitchford and Visser (1982) and De Bont *et al.,* (1995a) showed the prevalance of *Schistosoma* spp. in cattle as measured by fecal egg counts was higher in calves that in either the young or adults. Although a decline in fecal egg counts with increasing age may be expected in growing cattle as a result of greater egg dilution in increasing fecal masses (DeBont *et al.,* 1995a).Similar changes have been reported in cattle infections involving other schistosome species such as *S. bovis* (Bushara *et al.,* 1980) and *S. spindale* (DeBont *et al.,* 1991a).

3.8 Relationship of Schistosomiasis with Temperature and Rainfall

Considering the role of meteorological factors in the spread of schistosomiasis, it was noted that variation in the monthly distribution pattern was found in relation to temperature (0^0c) and rainfall (mm).Gradual increase in average temperature was associated with increased evaporation in May-June with an ultimate rapid increase of rainfall in July, August and September.It is commonly known that after rainfall humidity increases. Highest prevalence was noted in cattle in the month of September in four study areas (Mymensingh, Netrokona, Kishoregonj and Chittagong) except in Sirajgonj where highest prevalence was recorded in August when ambient temperature and rainfall was also high.Similarly lowest prevalence in all study areas was recorded in December and January and at that time temperature and rainfall was also low than other months. Co-rrelation co-efficient calculation showed positive relationship with temperature and rainfall with schistosomiasis in cattle. Similar suggestion has been given by Zhou *et al.,* (2008) who reported that the most important factor influencing the incidence of schistosomiasis is temperature that affects the hatching of schistosome ova, the viability of cercariae and snail population. Usually rainy season in Bangladesh starts in the month of June and changes the environmental temperature and humidity that favors the cercariae emerge from the snail (Qadir,1980).That's why after rainfall highest infection was noted in the present study. The influence of climate and environmental variables on the prevalence of schistosomiasis has been assessed in several previous studies which more or less support these results. (Appleton, 1978,Brown 1994, Cross *et al.,* 1994, Kristensen *et al.,* 2001, Malone *et al.,* 2001, Kabatereine *et al.,* 2004, Yang *et al.,* 2005, Song *et al.,* 2006, Zhou *et al.* , 2008).Rahman (1977) and Qadir (1980) also reported similar observations in Bangladesh.

CHAPTER 4

INVESTIGATION 2

PREVALENCE STUDY BY MESENTERIC BLOOD VESSELS EXAMINATION WITH INTENSITY OF *SCHISTOSOMA*

4.1 INVESTIGATION 2

PREVALENCE STUDY BY MESENTERIC BLOOD VESSELS EXAMINATION WITH INTENSITY OF *SCHISTOSOMA*

4.1.1 INTRODUCTION

Reports on the prevalence of infection have been principally based on samples collection during field survey doing fecal examination for egg of parasites or clinical symptoms. Only the method of fecal sample examination grossly underestimates the prevalence and thus interferes with treatment and control strategies.By the method of simple fecal examination in detection of *Schistosoma* eggs in chronic form has not achieved a level of reliability for use in animal.Therefore,the most direct and reliable technique for the diagnosis of schistosomiasis is post-mortem examination.At post-mortem,visceral schistosomes can easily be diagnosed by examining the mesenteric veins for the presence of paired and unpaired worms.During epidemiological studies precise data on worm burdens(intensity of infection) or accumulation of eggs in tissues (morbidity of infection) may be required, which can only be obtained by postmortem examination.Adult worms can be counted, in situ by examining the mesenteric veins over the entire length of the intestine (De Bont *et al.,*1991a,1994).This technique allow the detection of very light infections which could otherwise remain undetected with other techniques such as fecal egg or miracidial counts.Studies involving the systemic screening of slaughtered animal research to asses the actual status of infection and the incidence of the disease have not been carried out in detail in Bangladesh. Likewise, no attempt is made in slaughterhouses by veterinarians to search the mesentry of any animal for the presence of schistosomiasis except where scientist are engaged in work on schistosomiasis.

Hence, this study was undertaken with a view to fulfill the following objectives:

(i) To asses the prevalence with intensity of intestinal schistosomes by mesenteric blood vessels examination from slaughtered animals.

(ii) To findout the relationship of prevalence with temperature and rainfall.

4.1.2 MATERIALS and METHODS

4.1.2.1 Examination of mesenteric blood vessels for collection of schistosome parasites

To study the prevalence of schistosomiasis a total of 1097 of mesentery of both small and large intestines were collected weekly from the local municipal slaughter houses of the five earlier mentioned study areas for collection and identification of schistosomes.The age of the animals were determined by examining the teeth before/ or after slaughter (Sharma, 1981). The gender of the animal was recorded before slaughter.After slaughtering collected viscera was examined for any gross pathological changes caused by the schistosome parasites. Most of the slaughtered cattle were male except few were female as law of animal protection restricts to sacrifice the healthy female animals.At first the mesenteric veins were examined by exposing the mesentry against light to detect schistosomes, the mesentry is separated from its attachments and the mesenteries of both the small and large intestines were cut into small pieces and were immersed in large glass bowls containing normal saline and left undisturbed for 4-5 hours to recover schistosomes. The saline solution should then be searched for blood flukes.The parasitic identification and counts of washings of the mesenteric blood vessels were made by the method of (Taylor 1934, Bell, 1963 and Soulsby, 1965) with little modifications.

The intensity of infection was considered to be mild when 1-20 worm pairs were recovered (schistosome always occur in pairs as the female worm is carried by the male in the gynaecophoric canal), moderate when 21-100 worm pairs were observed and heavy when more than 100 worm pairs were observed.It can be mentioned here that the intensity of parasitic infection was expressed in number.

4.1.2.2 Study of gross lesions

To detect gross lesions and attachment of parasites the mesenteric blood vessels, mucosal surface of small and large intestine was examined before washing and detected gross lesions were recorded.

4.1.2.3 Identification and preservation of the parasites

The worms (schistosomes) were preliminary identified under microscope (x 10) using low power objectives. The schistosomes were then preserved in 10% formalin in separate vials. The parasites were finally identified following the standard key (Yamagutti, 1958) after preparation of permanent slides as described by Cable (1967).

4.1.2.4 Preparation of permanent slides

For making permanent slides of the collected parasites the following procedure was followed as described by Cable (1967).

After collection of adult parasites at first it was fixed in alcohol formal acid fixative solution for 20 minutes.

Then the parasite was sunk in 70% iodine alcohol for 1 to several hours.

After that the parasite was shifted into 70% alcohol until the specimen shows no iodine colour.

Then it was stained with semichons carmine solution for 1-8 hours.

After that the parasite was washed in 70% alcohol for twice.

Then it was de- stained in 0.5% HCl and 70% alcohol and it was passed through 70% alcohol to remove the acid.

Then it was changed successively in 80%, 90% and absolute alcohol for one hour.

Then the parasite was sunk in aniline oil or creosote until it sinks and then it was washed momentarily in xylene.

Finally it was mounted in DPX or Canada balsam.

After that the prepared samples were identified under low power magnification (×10).

Microphotograph of the identified parasite was taken with the help of digital camera.

4.1.2.5 Identity of the schistosomes encountered in the investigation

From the present investigation it is quite evident that the morphological differences among the species existed and the definite identity could be found out with respect to the following:

(i) Size and shape of the egg.

(ii) Number of the testicular glands.

(iii) Nature of the cuticle

(iv) Position of the ovary in female.

(v) Length of the paired intestines and the intestinal stem.

KEY TO THE SPECIES OF THE GENUS *SCHISTOSOMA* ENCOUNTERED IN THE PRESENT INVESTIGATION (Soulsby,1982)

1. Cuticle smooth, testes four to five in number, ovary posterior to median, eggs spindle shaped measuring 290.09 micron to 303.27 micron, the spine 16 micron to 18 micron long.---------- *Schistosoma spindale*

   Cuticle tuberculated, testes three to ten in number, ovary medium and anterior to median, eggs oval, and boomerang shaped. ----------2

2. Testes seven to ten in number, ovary anterior to median, eggs oval shaped measuring 92.30 micron to 125.26 micron x 32.96 micron to 52.74 micron, the spine 7.2 micron to 16 micron long. ---------- *Schistosoma indicum*

   Testes three to four in number, ovary median, eggs boomerang shaped measuring 334.21 micron to 502.3 micron x 48 micron to 70 micron, the spine 10 to 18 micron long. ---------- *Schistosoma nasale*

4.1.2.6 Examination of liver for collection of schistosome eggs

A representative 5% of the dissected liver were obtained when the carcasses were dressed and were screened for collection of eggs by the method of eggs per gram (Sumanth *et al.,* 2004).

The collected liver were mixed with 5 ml of 10% potassium hydroxide in wide bored pipettes and transferred to a test tube for heating (at 37^{0C} for 12 hours). After mixing it thoroughly, the sediment was drawn-off completely or in portions, depending upon the quantity, with the aid of long glass dropper and transferred to a slide, which was then covered by a cover slip.

4.2 RESULTS

4.2.1 Prevalence based on collection of *Schistosoma* from mesenteric blood vessel examination

Overall distribution of schistosomes collected from mesenteric blood vessels is presented in Figure 9.The prevalence and type of schistosomes recovered from mesenteric blood vessels is summarized in Table 13. The male worms are characterized by elongate flat with the body curved into a longitudinal fold, the gynaecophoric canal, in which the long and slender female lies (Plate5).The overall prevalence of adult schistosomes in cattle is 48.0%. Significantly higher prevalence of *S. indicum* (28.6%) is observed than that of *S. spindale* (12.7%). Mixed infection of *S. indicum* and *S. spindale* is found in 6.7% of the viscera. The highest number of total schistosomes collected from one viscera is 322 and lowest is18. Mean of paired schistosomes are (14.9) which are most frequently occured than male (Mean=12.2) and female (Mean=9.3). Mean of total schistosome is 50.3.Male schistosome population was more as compared to female in animals. Male and female parasite ratio is 12:10.The range of adult paired schistosome varied from 9 to110.Lowest recorded male schistosome is 6 and highest recorded is 73.Lowest recorded female schistosome is 2 and highest recorded is 125.

Districts, seasons, ages and sex wise distribution of adult schistosome's prevalence is shown in Table 14. Significantly ($p<0.01$) higher prevalence of adult schistosomes were recovered from Chittagong (52.3%,CI.46.1,58.4) and Sirajgonj (51.5%,CI.45.7,57.2) districts than those in Netrokona (44.9%,CI.37.0,53.0), Mymensingh (44.7%,CI.37.9,51.6) and Kishoregonj (41.4%,CI.33.6,49.5) districts. The prevalence of adult schistosomes was also significantly ($p<0.01$) higher in rainy (64.4%,CI.59.8,68.9)season in comparison to those in summer (40.3%,CI.34.6,46.2) and winter (34.9%,CI.29.9,40.0) seasons. The variation of adult schistosome prevalence's in different sex and age groups of slaughtered cattle were found insignificant.

4.2.2 Intensity of adult pair schistosomes

The intensities of infection with visceral schistosomes by *S.indicum* and *S.spindale* were determined based on the number of worm pairs obtained from the visceral cuttings. The intensity of adult paired schistosomes is presented in Table 15. About 52.1% viscera of infected animal were found adult schistosome free. Overall moderate intensity of paired schistosomes (21-100 pairs) was noted in 27.4% (CI.24.8,30.2) of the viscera which are statistically significant (P<0.001) than low (1-20 pairs) 0.1%,(CI.0.002,0.5,14.4) and heavy (>100 pairs) 20.6% (CI.18.2,23.1) worm intensity.Moderate intensity (21-100 pairs) of paired *S.indicum* was found significantly higher 17.6% (CI.15.4,19.9) than low (1-20 pairs) 0.1% (CI.0.002,0.05) and heavy (>100 pairs)10.9%(CI.9.2,12.9) worm intensity. But in case of *S.spindale* intensity of adult paired schistosomes did not show any significant variation.

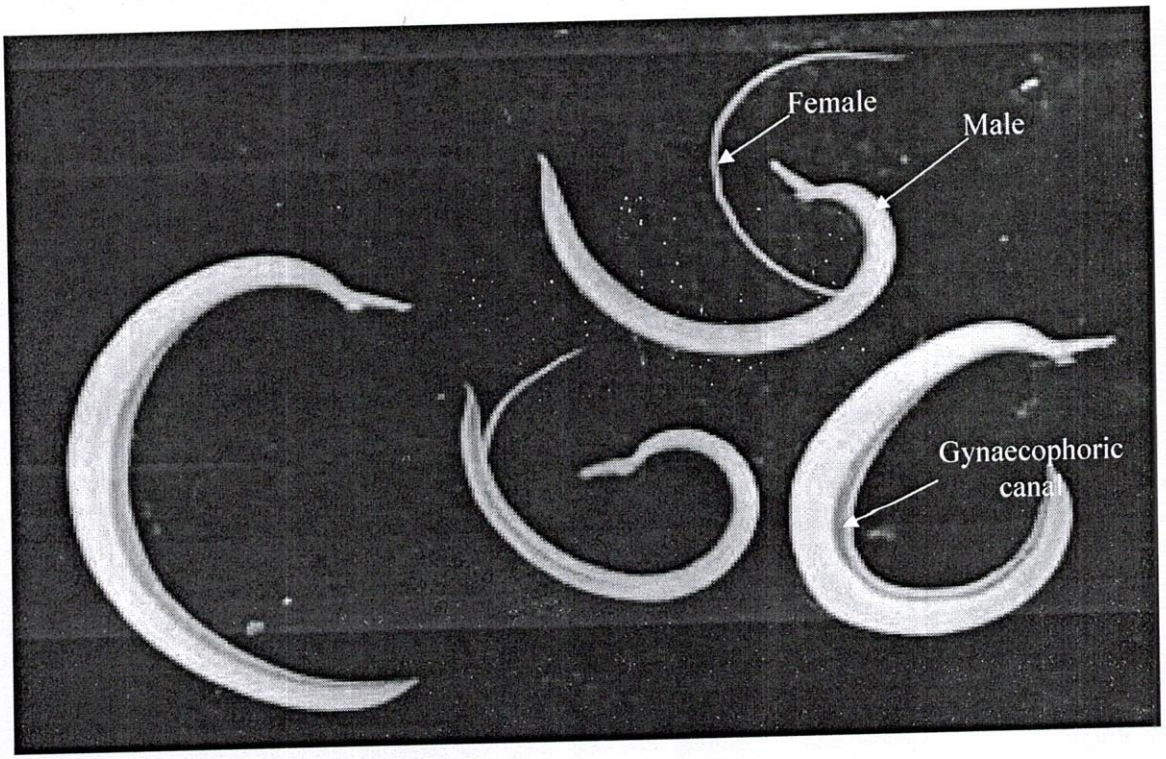

Plate 5. A male schistosome grasping a female worm by gynaecophoric canal

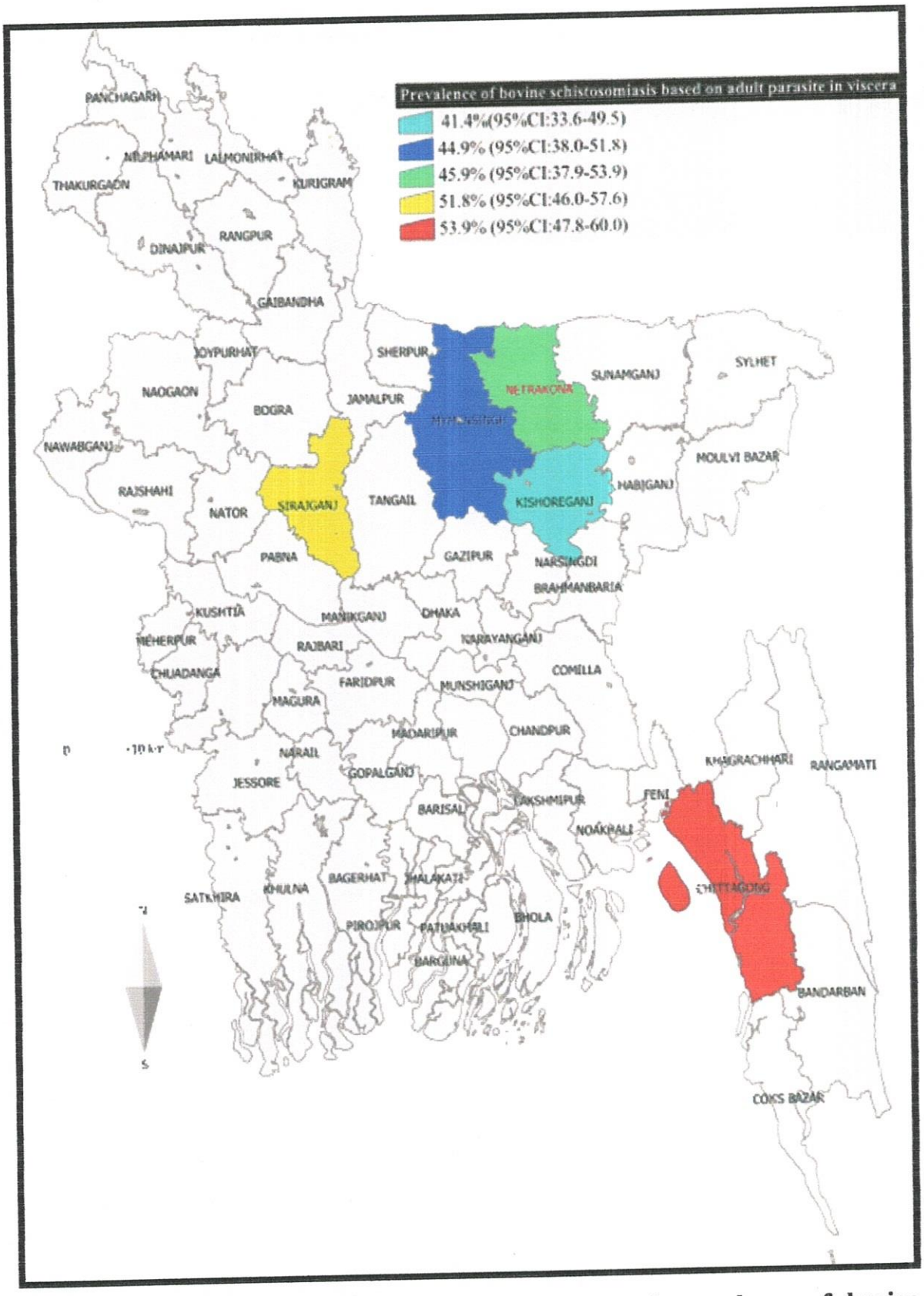

Figure9. Map of Bangladesh showing distribution of prevalence of bovine schistosomiasis based on schistosome parasites collected from mesenteric blood vessels

