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STATUS OF INTESTINAL SCHISTOSOMIASIS IN BUFFALOES AND CATTLE IN RAJSHAHI, BANGLADESH

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ABSTRACT

Schistosoma infection causes serious health impediment in livestock with poor productive and reproductive performances. This study was carried out to know the status of *Schistosoma* infection in buffaloes and cattle in Rajshahi, Bangladesh. The adult schistosomes were collected from mesenteries of buffaloes and cattle of Rajshahi. Out of 240 mesenteries (120 from each species) examined, 113 (47.08%) were found infected. The infection rate in buffaloes and cattle were 54.16% and 40%, respectively. Female buffalo and cattle (57.69% and 46%) were more prone to the infection than male (51.47% and 36.71%) although the differences were not statistically significant. Significant variations ($p<0.05$) were observed in infection rate in different season. The highest infection rate was observed in rainy season (85% and 65%) followed by summer (52.5% and 40%) and winter (25% and 15%) in both the animals. Intensity of the infection greatly varied. Mostly, half of the infected animal (55% buffalo and 46% cattle) showed moderate infection intensity (51 to 100 parasites/animal). High degree of infection (>100 parasites/animal) was found in 25% and 33% infected buffalo and cattle, respectively. Two species of intestinal schistosomes (*S. indicum* and *S. spindale*) were identified microscopically by observing egg of female parasites. *S. indicum* was present in all the infected mesenteries whereas only 29% buffaloes and 35% cattle samples contained *S. spindale*. The study determined the current scenario of intestinal schistosome infection in buffaloes and cattle of Rajshahi. The findings might be helpful to develop effective strategic plan to overcome the harmful effects of schistosomes in livestock of Bangladesh.

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INTRODUCTION

Livestock is an economically important sub-sector of agro-based Bangladesh. Cattle and buffaloes are the major part of livestock contributing significantly in meat and milk supply for huge population of Bangladesh. To-date, old traditional husbandry system is usually practiced for rearing of livestock in Bangladesh with inadequate and imbalanced ration (Sardar et al., 2006). Because of their habits, inadequate and imbalanced ration, most of the livestock population is suffering from a wide variety of infections. Parasitic infections are the most common constraint of livestock production which markedly contribute to reduce milk and meat production in cattle and buffaloes (Murthy and Rao, 2014). Helminth parasites are abundant in Bangladesh as geographical location and climatic condition are mostly favorable for their growth and development (Hossain et al., 2004). Parasitic helminths are common cause of health impediments in cattle and buffaloes as they can easily transmit from one host to another by several transmission methods. It is one of the leading causes of economic loss for livestock worldwide in different tropical and sub-tropical areas including Bangladesh (Hossain et al., 2021; Islam et al., 2017; Biswas et al., 2014; Barmon et al., 2014).

Schistosomes are commonly found in many tropical and subtropical countries of the world including Africa, Eastern-South America, Caribbean, Middle-East, and Southern-East Asia (Kassaw, 2007; Anisuzzaman and Tsuji, 2020). The disease is endemic in more than 78 countries, affecting both man and animals. Schistosomes cause debilitating disease of livestock mainly in cattle and buffaloes (Samad, 2008; Hovnanian et al., 2010; Frahm et al., 2021). Intestinal schistosomiosis, is one of the major veterinary problems in many countries of the world including Asia and Africa. In cattle and buffaloes, intestinal schistosomiosis is caused by *Schistosoma indicum*, *Schistosoma spindale, Schistosoma bovis and S. japonicum*. High degree of schistosome infection reduces productivity, increase mortality and causes chronic wasting illness in affected animals (Islam et al., 2011; Kerie and Seyoum, 2016; Cherian and D'Souza, 2009; Sudhakar et al., 2016). These parasites have been estimated to infect 530 million cattle worldwide, with more than 165 million cattle affected in the regions of Africa and Middle East (Islam et al., 2011). Cattle infected with *S. bovis* develop a syndrome characterized by liver damage, roughness of hair coat, pale mucous membrane, serious emaciation and a very poor reproductive performance (Aradaib et al., 1993), that results in a significant economic down turn and a threat to public health. Mc Cauley et al., (1983) mentioned that schistosomiasis hampers in economic traits like retarded growth, liver damage, poor reproductive performance, and increased susceptibility to other parasites or bacterial infection. It was reported that water buffaloes accounted for nearly 90% in the transmission of schistosomes in Chenqiao village, China (Wang et al., 2006). In recent studies, using qPCR a high endemicity of schistosomes (51–91%) was demonstrated in water buffalo of Philippines (Jiz et al., 2022). A number of snail's act as intermediate host for intestinal schistosomes among which *Indoplanorbis exustus* appears to be the most common and natural intermediate host (Kumar and Burbure, 1986). Different factors such as increased irrigation in agriculture and breeding of fish help in increasing snail number, and they carry cercariae which increase the prevalence of schistosomiosis in humans and animals (Islam et al., 2011).