Table13. Schistosomes recovered from mesenteric blood vessels in cattle of Bangladesh(n=1097)

Variable	Mean±SE	Median	Range
Age of slaughtered cattle (Years)	9.3±0.09	10.0	3.0-16.0
Pair of schistosomes	14.9±0.57		9-110
Male schistosomes	12.2±0.45		6-73
Female schistosomes	9.3±0.37		2-125
Total schistosomes	50.3±1.8		18-322
Species of *Schistosoma*	**Positive**	**Prevalence**	**95% CI**
Overall	**527**	**48.0**	**45.0, 51.0**
None	570	51.9	48.9, 54.9
S. indicum	314	28.6[a]	25.9, 31.4
S. spindale	140	12.7[b]	10.8, 14.8
S. indicum and *S. spindale*	73	6.7[c]	5.3, 8.3

Values with different letters within a column differ significantly ($p<0.01$)

Table14.Prevalence and distribution of bovine schistosomiasis based on schistosomes collected from mesenteric blood vessels (n=1097)

Category	Viscera with schistosomes			Total	Prevalence (95% CI)
District	*S. indicum*	*S. spindale*	Mixed		
Kishoreganj	37	16	12	65	41.4(33.6, 49.5)[a]
Netrokona	39	15	17	71	44.9 (37.0, 53.0)[a]
Mymensingh	59	26	11	96	44.7 (37.9, 51.6)[a]
Chittagong	81	44	14	139	52.3 (46.1, 58.4)[b]
Sirajgonj	98	39	19	156	51.5 (45.7, 57.2)[b]
Season					
Winter	70	29	23	123	34.9 (29.9, 40.0)[a]
Summer	71	33	12	116	40.3 (34.6, 46.2)[a]
Rainy	173	78	38	288	64.4 (59.8, 68.9)[b]
Age					
3-7 years	111	42	23	176	54.7 (49.0, 60.2)
8-11 years	132	52	30	214	45.1(40.6, 49.8)
12-16 years	77	43	24	144	47.8(42.1, 53.6)
Sex					
Male	240	106	56	402	50.3 (46.7, 53.8)
Female	80	31	21	132	44.4 (38.7, 50.3)

Values with different letters within a column differ significantly ($p<0.01$)

Table15. Intensity of adult pair schistosomes collected from bovine mesenteric blood vessels (n=1097)

Category	Viscera with (S.indicum and S.spindale)	Percentage	95% CI
Absence of schistosomes	571	52.1	49.0, 55.0
Low (1-20)	1	0.1	0.002, 0.5[a]
Moderate (21-100)	301	27.4	24.8, 30.2[b]
Heavy (>100)	226	20.6	18.2, 23.1[c]
	Viscera with *S. indicum*		
Low (1-20)	1	0.1	0.002, 0.5[a]
Moderate (21-100)	193	17.6	15.4, 19.9[b]
Heavy (>100)	120	10.9	9.2, 12.9[c]
	Viscera with *S. spindale*		
Low (1-20)	0	0	0, 0.3
Moderate (21-100)	69	6.3	4.9, 7.9
Heavy (>100)	71	6.4	5.1, 8.1

Values with different letters within a column differ significantly ($p<0.01$)

4.2.3 Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with mean temperature and rainfall at five study areas

Upon grading the level of prevalence, twelve months had high prevalences in all the five study areas without showing any significant differences except in Chittagong district.In Chittagong district the month of July,August and September showed significant higher ($P<0.05$) prevalence than the other months. The data presented in Table 16-20 and Figure 10-14 showed that there is positive significant relationship of temperature and rainfall with the prevalence of schistosomiasis.

Table16. Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with temperature and rainfall (mean) in Mymensingh

Month	Temperature (°C)	Rainfall (mm)	Prevalence (%)	95% CI
January[a]	18.5	0	40.7	22.4, 61.2
February[b]	21.7	2.0	25.9	11.1, 46.3
March[c]	25.6	17.2	25.9	11.1, 46.3
April[d]	28.1	86.3	44.4	25.5, 64.7
May[e]	28.3	373.4	53.8	25.1, 80.8
June[f]	28.8	247.5	57.1	28.9, 82.3
July[g]	29.4	443.0	60.0	26.2, 87.8
August[h]	29.3	358.0	62.5	35.4, 84.8
September[i]	28.9	218.3	71.4	41.9, 91.6
October[j]	27.8	25.9	64.3	35.1, 87.2
November[k]	23.8	2.0	41.7	15.2, 72.3
December[l]	19.5	0.65	28.5	8.3, 58.1
Correlation	r=0.75**	0.66*		

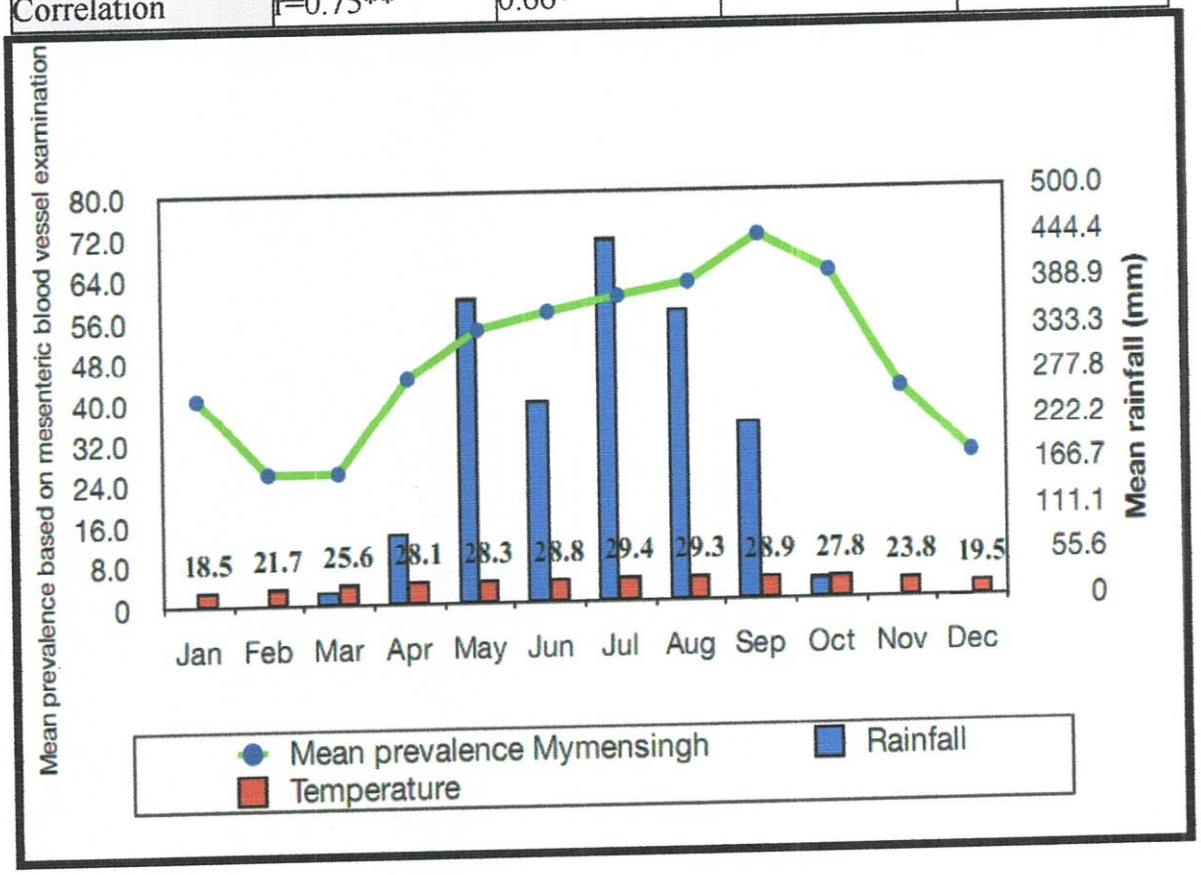

Figure10. Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with temperature and rainfall (mean) in Mymensingh

Table17.Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with temperature and rainfall (mean) in Netrokna

Month	Temperature (°C)	Rainfall (mm)	Prevalence (%)	95% CI
January[a]	18.5	0	25.0	5.5, 57.2
February[b]	21.7	2	21.4	4.7, 50.8
March[c]	25.6	17.2	26.7	7.8, 55.1
April[d]	28.1	86.3	36.4	10.9, 69.2
May[e]	28.3	373.4	42.8	17.7, 71.1
June[f]	28.8	247.5	58.3	27.7, 84.8
July[g]	29.4	443	60.0	32.3, 83.7
August[h]	29.3	358	69.2	38.6, 90.9
September[i]	28.9	218.3	76.9	46.2, 94.9
October[j]	27.8	25.9	61.5	31.6, 86.1
November[k]	23.8	2	37.5	15.2, 64.6
December[l]	19.5	0.65	30.0	6.7, 65.2
Correlation	r=0.78**	r=0.66*		

* Significant at p<0.02 **Significant at p<0.003

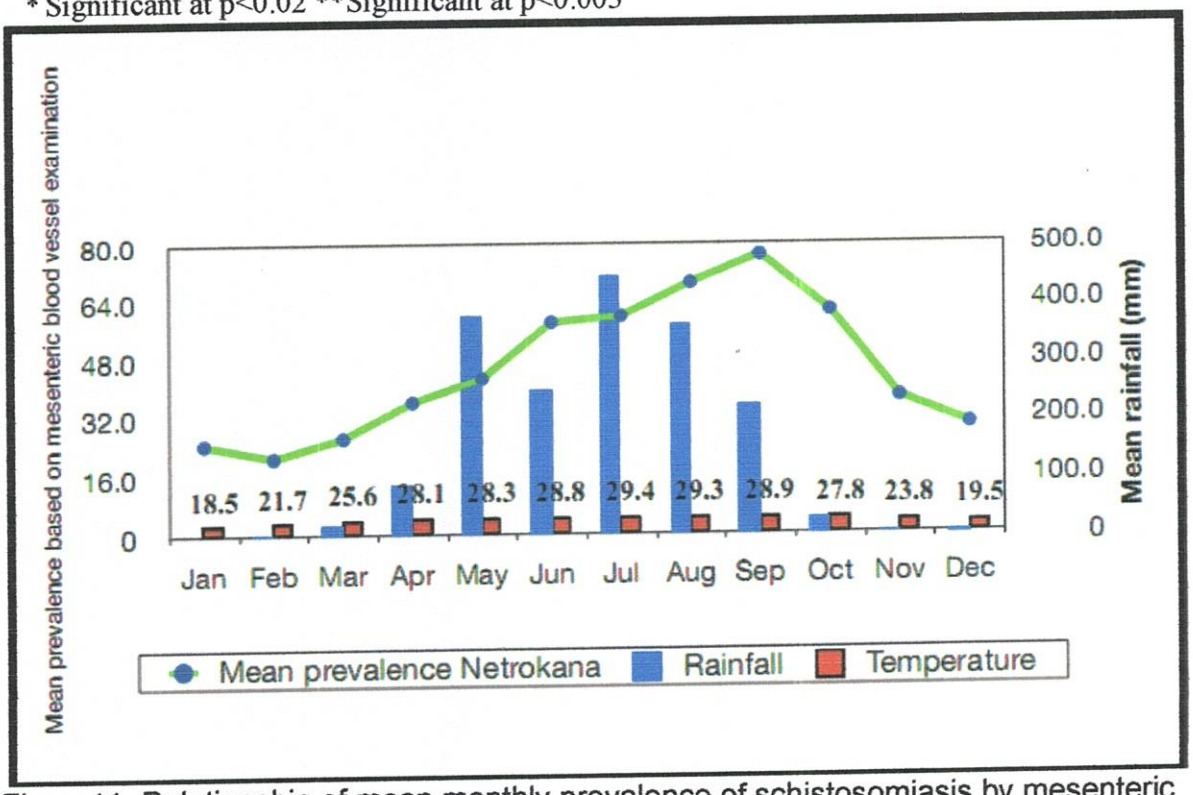

Figure11. Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with temperature and rainfall (mean) in Netrokona

Table18.Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with temperature and rainfall (mean) in Kishoregonj

Month	Temperature (°C)	Rainfall (mm)	Prevalence (%)	95% CI
January[a]	18.5	0	28.6	8.3, 58.8
February[b]	21.7	2.0	25.0	5.5, 57.2
March[c]	25.6	17.2	33.3	11.8, 61.6
April[d]	28.1	86.3	36.4	10.9, 69.2
May[e]	28.3	373.4	40.0	16.3, 67.7
June[f]	28.8	247.5	45.5	16.7, 76.6
July[g]	29.4	443	53.8	25.1, 80.8
August[h]	29.3	358	53.8	25.1, 80.8
September[i]	28.9	218.3	69.2	38.6, 90.9
October[j]	27.8	25.9	53.8	25.1, 80.8
November[k]	23.8	2.0	40.0	12.2, 73.8
December[l]	19.5	0.65	23.5	6.8, 49.9
Correlation	r=0.77**	r=0.53		

** Significant at $p<0.003$

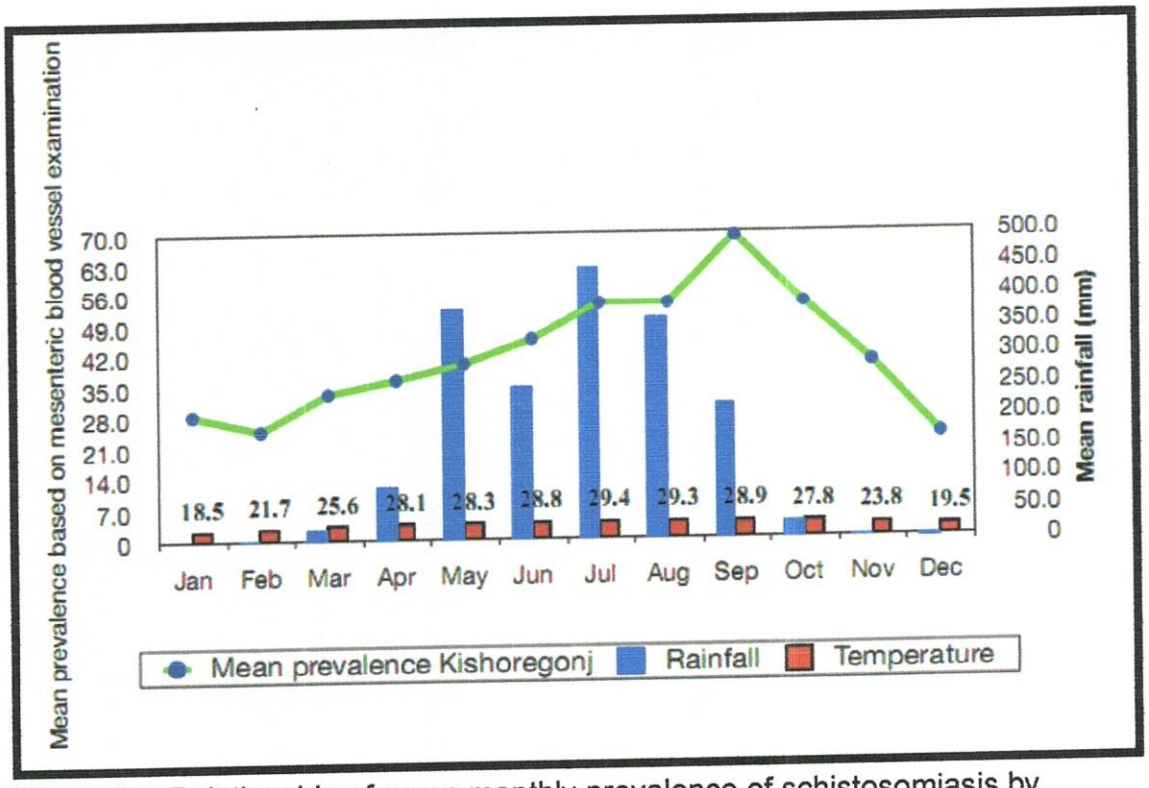

Figure12. Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with temperature and rainfall (mean) in Kishoregonj

Table19.Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with temperature and rainfall (mean) in Chittagong

Month	Temperature (°C)	Rainfall (mm)	Prevalence (%)	95% CI
January[a]	19.8	5.3	38.1	18.1, 61.6
February[b]	22.1	7.1	38.9	17.3, 64.3
March[c]	25.3	25.6	42.3	23.4, 63.1
April[d]	27.6	115.9	47.4	24.4, 71.1
May[e]	28.5	401.3	53.3	26.6, 78.7
June[f]	28.4	437.8	62.5	40.6, 81.2
July[g]	28	493.1	73.9[l]	51.6, 89.8
August[h]	28.1	386.5	72.3[l]	54.6, 92.2
September[i]	28.4	289.3	78.3[l]	56.3, 92.5
October[j]	27.8	89.9	51.9	31.9, 71.3
November[k]	25.2	53.3	47.6	25.7, 70.2
December[l]	20.8	6.0	22.2	8.6, 42.3
Correlation	r=0.79**	0.82**		

**Significant at p<0.002, letters [a-l] correspond to months from January to December. Month wise prevalence when significantly higher (p<0.05) than other month(s) are shown by that (those) month specific letters in superscript