Different species of *Schistosoma* cause schistosomiasis which is chronic in nature and it causes debilitating infections (Parijia, 2004). The degree of schistosomiasis in animals is divided into three stages: migratory, acute and chronic stage (Endris and Alemneh, 2017). A number of studies reported marked variation in infection rate (4.7% to 57.3%) of intestinal schistosome in cattle and buffaloes of different province of India. (Lakshmanan et al., 2011; Ravindran and Kumar, 2002; Kommu and Murthy, 2016; Ravindran et al., 2007). In Bangladesh, previous studies reported marked intestinal schistosome infection (11.5% to 62%) in cattle (Nazimuddin, 2007; Islam, 1975; Rahman and Mondal, 1983; Rahman, 2009; Islam et al., 2011; Abdullah and Mohanta, 2021; Hossain et al., 2015). Several studies on schistosomes indicated its abundance in cattle at different areas of Bangladesh but there is no information about schistosomes in buffaloes. Moreover, the problem is yet to be addressed in ruminants in northern area of Bangladesh. To meet this research gap, this study was focused to elucidate the infection status, identification and characterization of schistosomes of buffaloes and cattle in Rajshahi, Bangladesh.

MATERIALS AND METHODS

Experimental period and area of study

This study was conducted during the period of one year from March 2020 to February 2021. The experimental period of study was divided into three seasons as hot-humid dry summer (March to June), wet rainy (July to October) and cold-dry winter (November to February). Experimental samples were collected from slaughter houses of three different local markets of the Rajshahi City Corporation and its surrounding areas. The markets include Shaheb bazar, Talaimari, and Katakhali which were selected based on the availability of slaughtered cattle and buffaloes (Fig. 1). All the experimental works were conducted in Laboratory of Parasitology, Department of Veterinary and Animal Sciences, University of Rajshahi.

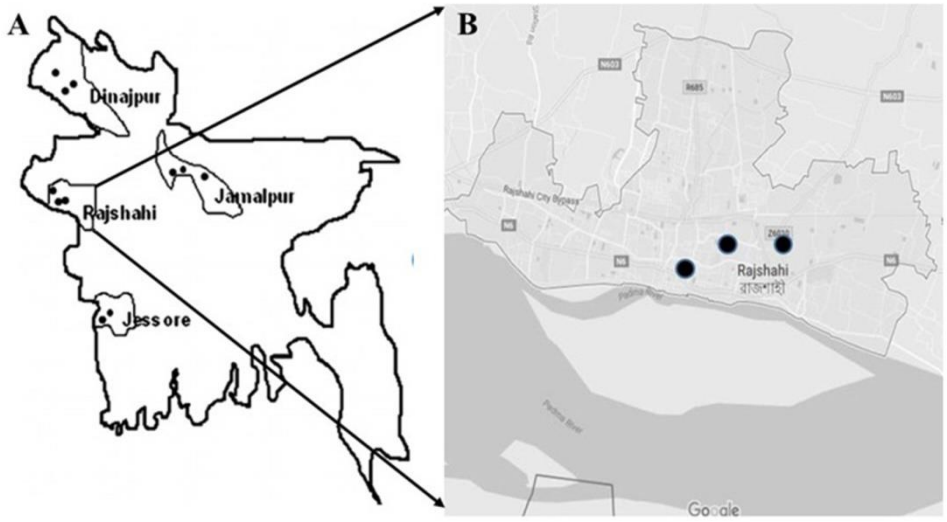

Figure. 1 Map of Study area
A) Map of Bangladesh B) Map of Rajshshi City Corporation indicating sample collection site (black circle)

Data and Sample Collection

A total 240 mesenteries (120 cattle and 120 buffaloes) were collected. The age of slaughtered cattle and buffaloes were between 2 to 8 years. The relevant data related to study were also collected during sample collection. The slaughtered animals imported from neighboring countries were excluded during the study. Each mesentery sample was kept in a separate polythene bag, tied carefully, marked by numbering and brought to the Laboratory of Parasitology in the Department of Veterinary and Animal Sciences, University of Rajshahi and examined immediately after processing.