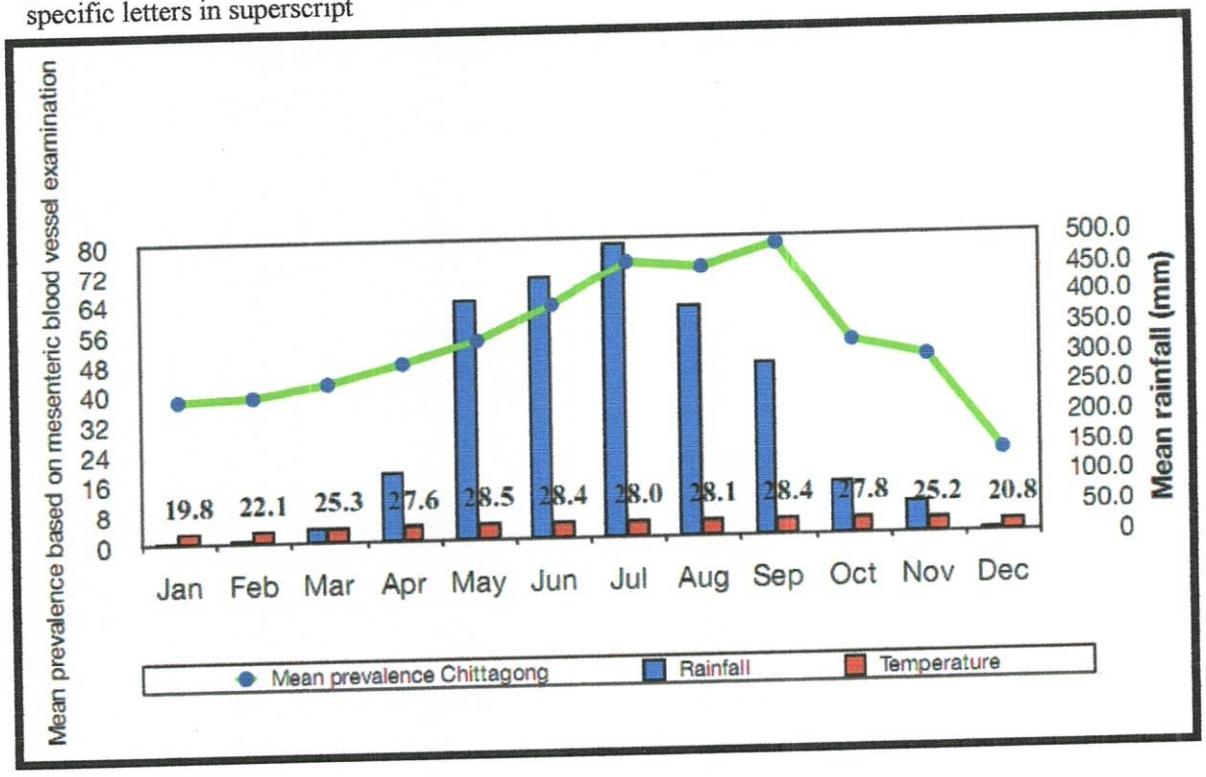

Figure13. Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with temperature and rainfall (mean) in Chittagong

Table20. Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with temperature and rainfall (mean) in Sirajgonj

Month	Temperature (°C)	Rainfall (mm)	Prevalence (%)	95% CI
January[a]	17.5	22.6	35.0	15.4, 59.2
February[b]	19.8	45.5	33.3	16.5, 53.9
March[c]	25	10.4	37.0	19.4, 57.6
April[d]	29.3	45.7	44.4	25.5, 64.7
May[e]	28.9	168.1	50.0	29.9, 70.1
June[f]	29.5	340.3	59.3	38.8, 77.6
July[g]	29	145.5	66.7	46.0, 83.5
August[h]	29.1	268.3	67.9	47.6, 84.1
September[i]	28.8	149.3	40.7	22.4, 61.2
October[j]	27.4	137.7	63.6	40.7, 82.8
November[k]	23.4	7.6	50.0	27.2, 72.8
December[l]	19.5	0.0	32.0	14.9, 53.5
Correlation	r=0.77**	r=0.69*		

*Significant at p<0.01 **Significant at p<0.004

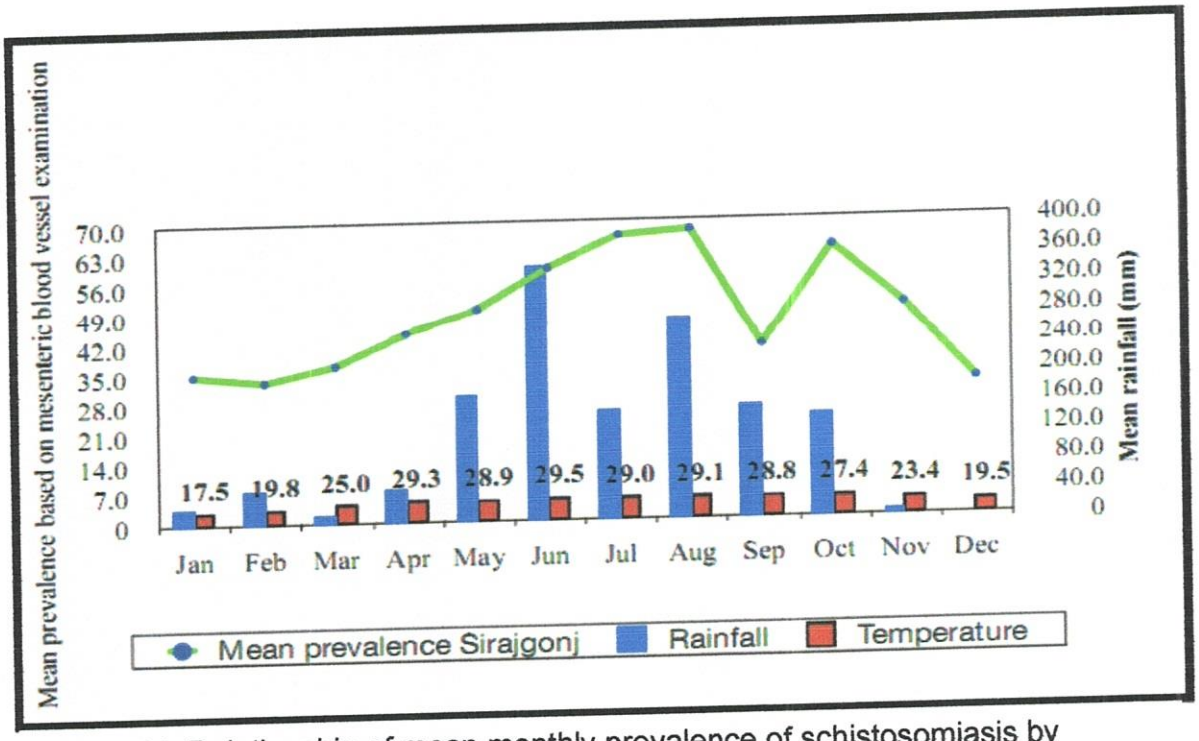

Figure14. Relationship of mean monthly prevalence of schistosomiasis by mesenteric blood vessels examination with temperature and rainfall (mean) Sirajgonj

4.3 DISCUSSION

4.3.1 Prevalence study by mesenteric blood vessels examination

In the present study overall prevalence was recorded (48.0%) with *S. indicum* 28.6%, *S.spindale* 12.7% and mixed of *S. indicum* and *S.spindale* 6.7% (Table15).In another observation by Islam (1975) examined the visceral organs of 224 cattle for the presence of *Schistosoma* spp. The overerall prevalence was found 62%, with 43% *S. indicum*, 33% *S. spindale* and 13% mixed infections which was higher than the present study.In north eastern Thailand, Upatoom *et al.,* (1988) reported a high prevalence of *S. spindale* of 60-80% sometimes associated with severe clinical symptoms and mortality, particularly in calves.

The incidence of *Schistosoma spindale* and *S.indicum* in Bangladesh based on detection of worms from visceral organs, indicated a lower incidence of 12.7% and 28.6% respectively in the present study when compared to the incidence rate of (Islam,1975).The infection incidence rate observed in the study of Sumanth *et al.,* (2004) which was based on the detection of eggs from intestinal scrapings and worms from the mesenteric veins was 59.16%. This is much higher incidence than observed in the present study indicating that the techniques used might not detect infection in subclinical and carrier animals. Similarly, a previous study, based on fecal examinations, reported lower prevalences of 0.41% and 0.1% of *S. spindale* in the Mysore and Mandy districts of Karnataka state (Muraleedharan *et al.,* 1990) respectively but the study by Sumanth *et al.,* (2004) based on the examinations of intestinal scrapings for the presence of eggs and worms indicated a higher percentage of infection of 59.16% for *S. spindale*. This strongly indicates that the incidence can be under diagnosed when based soley on fecal examination, as the sensitivity of diagnosis of this method is low.

Prevalence of schistosomiasis by parasite detection from slaughtered animals was found to be more prevalent throughout the year.There are many reports of higher fluke prevalence in adults than calves in various part of the world.In the present study all the slaughtered animals were adult (>3years) as animal showed higher occurrence

throught the year.Similar observations was revealed by (Cringloi *et al.*,2002, Molina *et al.*,2005).Lower prevalence (3.5%) of natural infections of *S. bovis* was observed by Rossa and Simoes (1999) in cattle slaughtered at Praia abaittoir of Santiago Island, Capeverde.But Ndifon *et al.,* (1988) noted higher prevalence (31.1%) of schistosomiasis by *S.curassoni* and *S.bovis* in northern Nigeria.

Schistosome infection also occured some part of the Sudan and Zambia throughout the year on the farm with average worm recoveries ranging from 71 worms during the cold dry season to 736 worms at the end of warmer rainy season. It is evident that there was a strong co-rrelation between worm burdens at a particular time and the infected snail densities recorded at the main transmission site (DeBont *et al.*, 1995b).The risk of infection is highest amongst those farms which are located near lakes or rivers. In Uganda, almost no transmission was found to have occurred at altitudes greater that 1400m or when the annual rainfall was less than 900 mm (Kabatereine *et al.* , 2004).Sumanth *et al.,* (2004) recorded 65.6% *S. nasale* and 50.3% *S. spindale* by examining 300 male cattle at an abaittior in Bangalore. A mild infection of *S. nasale* was observed in 43% animal and 41.3% animal was infected by *S. spindale*. Moderate infection of *S. nasale* was found in 25.7% animal and 2% animal was moderately infected by *S. spindale*. Visceral examination showed 72.7% and 68% prevalence by *S. nasale* and *S. spindale* infection respectively and mixed infection of both species occurred in 54.3% animals and it differs with the present study. Four percent of cattle were heavily infected with *S. nasale* and 4.7% cattle were heavily infected with *S. spindale*. The authors concluded that the incidence of nasal and visceral schistosomiasis in cattle of Bangalore is under diagnosed and the carrier status of these animals in the subclinical state is potentially high. Krishnasamy *et al.,* (2004) reported high prevalence rate (39.5%) of *S. spindale* infection in Malaysia and also showed that *S. spindale* had a high zoonotic potential in this area.

The prevalence and intensity of infection observed in Kandy, Srilanka increased with the age of the host which corroborates with the present findings (not significant difference). For cattle in Srilanka, there seems to be little age related variation in the amount of water contact as wherever possible, it is common practice for farmers to

bathe their stock daily in the local pond or river. It may therefore be assumed that bovines living in an endemic area would regulary be exposed to cercarial infection but may differ in attractiveness to cercariae, penetrability to skin etc.In this study although prevalence of infection increased with age, mean worm burdens remaind low (50.3)(Table13) considering the possible daily reinfection of some animals.The low worm burdens may be the result of either an innate resistance of the local breeds or an early development of protective immunity against infection. Similar acquired resistance was not observed by Lawrence (1973a) in *S. mattheei* infections under experimental conditions of moderate challenge, but was clearly demonstrated in both experimental (Bushara *et al.,* 1980, 1983a b) and natural infections of *S. bovis* (Majid *et al.,* 1980a, Kassuku *et al.,* 1986).

Jeyathilakan *et al* ., (2008) recoverd *S. spindale* from Chenni slaughter houses and in cattle the prevalence was 30.7% and higher than the present study while in buffalo the prevalence was 19.6%. The prevalence in goats was much lower at 9.2%. The difference in the prevalence of *S. spindale* in different species of animals is due to variation in the behaviour of domesticated animals (Agrawal and Sahasrabudhe, 1982b).The prevalence of *S. spindale* was higher in cattle followed by buffaloes and goats.

The prevalence of *S. spindale* in ruminants in Chennai is higher than that of Assam as Rajkhoa *et al.,* (1992) reported 2.9% in cattle, 2.7% in goat and 16% in buffalo. However, Banerjee and Agrawal (1992) reported higher prevalence of 41.2% in cattle and 20.5% in buffaloes. This variation is dependent upon the climatic factors present in their state suitable for the development of snail intermediate host. The incidence of *S. spindale* also depends upon the method used for collection of flukes, because liver press method detects more flukes than examination of mesenteries alone (Banerjee and Agrawal, 1989).The present study by examining the infection in slaughtered animals, provides a much clearer idea of the actual status of schistosomiasis in the study areas of Bangladesh.There was no statistical difference was found in prevalence between the age and sex of the slaughtered animal in the present study.As because law of animal protection restricts to sacrifice the healthy female and young animal.

It is known from the study (Rao, 1934) that *Schistosoma* shows a sex ratio of male bias (16:1).This observation corooborates the present finding of male female ratio (12:10). It could be hypothesize that the difference in size between sexes-the female being thinner than the male and thus less able to resist blood flow could underlie a difference in survival rates and so account for the male biased sex ratio found in schistosomes (Morand and Muller-Graf, 2000).The finding of the present investigation also coorborates with Qadir (1979) who reported only male schistosomes from a natural infection of goat.

4.3.2 Intensity or worm burden of infection

The frequency and intensity of infection are related to fecal egg counts, but they are all subject to methodological variations, immunogenetic predisposition and other confounding factors (Gryseels,1992).In the present study the moderate (21-100 pairs) intensity of paired schistosomes were higher than low and high intensity of infection.Male schistosome population was more as compared to female in all animals. This is accordance with the report of De Bont *et al.,* (1991a) who reported less than 100 worm pairs in the mesenteric veins. However Rao (1934) collected more than 500 flukes with higher male fluke population.Little information is available on the average worm burden in cattle. Dargie (1980) estimated that the overwhelming majority of cattle infected with *S. bovis* in Sudan had less than 500 worms. Abattior survery have shown that over 92% of the animals infected with *S. spindale.* In Srilanka DeBont *et al.,* 1994 noted that72% of the infected animals had less than 20 worm pairs in the mesenteric veins, 20% from 21-100pairs and only 8% more than100 worm counts in situ. No attempt was made during these abaittoir surveys to count worms in those 8% of animals infected with more than 100 pairs.

In contrast to fecal egg counts,worm burdens in naturally infected animals increase with the age of the host.In Srilanka,an age specific increase in prevalence and intensity was observed in bulls in an abaittoir,as measured by worm count in situ (De Bont *et al.,*1991a).In a total of 174 infected animals classified in five age groups(<2,2,3,4and ≥5 years), the average worm burdens increased by approximately20% for each step up in age group.In another study by perfusion from

young (9 months) and adult (7 years) from a herd in Zambia showed that adult cows had about 2.5 times more *S .mattheei* worms than calves (De Bont *et al,*1995a). However, large variations in worm burden have been observed in cattle. Other factors such as immunity development, age, sex, genetic predisposition, nutritional effects and stresses of field conditions, including gastro-intestinal nematode infection and having to graze over lagre tracts of land (Saad *et al.* , 1980) are certainly also playing a role in determining levels of disease.

In the great majority of cases, visceral schistosome infections in endemic areas occur in subclinical form, characterized by a high prevalence of low to moderate worm burdens in the cattle population (Condy, 1960, Lawrence, 1978a, Vercruysse *et. al,* 1985).Occasinal outbreaks of clinical intestinal schistosomiasis caused by *S. mattheei* (Strydom, 1963, Reinecke, 1970, Lawrence and Condy, 1970, Lawrence and Mckenzie, 1972,Van wyk *et al.,* 1974, Lawrence, 1977b), *S. bovis* (Eisa, 1966, Hussein 1968, Markovics *et al.,* 1993), *S. spindale* (Kalapesi and Puhorit, 1954, Kulkarni *et al.,* 1954) and *S.japonicum* (Wang *et al.,* 1988) have been reported. They are usually restricted to young livestock or adult animals undergoing relatively heavy primary infections under condition of intensive transmission. Worm burden may therefore be a better parameter for determining the epidemiological status for any parasitic disease than egg count, as pathophysiological changes in the host may result in fluctutations in fecal egg out puts (Boray, 1969).

The prevalenc of visceral schistosomiasis of cattle in Kerala, India was recorded 57.3% by Ravindran *et al.,* (2007) with the majority (58.1%) suffering from moderate infection. A considerable number of animals (41.8%) harbored a high intensity of infection. A similar observation was also made by Banerjee (1988) who recorded 41.2% infection rate in cattle, which buffaloes had only 26.9% infection rate.These findings are in close agreement with the present investigation.Even though the prevalence of schistosomiasis in domestic animals was high based on worm counts in the present observation, comparatively less number of clinical cases was notcied from the study.The symptoms, morbidity, and mortality of schistosomiasis are to some extent related to the intensity of the infection (De Bont and Vercruysse, 1998). In the study of Ravindran *et al.,* (2007) the majority of cattle

that were infected had moderate to heavy worm count and large number of bovine clinical schistosomiasis might have been due to high intensity of infection in this species.Vanwyk *et al.,*(1994)recovered an average of57721(range41654-71083) adult *S.mattheei* worms from five severely affected animals selected during an outbreak on a farm in SouthAfrica and Lawrence (1977) counted 40679 worms from the clinically affected aimals in Zimbabwe which is more higher than the present study.Similarly from the study of Islam (1975) found that animal had an average heavier burden of 147 worms/animal and one animal had a burdrn of 1665 *S.spindale* worms which are not in accordance of the present study.

Sing *et al.,*(2000) reported the seasonal predominance in the occurrence of schistosomiasis in cattle during rainy season (74.38%) followed by winter (57.2%) and summer (24.4%) in a study carried out by worm count from mesenteric blood vessels are in close agreement with the present study(Table 14). This was correlated with higher number of snails shedding *Furcocercus* cercariae was also highest during the rainy season (8.3%) followed by winter (6.9%) and summer (2.3%)(Yilma and Malone, 1998).This is attributed mainly to the variation in the climatic and ecological conditions such as altitude, rainfall, temperature and livestock management system The high prevalence was associated with the existence of favorable ecological biotopes for *L. auricularia.* Highest prevalence rate was analyzed during October, when the wet ecological condition prevailed. It has been described that the bionomic requirements for the breeding of *Lymnaea* snails and development of the intermolluscan stages of the flukes often reach the optimum threshold during the wet months of the year. During the dry periods, breeding of the snails and development of the larval fluke's slowdown or stops completely and snails undergo a state of aestivation (Yilma and Malone, 1998). The existence of permanent suitable ecological conditions in areas like slow flowing rivers, streams and low lying marshy areas may contribute to persistent but relatively low grade infection in dry season. The interaction between moisture and temperature determines the survival and reproduction rates of the snails and the parasites. The present study together with other investigations indicates that proportion of cattle in Bangladesh always suffers from sub-clinical schistosomiasis and farmers do not know because routine check is not in practice, which would otherwise maximize the production in farms.