Collection and Identification of *Schistosoma* sp.

Mesenteries were cut into small pieces, kept in a glass jar containing saline. After gentle stirring, the pieces of mesenteries were separated off and the saline was kept undisturbed for 20 minutes. The supernatant was poured off and the parasites were collected from the rest of the saline and stored/preserved for future study. During collection, the numbers of infected and non-infected samples were counted. The numbers of positive/negative male and female samples were also recorded. Fresh schistosomes were identified immediately by observing their morphological characteristics under microscope (LB, USA). Permanent slides were also prepared for detail morphological study according to the previously described procedures (Rahman et al., 1996). Briefly, properly washed schistosomes were pressed in between two slides and fixed by using slightly hot Alcohol-Formalin-Acetic Acid (AFA) solution for 30 minutes and dehydrated using alcohol at different concentrations (30-70%) and treated with 70% iodine alcohol. Then, the parasites were stained with Semichon's Carmine, dehydrated again with alcohol (50-100%), treated with aniline oil and washed in xylene. Finally, the parasites were mounted with canada balsam and observed under a microscope (4X and 10X; LB, USA).

Study on sex and season wise infection

During collection of sample, a male female ratio was considered to determine the sex wise infection status. The infection status in male and female animal was determined after examination of samples. Specific month and season wise study on infection status of schistosomes were also conducted. Equal number of samples (10 in each month and 40 in each season) for both cattle and buffaloes were collected to study the frequency of infection throughout the year. Seasonal influence was determined by comparing the infection status of schistosomes in buffalo and cattle in three different seasons.

Study on intensity of schistosome infection

Degree of schistosome infection was recorded based on number of parasites in each positive sample. It was determined by categorizing the presence of number schistosomes in each positive samples such as high (>100), moderate (51≤100) and low grade infection (10≤50). To determine the abundance of specific species of schistosome in buffaloes and cattle, the species wise (*S. indicum* and *S. spindale*) ratio in each positive sample was observed by the identification and counting the adult female schistosomes in each sample.

Statistical analysis

All recorded data in this research were entered into Microsoft excel 2007. According to the formula of Thrusfield (2005), the rate of prevalence was estimated. Co-relation between different variables such as sex, season and outcome variable (*Schistosoma* sp. infestation status) was done by using Chi-square test ($\chi 2$) test. All statistics in this research, were considered as significant at the level of $p<0.05$. According to the formula of Schesselman (1982), Odds ratio was calculated.

RESULTS

Infection status of intestinal schistosomes in buffaloes and cattle

The mesenteries of buffaloes and cattle examined in this study were considerably infected with the intestinal schistosomes. Sixty-five (54.16%) mesenteries of buffaloes were positive for schistosomes among the 120 mesentery examined. In cattle, out of 120 mesenteries, 48 were infected with schistosomes and infection rate was 40% (Table 1). The findings showed the buffaloes were a bit more susceptible to intestinal schistosome infection than cattle, however, the differences were statistically insignificant ($p<0.05$).

Table 1. Infection status of intestinal schistosomes in buffaloes and cattle

Animal Species	Mesentery examined	Infected	Non-infected	% Infection	Odd Ratio
Buffalo	120	65	55	54.16	1.77 (B vs C)
Cattle	120	48	72	40.00	
Total	140	113	127	47.08	

B: Buffalo; C: Cattle

Sex wise prevalence of intestinal schistosomes

The sex wise infection status of intestinal schistosomes in buffaloes and cattle was determined. In buffaloes, 33 were found infected out of 68 male samples. Among 52 female buffaloes, 30 were infected with schistosomes. Examination of 70 male cattle samples revealed 36.71% infection (25 infected samples) whereas out of 50 female cattle samples, 23 were found infected (46%) (Table 2). These results indicated that female of both buffalo and cattle were more prone to schistosome infection although the differences were statistically insignificant.

Table 2. Sex wise infection status of schistosomes in buffaloes and cattle

Sex	Buffaloes					Cattle				
	Examined	Infected	Non-infected	% Infection	Odd Ratio	Examined	Infected	Non-infected	% Infection	Odd ratio
Male	68	35	33	51.47	F vs M 1.28	70	25	45	36.71	F vs M 1.54
Female	52	30	22	57.69		50	23	27	46	

F: Female; M: Male

Seasonal variations in intestinal schistosome infection

During this study marked variations were found in infection status of intestinal schistosomes in different seasons even in different months in both the cattle and buffaloes. Highest infection rate (80-100%) was found in the month of July and August and lowest was in January and February (0-10%) in both the animals (Fig. 2). Study on seasonal prevalence showed the highest infection rate in rainy season in both buffaloes and cattle (85% and 65%) followed by summer (52.5% and 40%) and lowest in winter (25% and 15%) (Table 3), indicating variations in environmental conditions significantly (at $p<0.05$) influence the occurrence of intestinal schistosome infection in buffaloes and cattle.

Table 3. Seasonal occurrence of intestinal schistosomes in buffaloes and cattle

Season	Buffaloes				Cattle			
	Infected	Non-infected	% Infection	Odd ratio	Infected	Non-infected	% Infection	Odd ratio
Summer (n=40)	21	19	52.5	R vs S 5.15	16	24	40	R vs S 2.80
Rainy (n=40)	34	6	85	R vs W 16.67	26	14	65	R vs W 10.88
Winter (n=40)	10	30	25	S vs W 3.23	06	34	15	S vs W 3.88

S: Summer; R: Rainy; W: Winter

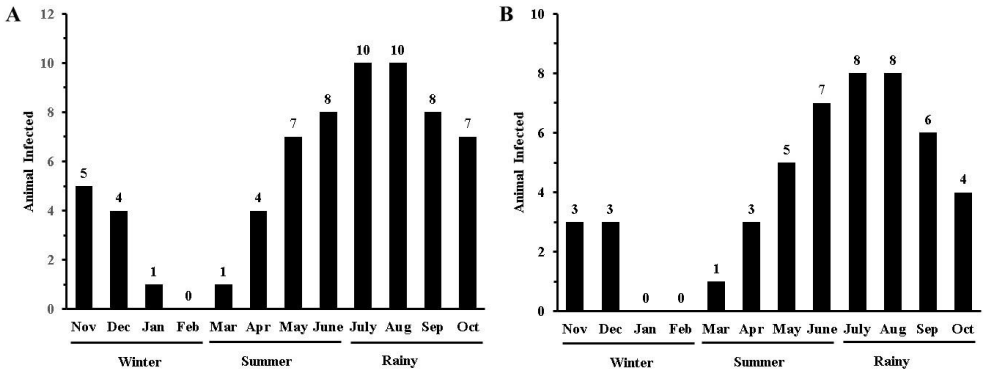

Figure 2. Month-wise occurrence of intestinal schistosomes in buffaloes (A) and Cattle (B)

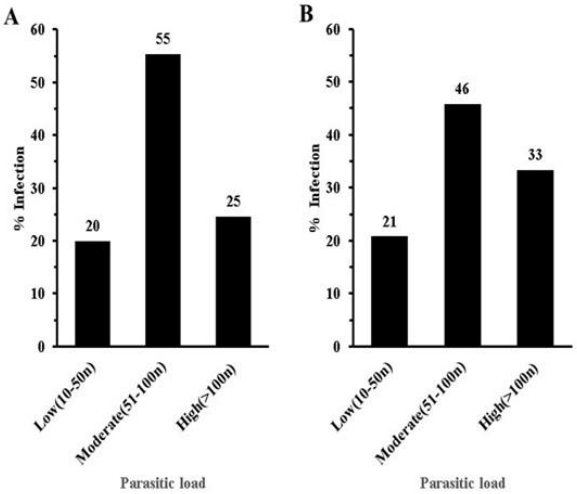

Figure 3. Intensity of intestinal schistosomes infection in buffaloes (A) and cattle (B)

Intensity of intestinal schistosomes infection

The parasites collected from infected animals were subjected to counting to determine the load of infections. The number of parasites found in each sample varied from 10 to several hundreds. High intensity of infection (>100 parasites/animal) was found in 25% buffaloes and 33% cattle. Moderate intensity (51 to 100) was recorded in 55% buffaloes and 46% cattle and low intensity (10 to 50) was observed in 20% buffaloes and 21% cattle (Fig. 3). The various degree of infection indicated the possibility of both clinical and subclinical disease in affected animals.