4.3.3 Relationship of prevalence with temperature and rainfall

Co-rrelation co-efficient calculation showed positive relationship with temperature and rainfall with schistosomiasis in cattle. Similar suggestion has been given by Zhou *et al.,* (2008) who reported that the most important factor influencing the prevalence of schistosomiasis is temperature that affects the hatching of schistosome ova, the viability of cercariae and snail population. Usually rainy season in Bangladesh starts in the month of June and changes the environmental temperature and humidity that favors the cercarial emergence from the snail (Qadir,1980) and more favorable conditions for survibility of cercariae is related with temperature and rainfall.That's why after rainfall highest infection in cattle was noted in the present study. The influence of climate and environmental variables on the prevalence of schistosomiasis has been assessed in several previous studies which more or less support these results. (Appleton, 1978, Brown, 1994, Cross *et.al.* ,1996, Malone *et al.,* 1997, Kristensen *et al.,* 2001,Kabatereine *et al.,* 2004, Yang *et al.,* 2005, Songliang *et. al,* 2007, Zhou *et. al,* 2008).Rahman (1977) and Qadir (1980) also reported similar observations in Bangladesh.

CHAPTER 5

INVESTIGATION 3

STUDY OF SNAILS AND FACTORS FOR THE DEVELOPMENT OF *FURCOCERCUS* CERCARIAE IN SNAILS AND THEIR RELEASE

5.1 INVESTIGATION 3

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5.1.1 INTRODUCTION

A through understanding of snail ecology is also a basic requirement for the success of control measures relying heavily on appropriate technology implementation. Understanding of snail ecology in relation to water resource development is greatly needed to guide preventive measures.Unfortunately, suitable snail habitats have increased as the requirements of increasing populations and development in several endemic countries have lead to large scale water impoundments for electricity and irrigation (Kay,1990 , Hunter *et al.,*1993).

Schistosomatidae need one intermediate host a freshwater gastropod from the super family of pulmonate snail family Lymnaeidae and Planorbidae in tropical regions. Besides *Schistosoma* spp. these snails also serve as an intermediate host of other trematodes (Basch and Lie, 1965, Boray, 1982). Little is known on the population dynamics of these snails in the area where agricultural activities are conducted intensively. Since it is known that the life cycle of this blood fluke is heavily dependent on snails then it is clear that the distribution of the disease will be determined by that of the snails.The dynamics of transmission are therefore intricate and exhibit variations dependent on the behaviour and habits of the host and the ecology of the molluscan intermediate hosts. So, transmission patterns would undoubtedly influence the incidence, intensity and pathology of the infection in cattle.

The duration and timing of the favorable period of snails may be only a few months during the wet season in closed water bodies (Mzembe and Chaudhry, 1979). Once the water level drops too fast during the dry season for the fringing aquatic vegetation to persist or the oxygen level drops too low, the habitat will be rendered unsuitable for these snails (Kendall, 1954). In irrigated rice fields, the population of snails is influenced by the availability of water for irrigation and the stage the previous crops

may colonies recently planted rice fields or they may enter with water introduced to flood the field after planting. Their numbers then increase over the next few months before declining again a few weeks before harvest (Widjajanti, 1989). Despite being aquatic, the snail hosts of *Schistosoma* spp. are able to survive desiccation in the shade on the surface of the soil for some weeks. Passive migration in streams formed by heavy rain is the most important way that snails are disseminated into new habitats such as paddy fields. In the dry season most snails die from lack of water, with the surviving snail is being mainly in streams, rivers, ponds and beel (large ditches).

All schistosomes use snails as their intermediate hosts. Within this host, they multiply asexually and produce relatively large number of infective stages known as cercariae. Typically, these free-swimming cercariae leave the snail host to penetrate their next vertebrate definitive host in which the worms mature. The production of cercariae in snails is a fundamental component of the parasites overall transmission success. This process is also directly influenced by temperature and rainfall. Within the range of temperatures in which snail host and parasite can live an increase in cercarial out put was recorded (Shostak and Esch, 1990, Lo and Lee, 1996, Umadevi and Madhavi, 1997, Mouritsen, 2002). The net outcome of increasing temperature and moisture will thus be a greater number of cercarial infective stages in aquatic habitats.

5.1.1.1 Ecology of snail and cercariae

In their distribution and different kinds of movement in water the cercariae of *S. indicum, S. spindale* and *S. nasale* are closely resemble as reported by Soparkar (1921), Srivastava and Dutt (1962) and Dutt and Srivastava (1968).

The cercariae of schistosomes remain in mid water and at water surface by their intermittent swimming behavior with a weak geo-negative, but not photopositive orientation (Haas *et al.,* 1990). When cercariae emerge from the snails, they are directly released into the fresh water environment. It is suggested that the main stimulus for their release is light intensity are non-feeding organism and they have a relatively short life span of between 36 and 48 hours although their infectivity diminishes rapidly. The daily cercarial productivity, which rarely exceeds2000 cercariae per snail (Webbe, 1965, Pitchford *et al.,* 1969, De Bont *et al.,*1991 b),

is a function of the same biotic and abiotic factors as those affecting the pre patent period. Schistosome parasites of man and cattle have a diurnal emission rhythm. In Bangladesh at least 9 species of freshwater mollusk are found including *Lymnaea luteola, L. auricularia, Indoplanorbis exustus, Planorbis corromandelicus* and T*hiara tuberculata* transmitting schistosomiasis in domestic ruminants (Shaikh and Rahman, 1968, Rahman, 1977, Rahman and Gillman, 1978). Because potential intermediate host for disease is widely distributed through out the country and dams and man made lakes are under construction in order to generate hydroelectric power, hence there may be new opportunities for the spread of this water borne disease in this region (Bangladesh). Scientist fears that global warming is expanding snail habitats and spread schistosomiasis into different regions previously protected by temperature too cold for the snails. On the other hand an absence of appropriate snails protects regions from schistosomiasis. Because the schistosomes must survive both in animal victims and in snails, the disease can only spread in areas when right mollusk live. It becomes a much greater problem after 1821 when the advent of perennial irrigation boosted agricultural production. Lager scale water projects during the 19th and specially the 20th century in many countries along with increasing use of irrigation multiplied both snail habitats and the number of schistosomiasis may also rise due to climate related changes in transmission dynamics and effects on the abundance of snail intermediate host.

Factors determining the availability, development and survival of intermediate hosts snail in the environment also influences the level and severity of infection. The optimum temperature range for the survival and development of *Lymnaea, Indoplanorbis* and *Planorbis* is 15-26^{0}c while temperature below 10^{0}c inhibits the development and reproductive activity of snails. *Lymnaea* requires permanent water bodies with abundant vegetation such as lakes, slow moving rivers, irrigation ditches and water tanks. Clean, clear and unpolluted water is the most favorable habitat for *Lymnaea* and *Indoplanorbis.Lymnaea* spp. may be either aquatic or amphibious.Some 350 snail species are estimated to be responsible for medical or veterinary importance(Sturrock *et al.*,2003). Moisture is the critical factor determining the presence and extent of snail habitats which serve as transmission foci for schistosomes. The interaction between moisture and temperature determines the survival and reproduction rates of the snails and the parasites. In lowland tropical areas *Lymnaea* breeds throughout the year in favorable habitats (Chartier *et al.,* 1990).

5.1.1.2 Snail habitants

Like all other vector borne disease the distribution of intestinal schistosomes depends on the spatial distribution of suitable intermediate host snails. Bangladesh being on the equator with a heavy long durated rainy season every year, the country has many fresh water bodies and climatic conditions that favor the transmission of different schistosomes in most areas. Snail habitats include almost all types of fresh water bodies ranging from small temporary pools, ponds and streams to large lakes and rivers. Within each habitat, snail distribution may be patchy and detection requires examination of different sites. Moreover snail densities vary significantly with seasons. In general, the aquatic snail hosts of schistosomes occur in shallow water near the shores of lakes, ponds, marshes, streams and irrigation channels. They live on water plants and mud that is rich in decaying organic matter. They can also be found on rock stones or concrete covered with algae or on various types of debris. They are most common in water where water plants are abundant and in water moderately polluted with organic matter, such as feces and urine, as is often the case near animal shade.Most aquatic snail species die when stranded on dry land in the dry season. However, a proportion of some snail species are able to withstand desiccation for months while buried in the mud bottom by sealing their shell opening with a layer of mucus. Most species can survive outside water for short periods. However, the epidemiology of schistosome is not only dependent on availability of suitable water bodies but also on the suitability of both climatic and environmental conditions for the schistosomes and the different intermediate host. Measurement of infection rate in field population of snails is a mainstay of studies on the epidemiology of schistosomiasis. The data obtained can facilitate planning of schistosome control and assessment of its outcome.

Cross-sectional and longitudinal determination of the distribution, density and infection rates of snail intermediate hosts showed is an integral component of all epidemiological studies. Previous worker Islam, (1977) investigated and established at least 3 Bangladeshi *Schistosoma* species with their intermediaries. While acting as intermediate host, these snails are spreading schistosomiasis through cercariae which

has an instant infectivity to domestic animals. Obviously, more attention is needed on the snails to understand epidemiology of the infection. More information on the ecology of snail intermediate host populations and schistosome transmission is needed in order to establish general guidelines for control that can be applied to a variety of habitats in broad climatic and geographical areas.

Hence, this study was undertaken with a view to fulfill the following objective:

(i) To determine the distribution and association for the development of *Furcocercus* cercariae in different snails and their release with ecological and temporal variables.

5.1.2 MATERIALS and METHODS

In conducting a research study or a scientific investigation methodological issues is one of the prime considerations for yielding of valid and reliable findings. In reality it is the base on which the research process rests upon. Appropriate methodology enables a researcher to collect and analyze the information purposively.However, the methods and procedures followed in conducting the study have been mentioned below.

5.1.2.1 Study areas

The study was conducted for 2 year from January 2010 to December 2011 in an around Mymensingh district.The snails were collected fortnightly following a non probability sampling technique (convenience sampling).The inhabitants of the snails are shown in Plate 6,7,8 and 9.Daily record of ambient temperature, humidity, rainfall and evaporation rate was collected from the Meterology section, BAU,Mymensingh.

5.1.2.2 Snail population

Ponds, large ditches (beel), local river viz Brammaputra and deep paddy field were visited; snails and water were collected to know aquatic fauna, turbidity and p^H of the water. Most of these snails were collected from the edges of water reservoirs where they were either moving freely or attached to the aquatic plants, grasses etc. The water reservoirs contained stagnant and slightly muddy water. The p^H of water from river and other sources ranged from 6.8-7.3 and 7.5-7.8 respectively. Snails were collected irrespective of their species and were isolated and counted.

To asses' season's effect, snails were picked up manually for a constant time (15 min) from a given area weekly for 2 year. Most of the collections were made in the morning (between 8.0 and 10.0 am) using scoop-net.

5.1.2.3 Screening of the snails

The collected snails were released in screw-top jars with wire fitted lids and were carried to the laboratory for detailed studies. A presumptive identification of the snails was made on the basis of shell characters as per methods of Malek and Cheng (1974).

Each snail was put in individual petri dish and exposed to artificial light (temperature 38-40^{0}c) for 15-30 minutes to release the cercariae (if present).Thereafter, snails with identical shell characters were de shelled for confirmatory identification on the basis of internal morphology.Parthinate eg.sporocyst to cercariae development were studied in 25% of the snails releasing *Furcocercus* cercariae. For confirming type of emerging schistosome cercariae the brevifurcated distome cercariae (Plate10a and Plate10b) were stained with iodine solution and examined under a compound microscope (100 x).The brevifurcated cercariae without eyespots were catagorized in the mammalian schistosome cercariae group (Yamagutti, 1958, Chauhan *et al.,* 1973). At times a method for identifying the cercariae of schistosome by studies their furcal tip after staining with mercuric bromophenol blue was used.

The routine staining with mercuric bromo phenol blue for demonstration of protein in the tissue sections (Pearse, 1968) was applied on the cercariae with some modifications. A drop of water containing 15-20 live cercariae was placed in the centre of the slide and allowed to dry at room temperature. These slides were used both without fixation and after fixing in 10% cold (4^{0C}) formalin for 24 hr. The slides were stained with mercuric bromo- phenol blue after standardizing the time to suit the cercarial staining. The procedure was as follows:

1. The slide was washed for 1 minute in distilled water to remove formalin (omitted for unfixed slides).
2. The slide was immersed in mercuric bromo-phenol blue staining solution for 20 minute. The staining solution was prepared by mixing 2ml glacial acetic acid in 100ml distilled water in which was dissolved 1g mercuric chloride and 0.05g bromo phenol blue.
3. Differentiated for 5 minute in 0.5% aqueous acetic acid.
4. Rinsed with distilled water and allowed to air dry.
5. Mounted in DPX and observed under oil immersion (1000 x).

Dehydration and clearing of the cercariae were omitted since these removed almost all the stain from the cercariae.The staining pattern of all the species of cercariae was similar and species variation was difficult to locate. However, differences were noted in the size and shape of the furcal rami as well in the furcal tips.The furcal rami of *S. indicum* and *S. spindale* were almost identical.Staining revealed consistent differences in the shape and size of the lightly stained tips of the furcal rami of the cercariae under oil immersion.

These morphological characters were consistent for each species of cercariae. Cercarial measurements in some cases also helped in identifying schistosome species. It is also of practical importance to differentiate the cercariae among three species of schistosomes (*S.indicum, S. spindale* and *S. nasale*). Since all the three parasites utilize the same species of snails' viz., *L.luteola, L auricularia* and *I. exustus* as their intermediate hosts are endemic in this country.This can be done by studying the flame cell formula, examining the tip of furcal rami and taking recourse of micrometry. The cercariae of *S. nasale* have a pair of flame cells less than those of *S. indicum* and *S. spindale.*

The morphology of the cercariae was studied in fresh unstained conditions. The identification of cercariae was based on tail type, position and number of suckers of salivary or cystogenous glands. Cercarial classification of Luhe (1909) as mentioned by benDawes (1968) was followed for the differentiation of cercariae into schistosome *Furcocercus* cercariae (Table21).The counts of cercariae were made as per method of Taylor(1934).

Table21. Classification of Cercariae according to Luhe (1909)

(A) Body of cercaria with a dorsal, longitudinal undulating fin-fold along the body	*Lophocercaria*
(B) Body without longitudinal membrane	
(I) Tail with two symmetrical, slender caudal appendages which arise separately and are several times longer than the body. Oral aperture at middle of ventral surface. Intestine simple, sac-like	*Gasterostome cercaria*
(II) Caudal appendage variable, absent or distall forked, but never split to the base. Oral aperture anterior. Intestine bifurcate	*Monostome cercaria*
(a) Ventral sucker absent	*Amphistome cercaria*
(b) Ventral sucker present	*Distome cercaria*
(1) Ventral sucker posterior, immediately anterior to base of slender tail	
(2) Ventral sucker anterior to hind end of body and well separated from base of tail if latter is present	
Distome Cercariae are further classified as follows:	
(A) Cercariae single	*Cystocercous cercaria*
(I) Tail well developed	
(a) Body retractile into a chamber formed by base of tail	*Phopalocercous cercaria*
(b) Body not retractile in to tail	*Leptocercous cercaria*
(1) Tail not bifurcate	*Trichocercous cercaria*
(a) Tail with bristles	
(i) Tail contractile to equal or exceed width of body	*Furcocercous cercaria*
(ii) Tail always distinctly narrower than body	
(2) Tail distally bifurcate	
(b) Tail with bristles (marine)	
(II) Tail stumpy of absent	*Microcercous cercaria*
(a) Tail stumpy	*Cercariaeum,*
(b) Tail absent	*Rat-king ercariaemarine*
(B) Cercariae joined into a colony by their tapering tail ends	

5.2 RESULTS

The prevalence of the snails according to habitat is shown in Table22. Of the 5272 snails examined from different locations and in different seasons *L. luteola* (26.2%), *L. auricularia* var *rufescens* (26.3%) and *I. exustus* (23.8%) were identified. However, other snails such as common apple snail *Pila globosa* and *Vivipara* sp. were collected during this period.But these were beyond the scope of the study as because they are not involving in transmission of schistosomes so no further discussion is made about these snails.Out of 5272 snails collected from Mymensingh region, 198 (3.8%) were found positive for cercariae of schistosomes of cattle with seasonal variation. The abundance of three vector snails were significantly higher (P<0.01) in river and deep paddy field than beel and pond (Table22).From the Table23 it is depicted that abundance of all three vector snails were significantly higher (P<0.01) in rainy season than summer and winter.

Prevalence of *Furcocercous* cercariae as tabulated in Table24 and Table25 showed that *I. exustus* played the dominant role followed by *L.luteola* and *L .auricularia.*The shedding of *Schistosoma* spp. cercariae was significantly higher (P<0.01) during rainy season by three vector snails except *L. luteola* also released significantly higher (P<0.01) number of cercariae in winter (Table24).Highest prevalence of cercariae was found in snails collected from deep paddy field than other three habitants of snails but the variation was found statistically insignificant (Table25).

The distribution and association between month and release rate of *Furcocercous* cercariae by snails have been shown in Table26.A highly significant (P<.01) association between months and release rate of *Furcocercous* cercariae by *L. auricularia,L.luteola* and *I. exustus* was noted.The release of *Furcocercus* cercariae was significantly (P<0.01) higher in October followed by September, August, July and November by the snail of *L. auricularia.*On the otherhand *L.luteola* released significantly higher (P<0.01) number of cercariae in the month of September followed by October,August,November and December.*I.exustus* sheded significantly higher (P<0.01) number of cercariae in the month of September followed byOctober, August,July and June (Table26). The number of cercariae collected from a single snail varied from 31to5061.A positive insignificant co-rrelation between the release of *Furcocercus* cercariae by the vector snails with air temperature, rainfall, evaporation and humidity was observed (Table27).The tip of the furcal ramus of *S. spindale* shows a bulbous expansion (Plate 11) whereas it is elongated in *S. indicum* (Plate 12).