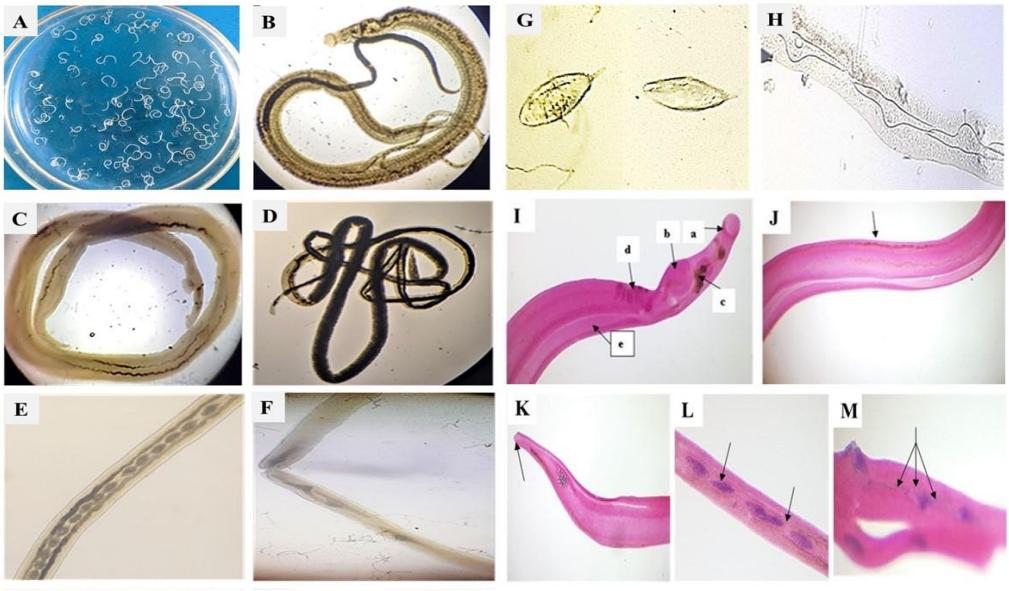

Figure 4. Gross and microscopic identification of fresh schistosomes

A) Collected gross sample, **B)** Male and female, **C)** Male, **D)** Female, **E)** Part of female *S. indicum*, **F)** Part of female *S. spindale,* **G)** Egg of *S. indicum*, **H)** Egg of *S. spindale*. **I)** Stained male anterior portion [(a) oral sucker (b) ventral sucker (c) oesophagus (d) testes (e) gynaecophoric canal], **J)** Stained middle portion with intestinal ceca, **K)** Stained posterior portion with blunt tail, **L)** Female *S. indicum* with eggs, **M)** Female *S. spindale* with eggs.

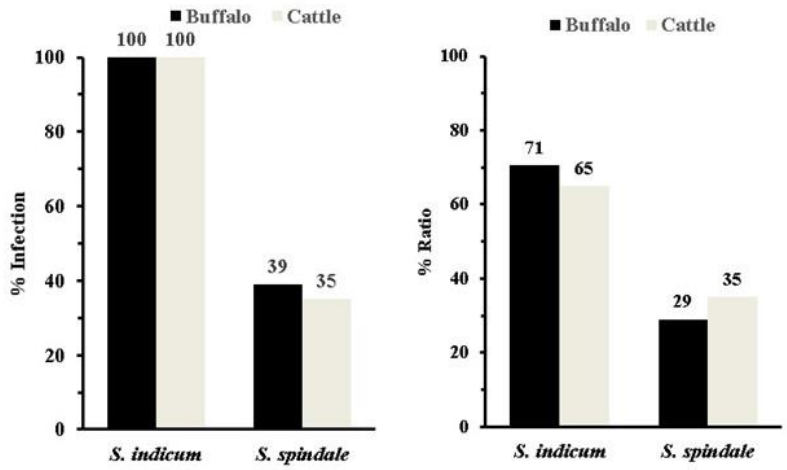

Figure 5. Species specific infection percentage (A) and ratio of two species in dual positive samples (B)

Morphological identification of two species of schistosomes

A considerable number of intestinal schistosomes were collected from the slaughtered buffaloes and cattle. Properly washed fresh schistosme samples were identified microscopically by observing the suckers, split body shape, characteristic intestinal caeca, testes, esophagus etc. The male was identified by the comparatively robust body, presence of gynaecophoric canal and absence of eggs. The female parasites were identified by the narrow long split body, presence of eggs and absence of gynaecophoric canal (Fig 4, A-H).