Table22. Prevalence of snails in different locations (n=5272)

Location	LA	Prevalence (95% CI)	LL	Prevalence (95% CI)	IE	Prevalence (95% CI)
Beel	378	20.2(18.4, 22.1)[a]	348	18.9(17.2, 20.8)[a]	348	22.3(20.2, 24.4)[b]
Pond	398	21.2(19.4, 23.2)[a]	349	18.9(17.2, 20.9)[a]	348	22.3(20.2, 24.4)[b]
DPF	513	27.4(25.4, 29.5)[b]	570	31.0(28.9, 33.2)[b]	414	26.5(24.3, 28.8)[a]
River	584	31.2(29.1, 33.3)[b]	570	31.0(28.9, 33.2)[b]	452	28.9(26.7, 31.3)[a]
Overall	1873		1837		1562	

Values with different letters within a column differ significantly ($p<0.01$) LA: *Lymnaea auricularia* LL: *Lymnaea luteola* IE: *Indoplanorbis exustus* CI: Confidence Interval DPF: Deep Paddy Field

Table23. Prevalence of snails in different seasons (n=5272)

Season	LA	Prevalence (95% CI)	LL	Prevalence (95% CI)	IE	Prevalence (95% CI)
Winter	494	26.4(24.4, 28.4)[a]	514	27.9(25.9, 30.1)[a]	384	24.6(22.5, 26.8)[a]
Summer	481	25.7(23.7, 27.7)[a]	425	23.1(21.2, 25.1)[b]	450	28.8 (26.6, 31.1)[a]
Rainy	898	47.9(45.7, 50.2)[b]	898	48.9 (46.6, 51.2)[c]	728	46.6(44.1, 49.1)[c]
Overall	1873		1837		1562	

Values with different letters within a column differ significantly ($p<0.01$) LA: *Lymnaea auricularia* LL: *Lymnaea luteola* IE: *Indoplanorbis exustus* CI: Confidence Interval

Table24. Cercaria recovered from different vector snails in different seasons

Category	Snail tested	Cercaria (%)	95% CI	Snail tested	Cercaria (%)	95% CI	Snail tested	Cercaria (%)	95% CI
Season	*L. auricularia*			*L. luteola*			*I. exustus*		
Winter	494	7 (1.4)[a]	0.6, 2.9	514	18 (3.5)[a]	2.1, 5.5	384	2 (0.5)[a]	0.06, 1.9
Summer	481	4 (0.8)[a]	0.2, 2.1	425	2 (0.5)[b]	0.06, 1.7	450	5 (1.1)[a]	0.4, 2.6
Rainy	898	52 (5.8)[b]	4.4, 7.5	898	49 (5.5)[a]	4.1, 7.2	728	59 (8.1)[b]	6.2, 10.3
Overall	1,873	63 (3.4)	2.6, 4.3	1,837	69(3.8)	2.9, 4.7	1,562	66 (4.2)	3.3, 5.3

Values with different letters within a column differ significantly ($p<0.01$)

Table25. Cercaria recovered from different vector snails in different locations

Category	Snail tested	Cercaria (%)	95% CI	Snail tested	Cercaria (%)	95% CI	Snail tested	Cercaria (%)	95% CI
	L.auricularia			*L.luteola*			*I.exustus*		
Habitant									
Beel	378	14 (3.7)	2.0, 6.1	348	15 (4.3)	2.4, 7.0	348	16 (4.6)	2.7, 7.4
Pond	398	11 (2.8)	1.4, 4.9	349	12 (3.4)	1.8, 5.9	348	12 (3.4)	1.8, 5.9
DPF	513	20 (3.9)	2.4, 5.9	570	26 (4.6)	3.0, 6.6	414	24 (5.8)	3.7, 8.5
River	584	18 (3.1)	1.8, 4.8	570	16 (2.8)	1.6, 4.5	452	14 (3.1)	1.7, 5.1
Overall	1,873	63 (3.4)	2.6, 4.3	1,837	69(3.8)	2.9, 4.7	1,562	66 (4.2)	3.3, 5.3
Overall prevalence of cercaria in snails:198/5272=3.8% (95% CI: 3.3-4.3)									

DPF=Deep Paddy Field

Table26. Monthly distribution of cercaria released by vector snails

Month	*L. auricularia*	Pr.Cer. LA (%)	95% CI	*L. luteola*	Pr.Cer.LL (%)	95% CI	*I. exustus*	Pr.Cer.IE (%)	95%CI
Jan	104	0 (0)	0, 34.5	86	0 (0)	0.0, 4.2	106	0 (0)	0, 3.4
Feb	106	0 (0)	0, 34.2	86	0 (0)	0.0, 4.2	106	0 (0)	0, 3.4
Mar	108	0 (0)	0, 34.6	86	0 (0)	0.0, 4.2	106	0 (0)	0, 3.4
Apr	200	2 (1.0)	0.1, 3.6	177	0 (0)	0.0, 2.1	172	1 (0.6)	0.0, 3.2
May	173	2(1.2)	0.1, 4.1	162	2 (1.2)	0.1, 4.4	172	4 (2.3)	0.6, 5.8
Jun	173	6 (3.4)	1.3, 7.3	196	4 (2.0)	0.6, 5.1	156	8 (5.1)	2.2, 9.8
Jul	167	10 (5.9)[a]	2.9, 10.7	186	6 (3.2)	1.2, 6.9	148	10 (6.8)	3.3, 12.1
Aug	173	10 (5.8)[a]	2.8, 10.4	172	11 (6.4)	3.2, 11.1	148	11 (7.4)[a]	2.2, 3.8
Sep	163	13 (7.9)[b]	4.3, 13.3	172	16 (9.3)[a]	5.4, 14.7	164	21 (12.8)[b]	8.1, 18.9
Oct	222	13(10.7)[b]	5.8, 17.5	172	12 (6.9)[a]	3.7, 11.9	112	9 (8.0)[a]	3.7, 14.7
Nov	165	6 (3.6)	1.3, 7.7	171	9 (5.3)[b]	2.4, 9.8	87	2(2.3)	2.8, 8.1
Dec	119	1 (0.8)	0.02, 4.6	171	9 (5.3)[b]	2.4, 9.8	85	0 (0)	0, 4.2
Total	5272	63 (1.2)	0.9, 1.5	5272	69 (1.3)	1.0, 1.7	5272	66 (1.2)	0.9, 1.6

Values with different letters within a column differ significantly ($p<0.01$), Pr.=Prevalence, Cr.=Cercariae

Table27. Relationship among ecologic variables (mean) with cercarial release rate by snails

Month	Temperature (°C)	Rainfall(mm)	Evaporation(mm)	Humidity (%)	*Furcocercous* cercaria release rate (%)	95% CI
Jan	18.5	2.0	65	84.6	0	0, 1.2
Feb	21.7	0	78.4	74.4	0	0, 1.2
Mar	25.6	17.2	126.7	74.9	0	0, 1.2
Apr	28.1	86.3	136.4	81.9	0.5^{a}	0.1, 1.6
May	28.3	373.4	135.7	82.6	1.6	0.7, 3.1
Jun	28.8	247.5	115.1	86.9	3.6^{b}	2.1, 5.6
Jul	29.4	443.0	112.7	75.6	5.2^{b}	3.4, 7.5
Aug	29.3	358.0	115.3	85.8	6.5^{b}	4.5, 9.0
Sep	28.9	218.3	102	86.9	8.2^{b}	5.9, 10.9
Oct	27.8	25.9	109.6	84.9	6.7^{b}	4.7, 9.3
Nov	23.8	2.0	82	83.1	4.0^{b}	2.4, 6.4
Dec	19.5	0.65	66.9	83.2	2.7	1.3, 4.8
COR	r=0.56	r=0.43	r=0.08	r=0.51	-	-

COR=Correlation. Values with different letters within a column differ significantly ($p<0.05$)

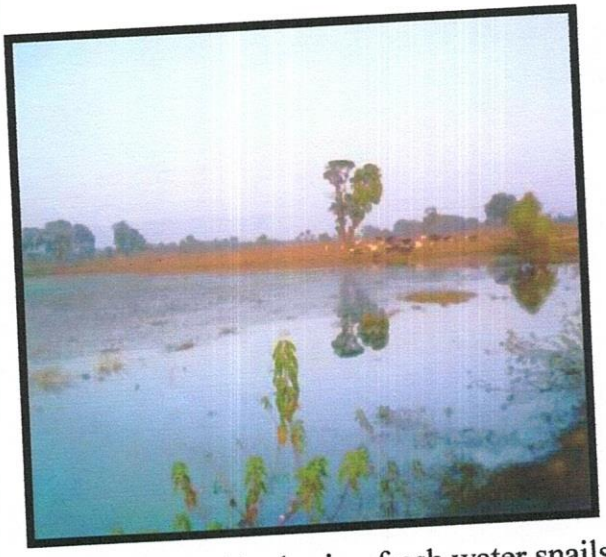

Plate6. A pond harboring fresh water snails including *Lymnaea* spp. and *Indoplanorbis exustus*

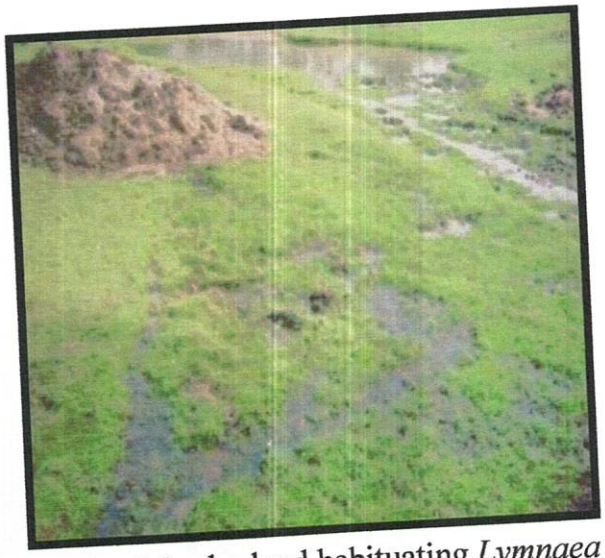

Plate7. Marshy land habituating *Lymnaea* spp. and *Indoplanorbis exustus*

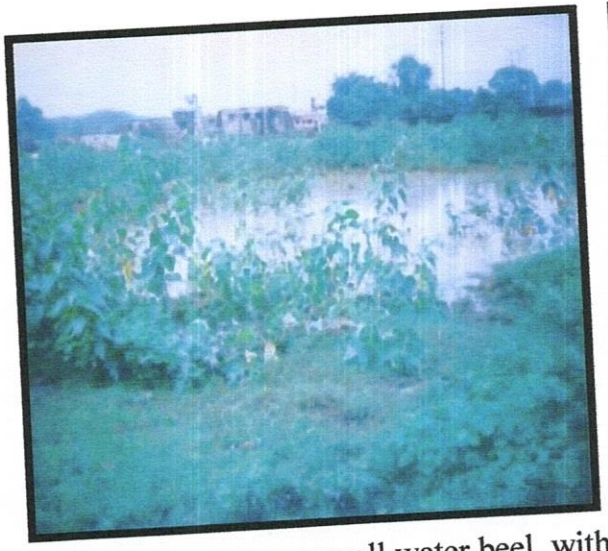

Plate8. A temporary small water beel with *Lymnaea* spp. and *Indoplanorbis exustus*

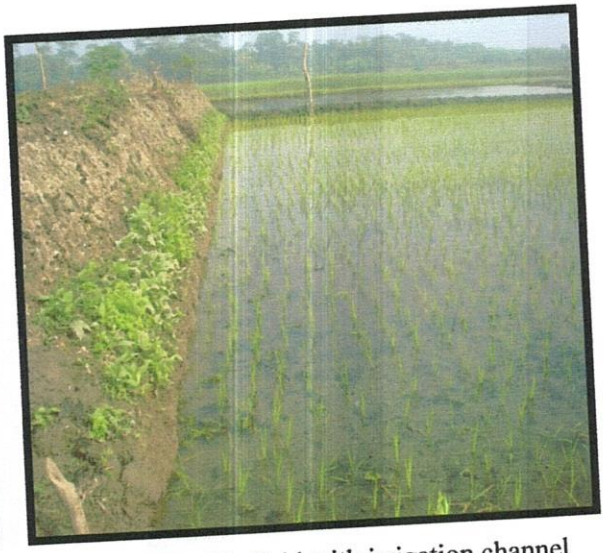

Plate9. Paddy field with irrigation channel habituating *Lymnaea* spp. and *Indoplanorbis exustus*

Plate10a. *Furcocercus* cercariae collected from *Lymnaea* sp. and *Indoplanorbis exustus*(x100)

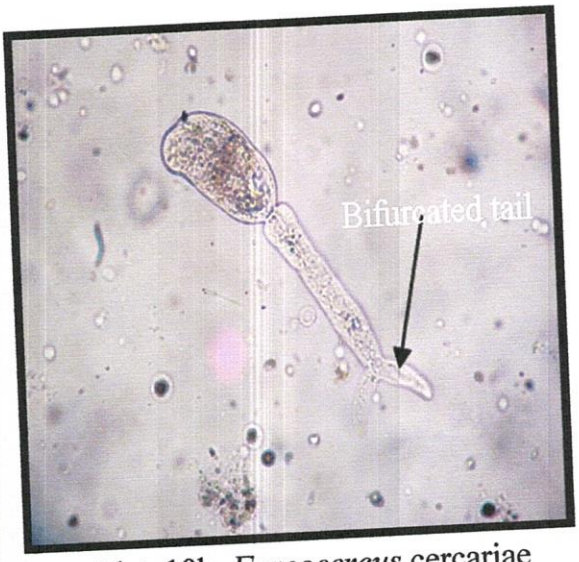

Plate10b. *Furcocercus* cercariae collected from *Lymnaea* sp. and *Indoplanorbis exustus*(x100)

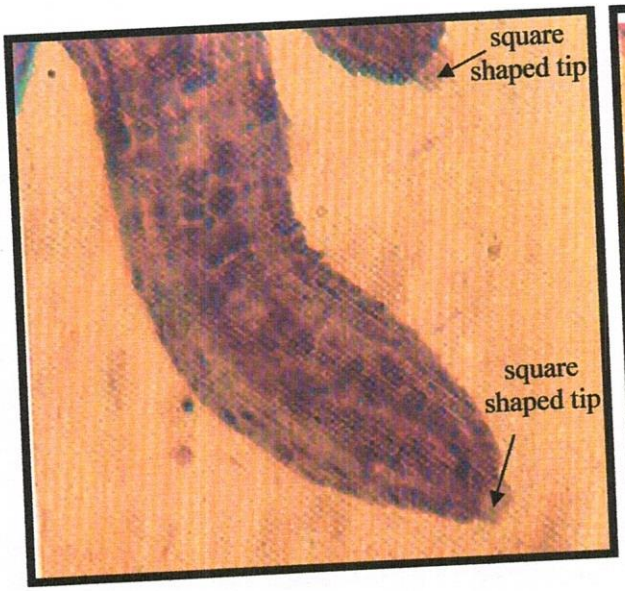

Plate11. Furcal ramus of *Schistosoma spindale* cercaria (square shaped tip, 100x)

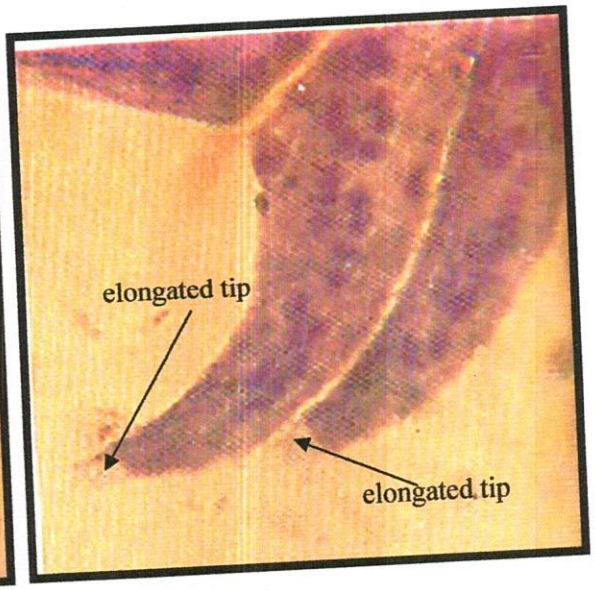

Plate12. Furcal ramus of *Schistosoma indicum* cercaria (elongated tip, 100x)

Plate13. *Lymnaea auricularia*

Plate14. *Lymnaea luteola*

Plate15. *Indoplanorbis exustus*

5.3 DISCUSSION

5.3.1 Prevalence of snail and cercariae

The distribution of schistosome infections within a population of intermediate host is likely to be influenced by a number of factors, including intermediate host snail and parasite genetics, snail age and environmental heterogeneity. Because these factors also determine prevalence, schistosome distribution among snail host is likely to vary with parasite prevalence at a transmission site. Thus it is important to determine the relationship between prevalence and parasite distribution with hosts. Without snails, there can be no schistosomes, because schistosome transmitting snails occur in vary particular ecological circumstances that are subject to rapid change in an increasingly human-dominated world one of the greatest challenges to understanding the future of this neglected yet persistent diseases.It is essential to try to understand how snails will be affected by global changes in climate, continued transport of exotic and invasive snail species, construction of dams and irrigation systems and mass movement of animals.

The present study has shown that the *Indoplanorbis* sp. and *Lymnaea* spp. snails are certainly transmitting the schistosome infection. It is interesting to mention that a good number of *Indoplanorbis* sp.and *Lymnaea* spp. were found to shed *Gymnoceplalus*, *Echinostome* and Schistosome cercariae at same time which indicates that snails may allow different species of parasite to development their life-cycle.This phenomenon helps to suggest that a large-scale study on various species of snails may be conducted to obtain the data on host-parasite relationship diversity.

This study showed that season has greatly influenced population of snail, *I. exustus, L. luteola* and *L. auricularia* which was lowest in winter and summer than rainy season (Table 24).The incidence of snails during the rainy season as recorded by Rolfe *et al.*, (1991), Abrous *et al.*, (1999), Kunitskii (2000) and Haridy *et al.*, (2006) was higher in monsoon than in summer due to some environmental factors and their findings shows close resemblance with the present results. The recently developed irrigation system causes water lodgement in the irrigated rice field and canals that breaks the temperature barrier (Kools *et al.*, 1978) and favors the reproduction of the vector snails especially in the winter months.