In this research, two species of intestinal schistosome *S. indicum* and *S. spindale* were identified by observing differential morphological characteristics of egg under microscope. In case of *S. spindale,* eggs were spindle shaped with long terminal spines at both ends. On the other hand, *S. indicum,* eggs were oval in shape with single terminal spine which was shorter than the eggs of *S. spindale*. However, it was not possible to identify the male of different species microscopically. Moreover, *S. indicum* and *S. spindale* were also differentiated microscopically by observing all the characteristics in permanent slide (Fig. 4, I-M).

The frequency of infection of *S. indicum* and *S. spindale*

Based on characteristics of female schistosomes, *S. indicum* and *S. spindale* were identified, and their species specific infection status was determined. Interestingly, it was found that 100% of positive samples contained *S. indicum* whereas only 29-35% of positive samples contain *S. spindale* in both buffalo and cattle (Fig. 5A). The ratio of *S. indicum* and *S. spindale* in dual positive samples was also determined. The number of *S. indicum* was much higher than *S. spindale* in both the cattle and buffalo samples. Figure 5B showed 71% and 65% *S. indicum* and 29% and 35% *S. spindale* in cattle and buffaloes, respectively. The high infection rate of *S. indicum* indicated survivability of their infective stage in the subtropical environment of Bangladesh in comparison to *S. spindale*.

DISCUSSION

Schistosomes are considered important helminth as they cause human and animal infections worldwide. Among animals, cattle and buffaloes are thought to be highly susceptible to schistosome infection due to their free grazing habit in water lodged areas where there may be the availability of infective cercaria. Like other helminth parasites, schistosomes are abundant in Bangladesh as geographical location and climatic conditions are highly favorable for their development, growth and multiplication. Many researchers conducted research in various aspects of schistosome infection in cattle and buffaloes throughout the world. A very few researches were conducted on *Schistosoma* infection in Bangladesh which were mainly on cattle schistosomes. This study mainly focused on prevalence of schistosome in buffaloes including their morphological identification. The cattle were also included as there is no data on intestinal schistosome infection in cattle of Rajshahi, and as well as to determine any variation in intestinal schistosome infection between two popular food animals.

The status of infection (54.16%) of intestinal schistosome in buffaloes was determined based on the presence of adult schistosomes in mesenteric veins, which is in agreement with the findings of Ravindran et al. (2007) where they stated 50% intestinal schistosome infection in buffaloes. In contrast, Banerjee and Agrawal, (1992) and Kommu and Murthy, (2016) reported 41.2% and 32.14% intestinal schistosomes in buffaloes, respectively. The schistosome infection rate (40%) in cattle found in this study showed the similarities with the findings of Islam et al. (2011), Banerjee and Agrawal, (1992) and Rahman and Mondal, (1983) where they mentioned 47.5%, 45.9% and 41.2% infection, respectively. In contrast, there is dissimilarities of the current findings with the report of some other researchers where they described 28.57% to 77.42% intestinal schstosomes in cattle (Abdullah and Mohanta, 2021; Kommu and Murthy, 2016; Ravindran et al., 2007; De Bont et al., 1991b; Agrawal and Sahasrabudhe, 1982a). Differences in geographic location, climatic condition, rearing and grazing system, body conditions of the examined animal and deworming status might have much effect on the occurrence of schistosomiasis in cattle and buffaloes. Types of sample examined may also greatly influence the infection rate as there is a noticeable difference in infection rate determined by examining the fecal sample and mesentery sample. The infection rate of intestinal schistosome was higher in buffalo (54.16%) than cattle (40%). The variation on behavior and feeding systems of different domesticated animals might influence the ratio of schistosome infection in susceptible animals (Agrawal and Sahastrabudhe, 1982) explained that the difference may be due to more tendencies of buffalo to keep more in touch with intermediate host snail by seeking pools, rivers or swamps for wallowing. It was noticed that the infection with schistosomes in female (57.69% in buffalo and 46% in cattle) was higher than male (51.47% in buffalo and 36.71% in cattle). This finding is supported by the report of Bachal et al. (2002) where he mentioned slightly higher infection rate of helminthes in female (48.30%) than male buffaloes (45.12%). Biswas et al. (2014) also reported higher prevalence of gastro-intestinal parasites in