Within the tropics rainfall is probably the most important climatic factor affecting snail population. Again in exceptionally heavy rainy seasons, flood dramatically alter snail sites, especially lentic (flowing) sites which may take months or years to return to their original sites. Conversely lotic (still water) sites shrink during droughts between rainy seasons and after dryout completely. If habitats stay unfilled in a succession of poor rainy seasons the snail will eventually succumb.

In the present study, relative occurence of the snail intermediate host varied among different sites. Such differences have been attributed by Woolhouse and Chandiwana (1990b). They commented the recent history of river condition, i.e.floods and drought and frequent contamination of the water by fecal materials are responsible for this phenomenon. In inhabitant wise data analysis showed that intermediate hosts of *Schistosoma* are tropical aquatic snails which thrive in clear, stagnant or slow moving water with high O_2 content and abundant aquatic vegetation. Such ecological situations are typically found at the fringes of rivers where the water levels are stable and irrigated rice fields throughout the humid tropics. So, prevalence of snails are found higher in local river and deep irrigated rice field in the present observation which are in close agreement with the results of Sturrock *et al.*,(2001).

In lowland tropical areas like Bangladesh *Lymnaea* spp. and *Indoplanorbis* sp. breeds in favorable habitants when temperature and moisture is optimum (Chartier *et al.*, 1990).The population of these snails in rivers may be negatively related to rainfall, reflecting the disruption of the habitat of the snails by flooding and also their disruption by flood water, as they spend upto 70% of their time floating at the surface (Widjajanti, 1989). In irrigated rice fields the population of snails is influenced by the availability of water for irrigation and the stage of growth of the crop. Thus the irrigated rice field and rivers provides more suitable habitats for the snail. As with schistosomes that infect animal and man, the degree of contamination of the habitat with cercariae is highly dependent upon ecological factors affecting the biology and survival of the intermediate host population, the rate of intermolluscan development and cercarial shedding patterns (Rollinson and Simpson, 1987, Jordan *et al.*, 1993). Higher prevalence of cercarial infection in *I. exustus* than *L. luteola* and *L.*

auricularia observed in the present study is in conformity with the earlier findings of Shaikh and Rahman (1968).But Rahman *et al.*, (1997) recorded 12.6% cercarial infection in *L. auricularia*, 7.3% in *L. luteola* and 5.7% in *I. exustus*.The reasons behind the variations of cercarial infections among different snail species are difficult to explain but it may be due to some unexplored physiological interactions between the respective parasitic development stages and vector snails.

Higher number of snail was found to shed cercariae of *Schistosoma bovis* (9%) reported by Lengy (1961) in Israel and is higher than the present investigation (3.8%). In Assam, mammalian *Furcocercous* cercariae were recorded in 2% snail (*I. exustus*) in October to November which 1.72% *I. exustus* were positive in the month of June (Rajkhowa, 1990). Soparkar (1921) studied the prevalence of *S. spindale* cercariae in *I. exustus* which was seasonal with a minimum in February to April (0.0-0.09%) and maximum from June to October (2.0-8.6%). Muraleedharan *et al.*, (1977) obtained *S. spindale* cercariae from *I. exustus* (0.58%) with the highest prevalence from September to October. Singh (1959) and Raut (1983) recorded highest prevalence of cercariae in May and, December and July and August, respectively. In the present study the cercarial infection in snails turned negative in dry winter and positiveness attained in May (Table27) after completion of the snail's hibernation in April. This work has confirmed seasonality of cercarial output which is in accordance to the work of Soparkar (1921) and Raut (1983). The seasonality is of great epizootiologic significance but also of importance in any survey work in an unknown area, as season of survey may greatly influence result with chances of false negativeness if work is carried out in adverse condition.

Schistosome eggs hatch out only if they come in contact with fresh water and the mature cercariae comes out from the day 38 of infection upto 126 in monsoon and 154 days in winter (Pitchford and Visser, 1969).So, the overwinterd snails infected with schistosome miracidia in the summer release mature cercariae in the monsoon. The lower incidence of schistosome cercariae in winter reflects less contamination of pasture in monsoon by schistosome infected cattle feces as the animals are mostly confined in the stable yards in the rains. Apart from this, low environmental

temperature hampers both the hatching of schistosome eggs and their larval development in the snails. Moreover, in winter the snails undergo aestivation, which also keeps the snails away from schistosome miracidial infection. This finding is in agreement with the observation of Lim and Lie (1969). Owing to the ability of larval schistosomes to reproduce asexually in their intermediate host, the number of infected snails at the transmission site does not need to be high to secure significant levels of transmission.

In overall month wise data analysis it showed that, infection of schistosome cercariae in snail was highest in September,October,August and July while tended to lower from November to December and turned zero from January to March.These findings are in support of the result of Rahman (1977).Arijo *et al.,* (2005) screened naturally infected snails from Pakistan and reported that snails did not shed *Schistosoma* cercariae in the months of January to April.During the present study low prevalence of *Schistosoma* cercariae in the month of January to April can also get favor from results of various researches as they stated that there was no development and hatching of trematode eggs, no development of flukes in snails nor even emergence of cercariae from snails at temperature below 10°C (Dekock and Wolmaranas,2005,Joao *et al.,*2006,Dreyfuss *et al.*,2007, Morley *etal.*,2007 Rondeland *etal.,*2007) which prevail during winter not only Bangladesh but also other parts of the world.

Considering the role of seasons and environmental variables in cercarial prevalence it was observed that it varied in relation to temperature and rainfall. Gradual increase in mean temperature was associated with evaporation in May, June with an ultimate rapid increase of mean rainfall in July to Septemberand October which coincides with highest prevalence of cercariae.In Bangladesh rainy season begins in the month of June and changes the environmental temperature and humidity that favors the cercariae to emerge from snail. That is the reason during and after rainfall highest infection of schistosome parasite with its vector and cercariae was reported in present study.The influence of temperature and rainfall on prevalence of cercariae has been assesed in various previous studies, which have more or less similarity with the present works (Appleton,1978,Brown,1994,Cross *et al.,*1994,Melone *et al.,*2001, Kristensen *et al.,*Yang *et al.,*2005).Temperature during May and September ranged

between 28.3°C and 28.9°Cwhen snails were in abundance;similarly rainfall ranged between 373.4mm and218.3mm during the same period.Less availability leading to reduced activity of snails started from November and those who survived rigorous of winter releasing *Furcocercous* cercariae in April when the temperature is 28.1°C and the rainfall is 86.3 mm.(Table27).Consequently both humidity and evaporation rate also affected snail breeding ;both were at higher sides during maximum snail breeding.As stated earlier, most *Furcocercous* cercariae started to emerge between July and October and it was directly influenced by the temperature and it started to rise gradually 29.4°C in July and in August 29.3°C from 28.8°C in June. But the temperature went down to 19.5°C in December and to18.5°C in January.This indicated that snails had gone to probable aestivation during this period.When the temperature started to rise again in April (28.1°C) and May (28.3°C) *Furcocercous* cercariae were again released by the snails.From the above and considering the observed fact that normal parthinate of trematodes is of 4-6 weeks period it is assumed that infection of snails by miracidium probably occurs between April and May when animals grazed at edges of water reservoirs and development of asexual stages e.g. sporocyst to cercariae continues for 3-4 months with emergence of cercariae coinciding with rise of rainfall and temperature.However, few snails releasing cercariae in April and May may be the infected snails which withstood rigorous winter and released leftover cercariae. Therefore, it could be observed that snails once infected harbor the parthinate for one generation only.

The effects of rainfall and temparature are the most important factors, resulting in clear seasonal patterns of transmission in most habitats (Majid *et al.,* 1980a, Chandiwana *et al.,* 1987b ,DeBont *et al.,*1995a).From the present study it appeared that both temperature,rainfall and seasons had marked influence on the prevalence of snails and release of *Furcocercus* cercariae.

Like the present investigator some experts also emphasized on the impact of climate change on the distribution of snails that act as intermediate host for schsitosomiasis (Morgan *et al.,* 2001).Previous research has shown that the development of schistosome larvae in the intermediate snails is closely influenced by temperature (Nagasaki,1960).It has been shown that the survival rates of parasitic trematode and its intermediate snail varied between the different stages of the parasite larvae and

snails during aestivation.However, snails carrying early stage larvae had a better survival rate than snails with later stage larvae. The authors concluded that if the development of the parasite started before aestivation, the risk of dying is high (Schjetlein and Skorping, 1995). Ataev, (1991) showed that higher temperature not only trigger the emergence of cercariae from the snails, but they also accelerate the production of cercariae within snails.In some species, although cercarial production is inhibited below a certain temperature, the production of rediae or sporocyst continues and there generative stages accumulate inside the snail (Galaktionov and Dobrovolskij, 2003, Mouritsen and Poulin, 2002).Generally cercarial survival decreases steadily with increasing temperature (Evans, 1985,Mouritsen, 2002, Fried and Ponder, 2003), although this phenomenon is not universal (Lo and Lee, 1996).

CHAPTER 6

INVESTIGATION 4

MIRACIDIAL HATCHABILITY

6.1 INVESTIGATION 4

MIRACIDIAL HATCHABILITY

6.1.1 INTRODUCTION

Miracidial hatching techniques have sometimes been preferred to fecal egg counts for the diagnosis and quantification of schistosome infections in cattle (Kruger and Heitmann,1967,Saeed *et al.*,1969,Kassuku *et al.*,1986,Banerjee and Agrawal,1989, 1990, Banerjee *et al.*, 1990,De Bont *et al.*,1991a).By these methods much larger fecal material can be used,which improves the sensitivity and facilitates the detection of light or old infections.In addition, pooled samples from several animals may be examined to detect infection at the herd level. Miracidia counts may also be used in combination with fecal egg counts to measure the viability of the eggs found in the feces.

Hence, this study was done with a view to fulfill the following objective:

To know the miracidial hatching prevalence of different age groups of cattle.

6.1.2 MATERIALS and METHODS

For this study a total of 114 fecal samples were collected from three different age groups of cattle following the method described by Samad (2001).Miracidial hatching test was performed as the method described by Vohra and Agrawal(2006).To obtain miracidia from fecal material(100gm/ animal),eggs were extracted by washing the feces with a saline solution (to prevent premature hatching), sieved and concentrated atleast thrice.The sediment was diluted with distilled water to stimulate egg hatching in a conical flask.The flask was then covered, except at the brim with a black cloth and was further exposed to light for half an hour.Ten ml. of water from the upper surface taken in a Petri dish was examined under stereoscopic microscope for miracidia.If the first sample was found negative, an additional 10 ml of water was checked and collected miracidia was counted. The presumptive identification of miracidia were made on the basis of their morphological characteristics as descrbed by Soulsby (1982).The hatching intensity was considered to be mild when 1-20 miracidia were recorded and moderate when 21-100 miracidia were obtained and high when >100 miracidia were observed. It can be noted here, that the hatching intensity of miracidia was expressed in number.

6.3 RESULTS

Miracidial hatchability from fecal samples of different ages of cattle is shown in Table28. Overall hatatcability was found in 66.7% cattle. The data in this Table clearly shows that miracidial hatchability increases with decreasing age of the cattle. But the difference is statistically insignificant as indicated by overlapping of the 95% confidence intervals. The highest miracidial hatchability (81.3%) was found in the age group of upto 2 years.

Table28. Miracidal hatchability from fecal samples collected from different ages of cattle (n=114)

Age group	Hatching Intensity				Total	Hatching intensity (95% CI)
	Nil (%)	Mild 1-20	Moderate 21-100	High >1000		
Up to 2 years	9	0	31	8	48	81.3 (67.4, 91.1)
2 years to 6 years	11	6	9	2	28	60.7 (40.6, 78.5)
6 years and above	18	10	10	0	38	52.6 (35.8, 69.0)
Total	38(33.3)	16(14)	50(43.9)	10(8.8)	114(66.7%)	

6.4 DISCUSSION

Hatching technique is a simple test; it does not require the use of chemicals and it is more sensitive than fecal egg counts. By measuring the output of viable eggs a clearer indication of the actual transmission potential of each age group could be provided.In spite of the high sensitivity of the technique 33.3% of the examined samples were found negative and only 8.8% contained more than 100 miracidia which correspond to the findings of De Bont *et al.,*(1991a).Age and intensity of infection both had a significant effect on miracidial counts. It is showed that viable egg output decreased with age. Lower miracidial counts in older animals could be due to a higher egg dilution in larger fecal masses and could be the result of a reduction in egg production of the female worms, a lower fertility of the eggs produced or an increased destruction of eggs during their visceral migration.Evidence of a host immune response acting through suppression of female worm fecundity as soon as 10-14 weeks post infection was clearly demonastrated in *S.mattheei* (Lawrence,1973a,1977a,1978a) and *S.bovis* (Saad *et al.,*1980,Majid *et al.,*1980a,Bushara *et al.,*1980) infections in cattle. A similar phemenon seemed to be has occurred in the present infections.

In Srilanka, the fecal egg output of viable eggs by *S. spindale* was highest (96 miracidia / 100g) in animals of 2 years and younger, thereafter decreasing with age to 11 miracidia / 100g in cattle of 3 years and older (De Bont *et al.,* 1991a). In Zambia, 50% of the eggs of *S.mattheei* from calves hatched in water, compared with only 15% of eggs from adult cows (DeBont *et al.*, 1976). The findings of the present study support the notion that young animals play an important role in the contamination of the environment with eggs.The potential importance of older age groups in the spread of schistosomiasis is further reduced by an age related decrease in egg hatchability.This decline in egg hatchability is most probably immunologically induced.This type of decline has been noted in human *S. mansoni* infections (Upatham,1984) as well as after vaccination against *S.mattheei* in cattle and *S.mansoni* in laboratory animals (Xu *et al.,*1993).From the Table 28 it is shows that miracidial hatching test is superior as it is detected 66.7% positive cases than the fecal examnation and mesenteric blood vessels examination.This finding is closely related with the results of Agrawal(1999) who recorded30% positive cases as direct smear method of fecal examination but miracidial hatching test shows72.7% positive cases.

CHAPTER 7

INVESTIGATION 5

PATHOLOGY OF NATURALLY INFECTED SCHISTOSOMIASIS OF CATTLE BY *SCHISTOSOMA INDICUM*

7.1 INVESTIGATION 5

PATHOLOGY OF NATURALLY INFECTED SCHISTOSOMIASIS OF CATTLE BY *SCHISTOSOMA INDICUM*

7.1.1 INTRODUCTION

The development of pathological lesions in schistosomiasis is the result of complex interaction between the host and parasite. Although different development stages of the parasite induce lesions in the skin and other tissues during their migration through the body of the host, the most pathogenic stage is the egg (Taylor,1987).Eggs that do not succeded in penetrating the intestinal mucosa or eggs that are carried to other organs by the portal blood flow, remain trapped in the tissues, where secretions from the miracidia provoke strong local inflammatory responses (Lichtenberg,1964, Dunne *etal*.,1981).Schistosomiasis in bovine the most severe pathology is located to the intestines and liver where perioval granulomatous inflammatory lesions are formed around deposited eggs and egg antigens also induce increased fibrosis of the portal tracts.The marked periportal fibrosis seen in chronic hepatospleenic schistosomiasis was described by Symmers and Stuart(1904).These were based on studies in experimental animals. Recently, a major liver research unit which has had a long interest in hepatic fibrosis has adopted murine *S.mansoni* as a model. Eminent scientists of both the developed and developing world have neglected this widespread major disease the quality of work on this problem has suffered. Detailed descriptions on the pathology of schistosomiasis in cattle are available mainly for *S. mattheei* (McCully and Kruger, 1969, Hussein, 1971, Vanwyk *et al.,* 1974, Bartsch and Vanwyk, 1977, Lawrence, 1978 b,c) and *S. bovis* (Hussein 1971, Massoud, 1973, Hussein *et al.,* 1975, Saad *et al* .,1984a, Lindberg *et al.,* 1993, 1995, 1997, Johansen *et al.,*1996a, Ferreras-Estrada *et al.,*1998).For *S.spindale* (Rao,1934,Kalapesi and Puhorit1954,Fransen *et al.,* 1990*),S nasale* (De Bont *et al.,*1989a),and *S japonicum* (Wang *et al*.,1988) only a limited number of studies have been published. The pathology of *S. curassoni*, *S. leiperi* and *S. margrebowiei* has yet not been studied in cattle, however from observations in other ruminants it is thought to be similar to

infections with *S. bovis* or *S. mattheei*. Pathology of schistosomiasis in cattle is largely caused by schistosome eggs rather than worms.Adult worms are able to evade host immune attacks (Butterworth,1993) although dead worms swept into the liver and lungs do give rise to focal lesions (Lawrence,1978c). In most cases lesions caused by worms are few in number and have a limited clinical importance, compared with the lesions caused by the million of eggs generated during the course of infection. Lesions caused by eggs are seen mainly in the intestine wall and the liver (for *S. nasale* in nasal tissues) and more rarely in other downstream paranchymal organs such as the lungs, spleen, bladder, genital tract or ectopic sites (abomasum). The inflammatory response around eggs is an immune mediated event in which hypersensitivity to egg plays a vital role (Weinstock, 1992). In early patency of primary infections tissue reactivity to eggs is low compared with later stages (Lawrence, 1978b, Saad *et al.*, 1980). It is well known that granuloma formation progress concurrently with the development of delayed hypersensitivity (Hirata *et al.*,1993). The live worms in the blood vessels caused little reaction but the dead and degenerating worms in the blood vessels caused phlebitis and thrombosis.Schistosomiasis involving livers of man and animal are among the great neglected diseases on the basis of both of their prevalence and relative lack of interest shown in them by clinicians, veterinarians and biomedical investigators. Schistosomiasis is not only an interesting disease in itself but has the added advantage of providing unique human and animal models of hepatic pathophysiology, immunopathogenesis and collagen metabolism.Ths disease is great in terms of enormous numbers of animal and human suffering from it, and are neglected both financially and scientifically.

The present study was undertaken to know the histopathology of the infection caused by the *S.indicum* in cattle.

7.1.2 MATERIALS and METHODS

Importance of methodology for conducting any research or scientific investigation can hardly be overemphasized. Keeping this point in view, the researcher took great care to collect valid and reliable data, to analyze the same properly interprete those to arrive at a meaningful conclusion and the research design, method and procedure followed in conducting this study have been described in this Chapter.