female (87.53%) than male (84.37%). In contrast, there are disunities to the findings of Islam et al. (2011) where he reported higher infection rate of schistosome in male (65.28%) than female (43.29%) cattle. Mamun et al. (2011) described higher prevalence of gastro-intestinal parasitic infection in male buffaloes (61.34%) than females (59.52%) in Bangladesh. It was also described in some report that there was no difference in the infection of gastro-intestinal parasites in between sexes (Azhar et al., 2002; Aylate et al., 2017; Kerie and Seyoum, 2016). Biswas et al. (2014) mentioned that the variation of infection of helminth in female and male may be due to alteration of physiological conditions in female during pregnancy and lactation period. Lioyd, (1983) described that higher level of prolactin and progesterone makes female more susceptible to any kind of infection. Islam et al. (2011) mentioned that hormonal control may have influence for this reason. During parturient and pre-parturient period, parasitic infections easily increase in females than male due to their stress and reduced immune status (Urquhart et al., 1996).

Season has great influence on the prevalence of intestinal schistosome infection in buffaloes and cattle. Higher prevalence of schistosome infection was observed in rainy season in both animals followed by summer and winter (Table 3). This result are in agreement with the findings of Islam et al. (2011); Mamun et al. (2011) where they reported higher infection of parasites in rainy season followed by summer and lowest in winter. On other hand, there are dissimilarities with the findings of Jeyathilakan et al. (2008) where he found higher prevalence of schistosome in cattle in monsoon and in winter season in case of buffaloes, and low in summer for both animals. Bedarkar et al. (2000) also found higher prevalence of *Schistosoma* species in monsoon and low in summer for ruminants. Azhar et al. (2002) reported higher prevalence of gastro-intestinal parasites in buffaloes in autumn followed by spring (20%), winter (13%) and lowest in summer (9%) in Pakistan. For both animals, the highest prevalence was in July and August month and the lowest in January-February (Fig. 2). This finding is an agreement with the report of Arshad et al., (2022) where he recorded highest prevalence of schistosomes in buffaloes during August (25.5%) followed by July and the lowest during December and January. The similarities and dissimilarities between current and earlier studies in regard to this seasonal difference of infection by schistosome may be due to geographical location, study methods and other factors which have the influence on the occurrence of infections in different seasons. The abundance of intermediate host (snail) and dispersion of faeces by rain, heavy rainfall, flood and humidity, dry conditions etc. might influence the infection rate.

Intestinal schistosome infection intensity was different among the animals (Fig 5). Availability of intermediate and final host, climatic conditions, collection and counting methods may influence the infection intensity. Two species of intestinal schistosomes, *S. indicum* and *S. spindale*, were identified morphologically which are the most common in cattle and buffaloes of Indian subcontinent. Morphological characteristics of specific schistosomes observed in this study were similar to that characteristics described by Soulsby et al., 1992. Species specific infection percentage is very important to determine the abundance of different species of *Schistosoma*. In this study, in both cattle and buffaloes, infection percentage of *S. indicum* was higher than *S. spindale* which was determined by observing female schistosome. The difference between the ratio of *S. indicum* and *S. spindale* may be due to the availability of intermediate host, climatic conditions, availability of final host and development, growth and multiplication of infective cercaria of specific schistosome. There was a limitations regarding identification of different species under microscope. The ratio was determined only based on the female parasites. It was not possible to differentiate the male of different species. Further detail study is necessary using the more appropriate and molecular techniques to identify the male of different species.

CONCLUSIONS

Schistosomes infections are harmful in cattle and buffaloes in respect of growth, development, production and reproduction. This study indicated the high infection status of intestinal schistosomes in buffaloes and cattle. The findings might be helpful to identify the risk factors regarding schistosomiosis and to determine the availability of different species of schistosomes in Bangladesh. A large scale epidemiological and molecular study is necessary to develop a strategic plan to prevent the cattle and buffaloes from the harmful effects of this economically important helminth.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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