In order to study the pathology of cattle schistosomiasis a study was conducted with the co-operation of the Department of Pathology, Bangladesh Agricultural University (BAU), Mymensingh.

7.1.2.1 Sample collection

Pathological observations were made on 50 indigenous cattle naturally infected with intestinal schistosome (*S. indicum*).The animals were selected from infected cattle of different age groups (judged by the number of permanent incisors).They were examined at the different slaughter house of Mymensingh district where a survey showed that *S. indicum* was identified by microscopic examination of eggs found in utero.

Representative samples of liver, small intestine and large intestine were fixed in 10% buffered formalin. Tissue embedded in paraffin wax was sectioned 5 μm thick and slides were stained with haematoxylin and eosin following the method described by (Luna, 1968).

The prepared slides were examined for the presence of granulomas, viable and dead eggs, giant cell reaction and infiltrations of eosinophils both in the mucosa and sub mucosa. Seemingly viable eggs were identified by their intact shell containing a miracidium. Clinical schistosomiasis was not observed in the infected animals.

7.1.2.2 Histopathology

Formalin-fixed tissues of small, large intestines and liver were processed, sectioned and stained with haematoxylin and eosin as per standard procedure of Luna (1968).

7.1.2.3 Processing of tissues and sectioned:

Collected tissues were properly trimmed to obtain a good cross -section of tissues. Washingof trimmed tissues under running tap water for overnight to remove the fixative.Dehydration of tissues in ascending grades of alcohol using 50%, 70%, 80%, 90% alcohol and three changes in absolute alcohol, for 1 hr.in each. Clearing of tissues in two changes of chloroform, 1.5 hr.in each.Embedding with molten paraffin wax at 56°C.Preparation of paraffin blocks containing tissue pieces using templates and molten paraffin.Sectioning of tissues with a microtome at 5μm thickness, which were allowed to spread on warm water bath (45°C) containing small amount of gelatin and taken on oil and grease-free glass slides. The slides were air dried and kept at a cool place until staining.

7.1.2.4 Haematoxylin and Eosin staining:

De-paraffinization of the tissue sections in three changes of xylene (3 minute in each). Rehydration of the sectioned tissues through descending grades of alcohol (3 changes in absolute alcohol, 3 minute in each; 95%alcohol for 2 minute; 70% alcohol for 2 minute;80% alcohol for 2 minute) and distilled water for 5 minute. Staining with Harris' haematoxylin for 15 minute.Washing in running tap water for 10-15 minute. Differentiation in acid alcohol (l part HCl and 99 parts 70%alcohol),2-4 dips. Washing in tap water for 5 minute and dipping in ammonia water (2-4 times) until sections become bright blue.Staining with eosin for 1 minute and differentiation and dehydration in alcohol (95%alcohol, 3 changes, 2-4 dips in each; absolute alcohol 3 changes, 2-3 minute in each). Cleaning in 3 changes of xylene, 5 minute in each and finally mounting of the sections with cover slip using DPX. Then the prepared slides were observed on a light microscope (x100) and interpretations were made.

7.3 RESULTS

7.3.1 Gross pathology:

Haemorrhagic lesions were found in the intestinal mucosa (Plate16).Numerous minute white necrotic foci and granulomas were seen at the surface and in the parenchyma of the liver (Plate17).Occasional edema was found in the liver. Egg of *S. indicum* recovered from liver is presented in (Plate 18).

7.3.2 Histopathologyof Liver, small intestine and Large intestine

7.3.2.1 Liver:

Total of 50 samples examined 48 (96%) showed slight to moderate parasitic lesions but adult worms were not detected. Within the perilobular zones a moderate number of small and medium size encapsulated epitheloid cell granulomas were found. They contained eggs which were seemingly viable, degenerating or dead. Giant cells containing eggs or remnants of eggs were present within the gramulomas (Plate19 a,b). Most granulomas develop at the sites of maximal accumulation of eggs. The granulomas were infiltrated and surrounded by eosinophils, lymphocytes and plasma cells. The hepatocytes adjacent to the granulomas were necrotic. Small foci of necrotic hepatocytes with imflammatory cells were observed diffusely through out the liver parenchyma.Perilobular zones were infiltrated by lymphocytes, neutrophils and eosinophils.These cells together with reticulo-endothelial proliferation were also found with sinusoids which were sometimes dilated.

7.3.2.2 Small intestine:

Parasitic lesions varying from slight to severe were found in 38 (76%) cattle out of 50 examined. Adult parasites were detected within the serosal veins of some animals, which showed perivascular infiltration by eosinophils and neutrophils. When parasitic involvement was severe, the interstitium of tunica muscularis was perivascularly infiltrated by eosinophils.The granulomas were infiltrated and surrounded by eosinophils and neutrophils, lymphocytes and plasma cells. Adult parasites were found within the granuloma (Plate20).The blood vessel walls were irregularly thickened and surrounded by polymorphonuclear leukocytes, lymphocytes and plasma cells. Some vessels showed endothelial cell proliferation, subendothelial granulomatous reaction.Mucoid material and inflamatory cells were found within the lumina of the small intestine.

7.3.2.3 Large intestine:

Adult parasites were sometimes detected within serosal veins without inflammatory cell reaction but interstitium of the tunica muscularis was focally, slightly to moderately, infiltrated by eosinophils and neutrophils. The lesions in the sub mucosa and mucosa were variable. Encapsulated, epitheloid cell granulomas containing seemingly adult parasites were present in 80% (40) examined cattle.

Depending upon the severity of infection, the muscularis mucosa was inflamed and focal smooth muscle degeneration was observed due to the penetration and migration of eggs. Perivascular localized parasitic granulomas were also observed. Within the mucosa adult parasites, were surrounded by various types of infiltrating cells or enclosed within epitheloid cell granulomas (Plate21). Secretory materials were found withinthe lumina of the large intestine.

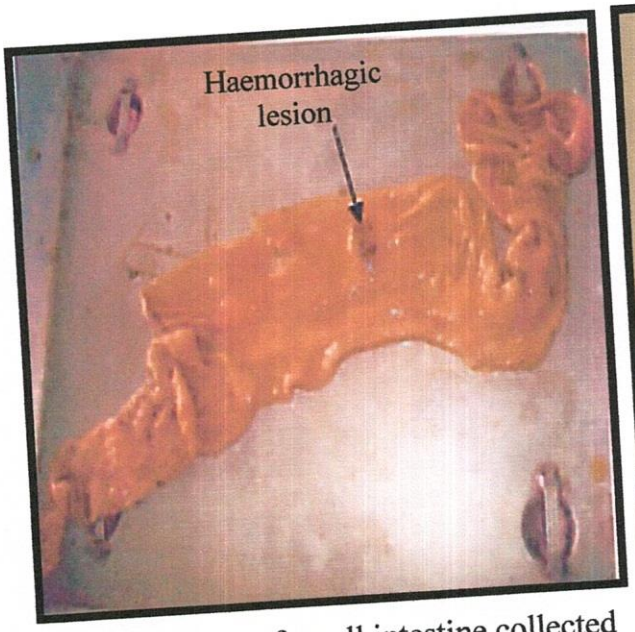

Plate16. Piece of small intestine collected from infected slaughtered cattle

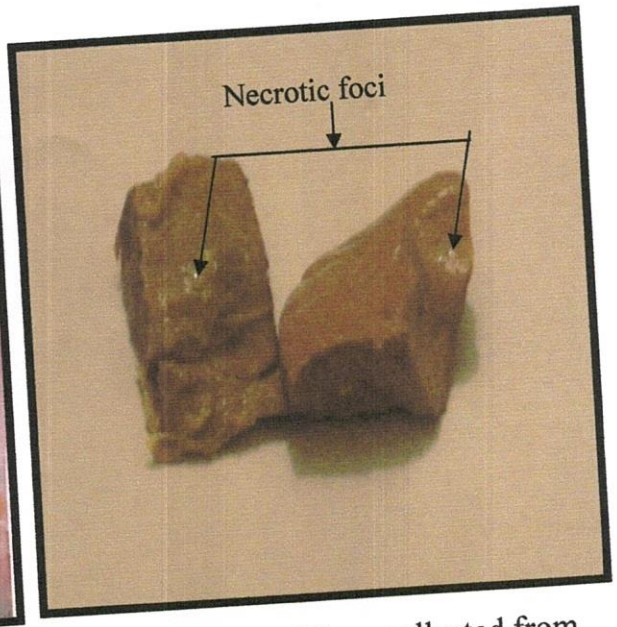

Plate17. Piece of liver collected from infected slaughtered cattle

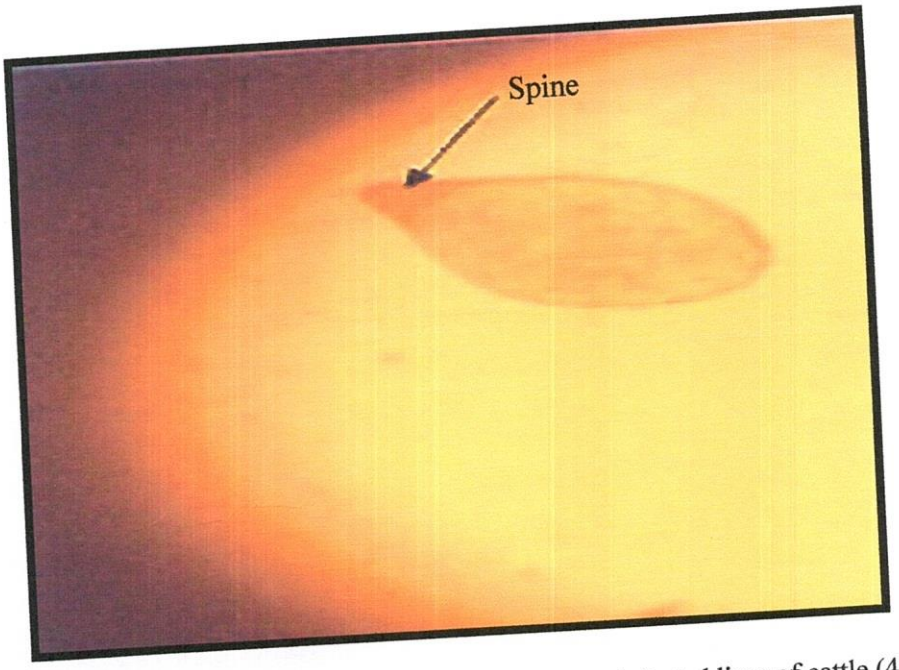

Plate18. *Schistosoma indicum* egg collected from infected liver of cattle (40 x)

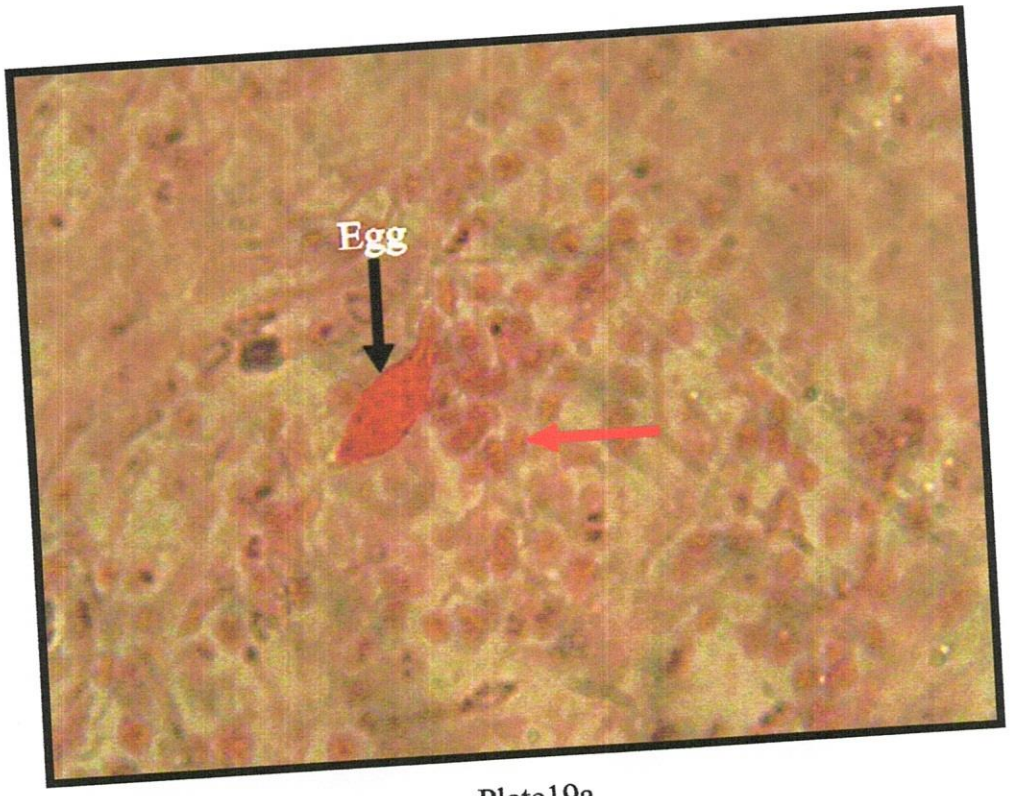

Plate19a

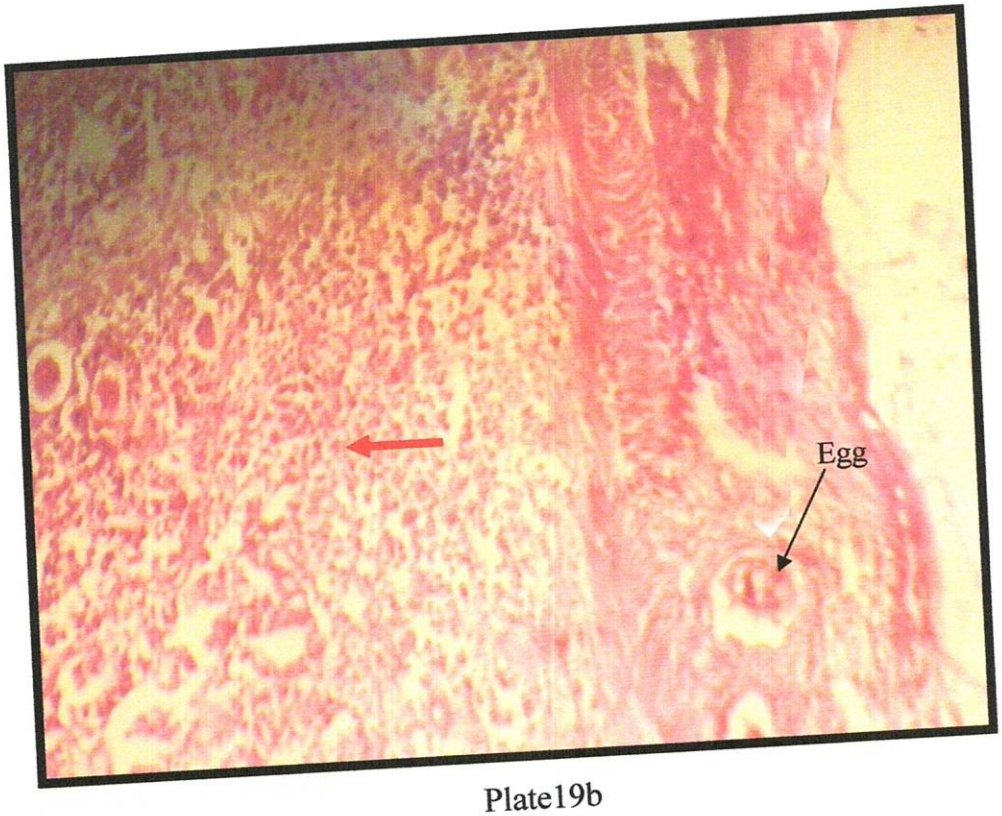

Plate19b

Plate19a,b Section of liver showing egg of *Schistosoma indicum* with parasitic granuloma (red arrow) which are surrounded by lymphocytes, neutrophils and macrophages(x82.5)

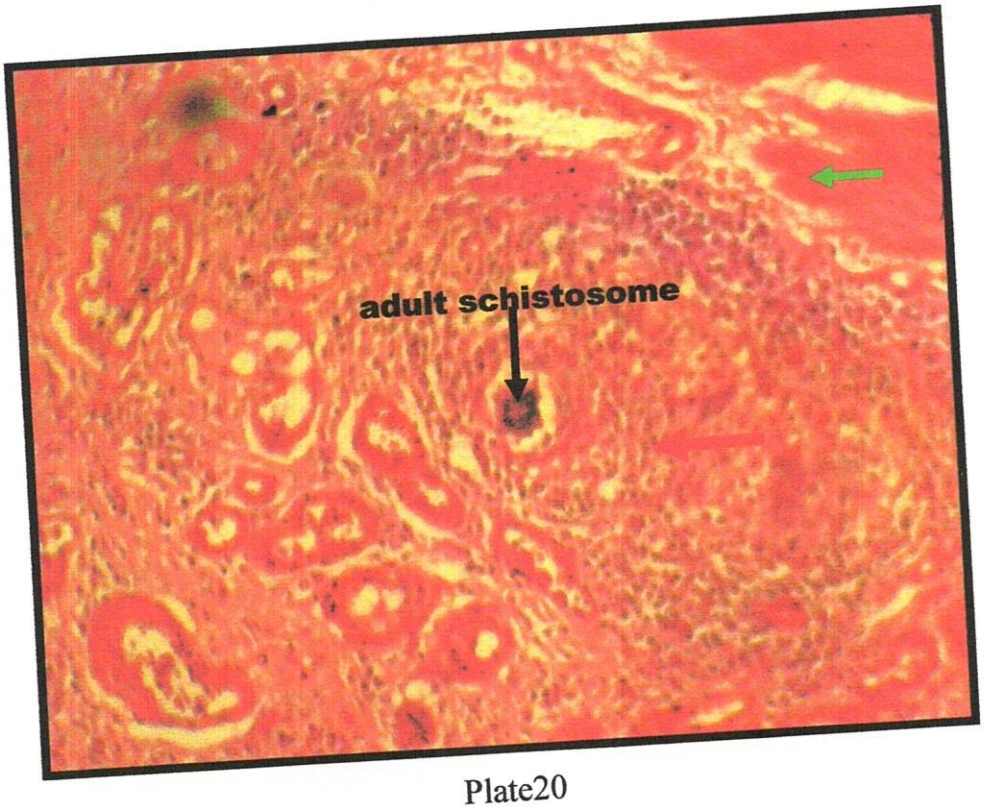

Plate20

Plate20.Section of small intestine showing adult schistosome with parasitic granuloma (red arrow) with infiltration of lymphocytes, neutrophils and macrophages in the lamina propia of small intestine (x82.5). Green arrow indicates mucoid material with in the lumina of small intestine.

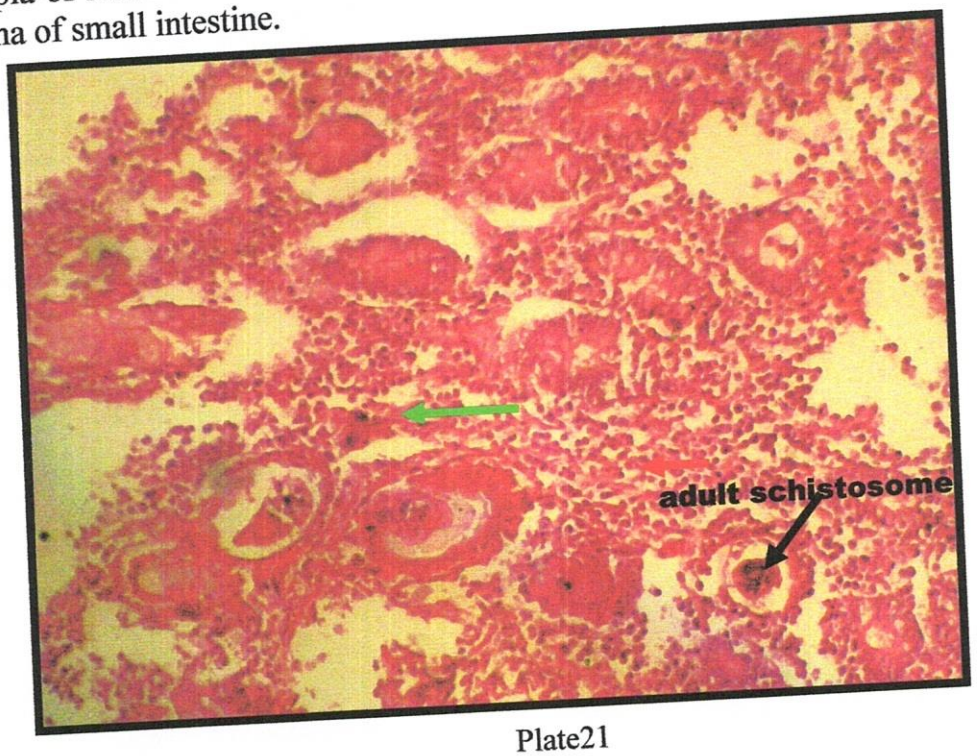

Plate21

Plate21.Section of large intestine showing adult schistosomes with parasitic granuloma (red arrow) with infiltration of lymphocytes, neutrophils and macrophages in the lamina propia of large intestine (x82.5). Green arrow indicates secretory material within lumina of small intestine

7.4 DISCUSSION

In schistosomiasis, pathology is mainly a result of host immune responses to the eggs of the parasite.Adult worms are not directly pathogenic, but yet strongly immunogenic (Dunne, *et al.*,1988). However, they manage to evade host immune responses by incorporating host-derived macronucleus into their tegumental membrane and by losing expression of their own antigens (Smithers and Terry,1969, Pearse *et al.*,1986). It is assumed that approximately 50% of the eggs pass through feces. The remaining 50% of the eggs are trapped within the tissues of the various organs (Davis, 1996) and finally trapped in the portal system of the liver and may cause hepatospleenomegaly.

Despite the clear pathological changes, clinical manifestations by *S. indicum* were not observed in this study.Except for some minute granuloma in the liver and haemorrhage with some occasional oedema in the intestines, no macroscopic lesions were observed in the animal's examined.Fairly and Jasudasan (1927) studied pathology of *S. spindale* in experimentally infected buffaloes. Thrombosis in periportal veins and pseudotubercles in the liver with leukocytic infiltration were the main features. Burggraaf (1935) noted endophlebitis, necrosis of hepatic cells and increase in interstitial connective tissue in a bull calf which were infected with *S. spindale*. The liver, lung and intestine of guineapig showed macroscopic and microscopic lesions attributed by pseudotubercles of pin head size whitish narrow areas of irregular shape which were experimentally infected with $8 \times 10^3 - 5 \times 10^4$ *S. spindale* cercariae (Dutt, 1962). The lesions in liver consisted of coagulative necrosis of parenchymal cells, preudotubercles, phlebitis and thrombosis. The pseudotubercles with different stages of development contained only egg.These type of lesions were not detected during the present study which was caused by the *S. indicum*. The live worms in the blood vessels caused phlebitis and thrombosis. Detailed studies by (Lawrence, 1973 a, b, 1978 a, b) on experimental infections of *S.mattheei* in cattle have shown that the course of infection and its related pathology follow a characteristic sequential development. In early stage, lesions are mainly localized in the intestinal wall as a result of massive egg laying by the adult worms. Later these lesions resolve following an immunological response of the host, which causes the

elimination of most of the adult and suppression of egg-laying in those which survive. This elimination coincides with the appearance of vascular responses. They are either localized around dead worms lying in the blood vessels of intestines, liver and lung eventually leading to lymphoid nodule formation. The lesions detected in the present study infected with *S. indicum* were seen to be similar with the findings of Fransen *et al.,* (1990) where they studied the lesions in *S. spindale* infected animals examined in Kandy were mainly associated with eggs. The deposits of ova occurred almost invariably in the liver and intestines. Fransen *et al.,* (1990) also reported lesions to a much lesser extent in the lung and involvement is commom in *S. mattheei* infections and their findings are in close agreement with the findings of (McCauly and Kruger, 1969, Van wyk *et al.,*1974, Lawrence, 1978a). However, it was not observed by Hussein (1971) in experimental infection with *S. mattheei* and *S. bovis*. Fairly *et al.,* (1930) showed that pancreas, mesenteric lymph nodes and spleen were also often involved in goats with a heavy (> 100,000 cercariae) experimental infection. The granuloma formation around eggs was similar to that described for other species of schistosomes (McCauly and Kruger, 1969, Hussein, 1971, Lawrence 1978a). The portal and mesenteric veins contained clusters of blood flukes which were indentified as *S. spindale*.From the various observations it can be inferred that eggs release immunogens to mediate the inflammatory response which assist their tissue migration. Furthermore, it is plausible that such secretions from viable eggs embolized in liver might initiate granuloma formation (Hang *et al.,* 1974) which drives the pathogenic process of schistosomiasis.

The "Hoeppli" reactions observed around mature eggs in granulomas was not detected in the present observation in comparison with those observed in *S. bovis* infection, (Hussein, 1971) and in *S. mattheei* infections (McCauly and Kruger, 1969). Blood vessel lesions attributable to the presence of adult worms as described in *S. mattheei* and *S. bovis* infections were not observed in this study by *S. indicum* natural infections. These vascular lesions, described in animals experimentally infected with large number of cercariae or in naturally infected animals often treatment (McCauly and Kruger, 1969) are attributable to the presence of dead or dying worms within the

blood vessels. As adults worms are able to evade host immune attacks (Butteroworth, 1993), although dead worms swept into the liver and lungs do give rise to focal lesions (Lawernce, 1978c). In most cases lesions caused by worms are few in number and have a limited clinical importance, compared with the lesions caused by the millions of eggs generated with the course of infection. Although the age of infections in slaughtered cattle in this study was unknown, the intensity of infection was generally low. The few worms which might have died did not cause detectable blood vessel lesions.

It is known that the amorphous eosinophilic precipitations develop inside or outside the shell of mature eggs and consist largely of ImmunoglobulinG(IgG) complement and gramulocyte debris (Hirata, *et al.* ,1987) and the inflammatory response around eggs is an immune mediated events in which hypersensitivity to egg plays a vital role (Weinstock, 1992). The phenomenon represents an antigen-antibody complex analogous to the diagnostic cirumoval precipitation formed around eggs by immune sera *invitro* (Smith and von Lichtenberg, 1967). In early patentcy of primary infections tissue reactivity to eggs is low compared with later stages (Lawrence, 1978, b; Saad *et al.,* 1980). Lawrence (1978, a,b) concludes that histopathological findings, more specially the relative distribution of eggs between the different layers of the intestinal wall and the presence or absence of intrahepatic vascular lesions, could be of value in assessing the age of natural infections. Similar pathological observations, although not classified sequentially, were reported by Hussein (1971) for *S. bovis* experimental infection in cattle.

CHAPTER 8

SUMMARY AND CONCLUSIONS

SUMMARY

A series of on-farm and abaittor study was conducted during the field work on the epidemiology of bovine schistosomiasis to identify the risk factors, abundance of vectors with their infective cercariae and their relationship with ecological variables during the period of September 2009 to August 2011. In order to study the epidemiology of bovine schistosomiasis a survey was carried out in five different agroecological areas of Bangladesh. The first study of the series was on the 330 dairy farms (two hundreds and seventy subsistence and sixty commercial) were surveyed for fecal collection with a pre-tested questionnaire. A total of 2514 fecal sample were collected from the study areas and examined microscopically using the concentration technique of Lawrence (1970). Among the three species of mostly available in Indian subcontinent two species of *Schistosoma* were detected during this time. *S. indicum* were the most common species than *S. spindale* recorded. The overall prevalence of bovine schistosomiasis in Bangladesh was 33.2%. About 29.9% and 10.6% cattle were found to be *S.indicum* and *S. spindale* egg positive respectively. A significant variation in the prevalence of schistosomiasis in cattle were observed according to districts, ages, breeds, sexes, physical conditions, fecal consistency, sanitations, grazing, diets, de-worming, farming systems and seasons. A high rate of prevalence was noticed in Sirajgonj (49.5%) followed by Chittagong (41.7%), Mymensingh (27.9%) and Netrokona (24.6%) and lowest in Kishoregonj (21.1%). In the age groups the prevalence was the highest in adult cattle of >3 years (35.6%) followed by young cattle 1.5 years to 3 years (31.9%) and lowest in calves of ≤ 1 year (27.2%).

Between two breeds indigenous cattle showed high level of prevalence (38.4%) than cross breed cattle (19.5%). The prevalence of infection was recorded higher in male animals (40.1%) than female (25.9%). In respect of health condition the prevalence of infection was significantly higher (39.6%) in very thin animal than slightly thin animal (38.2%) and normal healthy animal (21.6%).

The prevalence of infections varied in different seasons of the year and the highest prevalence was recored in rainy seasons (44.7%) follwed by summer (30.3%) and winter (21.4%). By overall monthwise data analyses it showed highest prevalence in the month of September (59.0%) which gradually decreased in December (17.9%) and Janaury (17.5%).Prevalence of bovine schistosomiasis increased with the increase of herd size.Significantly higher prevalence ($p<0.002$) were observed in herds containing 5-9 and 10-15 animals ($P<0.006$) than those herds having less than 5 animals.

To find out the risk factors associated with schistosome infection in catte EPG was calculated. Topographic regions, ages, sexes, grazing, fecal consistency and seasons were identified as significant risk factors for bovine schistosomiasis by egg count in Bangladesh.

In the 2nd study relationship with mean prevalence of bovine schistosomiasis by fecal examination with mean temperature and mean rainfall of the five study areas were carried out. The study revealed significant higher prevalence in the month of September and August and showed significant positive correlationship with temperature and rainfall in the five agroecological regions of Bangladesh.

In the 3rd study of the series the prevalence with intensity of intestinal schistosomes by mesenteric blood vessels examination from slaughtered cattle was done.The study also findout the relationship of prevalence with ecological variables (temperature and rainfall). The overall prevalence of adult schistosomes in cattle was 48%. The highest number of total schistosomes collected from one viscera was 322 and lowest was 18. Mean of total schistosome was 50.3. Male and female parasite ratio was 12:10. A significant variation in the prevalence of bovine schistosomiasis was observed according to regions and seasons. But the variation of adult schistosome's prevalence with different sex and age group of slaughtered cattle were found insignificant. In the study of intensity 27.2% of the mesenteric blood vessels showed moderate intensity (21-100 pairs) which were statistically significant than low and heavy worm intensity. To study the relationship of the regional prevalence with month it showed that the prevalence were more or less high throughout the year in four regions except in Chittagong where July, August and September showed significant higher prevalence.Correlation studies showed a positive significant relationship between schistosomiasis in cattle with temperature and rainfall in all studied areas.

In the 4th study of the series a survery for schistosomiasis vectors (snails) was carried out in four different inhabitants of Mymensingh region from January 2010 to December 2011. To find out the role of snails in the spread of schistosomiasis overall 5272 snails were examined from different inhabitants and in different seasons.*L.luteola* (26.2%), *L. auricularia* (26.3%), and *I. exustus* (29.8%) were identified. The abundance of all three vector snails were significantly higher ($P<0.01$) in rainy season than summer and winter. The abundance of three vector snails was significantly higher in deep paddy field and river than beel and pond. Out of 5272 snails was examined 3.8% were found infected with *Furcocercus* cercariae. For shedding of *Furcocercus* cercariae *I.exustus* played the dominant role followed by *L.luteola* and *L. auricularia.*The releasing of *Schistosoma* spp. cercariae was significantly higher ($P<0.01$) during rainy season by three vector snails except *L. luteola* which also released higher number of cercariae in winter (Table 24). Highest prevalence of cercariae was found in snails collected from deep paddy field than other three locations but the variation was found statistically insignificant. The release of *Furcocercus* cercariae was higher in September followed by October and May by the snail of *L. auricularia, L. luteola* and *I. exustus.* A positive correlation was noticed between the releases of *Furcocercus* cercariae by the vector snails with ecological variables.

The 5th study was carried out to observe the first larval stage of S*chistosoma* spp. the miracidial hatching ability. This study clearly showed that miracidial hatchability increases with the decreasing age of cattle. The highest miracidial hatchability (81.3%) was found in the age group of upto 2 years.

In the 6th study a total of 50 liver, 50 small intestines and 50 large intestines were examined for pathological study. Among there 96% liver showed slight to moderate parasitic granulomas produced by the schistosome eggs. The granulomas were infiltrated and surrounded by eosinophils, lymphocytes and plasma cells. In the small intestines parasitic lesions were found in 76% of examined cattle. Adult parasites were detected within the serosal veins of some animals, which showed perivascular infiltration by eosinophils and neutrophils. In the large intestine encapsulated, epitheloid cell granulomas containing adult parasites were present in 80% examined animals.

CONCLUSIONS

It is concluded that quantitative measures of fecal egg output alone may not be regarded as reliable method for the assessment of the prevalence and intensity of natural infections in bovine visceral schistosomiasis, where the duration of infection is unknown.High prevalence of fluke infection in this country indicated the favorable environments are prevailing for these parasites. Age groups, breeds, sexes, topographical positions, seasons and management of cattle will determine the magnitude of the infection acquired. The nutritional status of the livestock may determine how adversely an individual will be affected by the parasites they acquire. Because of the way each type of cattle is managed it is likely that parasitic diseases vary greatly among them. Therefore, the parasites of cattle should be approached as a herd or group problem rather than a problem of an individual animal. A successful grazing management system needs to be based on appropriate knowledge of the epidemiological conditions for the prevailing parasite infections (Dimander *et al.,* 2003). It may be hypothesize that poor management by the farmers, such as sharing the same grazing by the farmers, poor sanitation of the animals might be major factors that cause increasing parasitic infection. Climate variables are able to affect the prevalence, intensity and geographical distribution of schistosomiasis, directly influencing free-living larval stages and indirectly influencing mainly invertebrate hosts. The impact of climate change appears to be more pronounced in trematodes, and is mainly shown by increased cercarial production and emergence associated with global warming. However, fecal examinations only provide an indirect measure of the intensity of infection. In this method light infections may escape detection, and it may therefore be necessary to use postmortem examinations to obtain accurate data on transmission.

In the opinion of the present worker the two simple methods egg detection method along with miracidia hatching test will be of great help in performing a proper diagnosis and fair assessment of schistosomiais in the field. Recent progress in characterizing potentially protective parasite antigens has opened new perspectives in the control of schistosomiasis. Whatever the means, it will be necessary to findout the sustainable strategy for prevention and control of schistosomiasis on a profound knowledge of the epidemiology of the disease.Furthermore, the present study addresses several key epidemiological questions for schistosomiasis and thus creating a basis for further epidemiological investigation.Research works focus on molecular approaches for diagnosis and epidemiological study of schistosomiasis is warranted. It may be concluded that schistosomiasis should be listed among the infectious diseases with which special care because of climate change in the future, especially in temperate and colder northern latitudes and in areas of high altitude.It is evident from the study that the planning of an effective strategy for the control of schistosomiasis require on the basis of it's geographic and seasonal distribution. The influence of management practices will also used to be considered and the timing of treatment-prior the release of *Furcocercus* cercariae from snails is important in order to achieve the greatest efficacy.More research activities are recommended with the assistance of collaborative institutions. Technical and financial support provided by WHO, FAO and other blilateral donars should be solicited.

CHAPTER 9
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APPENDIX-1

Questionnaire for sample collection

1. Farmer ID 2. Date:

District: Upazila: Union: Village: Post office:

10. Status of Nutrition: Good/Poor

11. Where do your animals graze? Fallow land /Char/ Haor/ Beel/ Tethering in road side or field/Zero grazing.

12. Type of animal house used: Kaccha floor/Brick floor/ Cemented.

13. Level of sanitation in the animal shed: Poor/Moderate/ Good.

14. How do you manage health related problems? By Vets/Veterinary field assistants/ Village doctors/Ethno Veterinary/

15. How frequently you de worm your animals? As recommended by Vets / When sick / Never.

16. Sample taken: Feces/ Mesenteric blood vessels/ Visceral organ.

17. Fecal sample: Hard/Soft/Watery

18. Test Results: Positive/Negative/In conclusive

3. Particulars of the location

4. What is the purpose of animal production? Subsistence (own consumption)/ commercial/mixed

5. Breed of cattle: Local/Cross-breed/Exotic

6. Age of cattle: (in year)

7. Sex of cattle: M/F

8. General health condition of cattle: Normal/Thin/ Moderate

9. What are the types of feeds provided to your animals? Cut grass/Straw/Rice polish/Wheat bran/Oil cake/Vitamin mineral premix/Urea molasses block/Urea treated straw/ Urea molasses treated straw.