



Prevalence and associated risk factors of *Schistosoma indicum* infection in cattle of Assam, India

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ABSTRACT

The hepato-intestinal schistosomiasis, a chronic debilitating infection caused by *Schistosoma indicum* is one of the major helminthic problems which cause considerable reduction in production and productivity, and economic losses in Assam, India. Hence their accurate diagnosis by traditional and molecular methods is the key to its management. A total of 420 faecal samples and liver and intestinal mesenteries from different slaughter houses of Assam were collected and examined during March 2015 to February 2016 to record the prevalence of visceral schistosomiasis. It was observed that prevalence rate of schistosomiasis was found to be more in mesenteric and hepatic portal vein examination (12.38%) than the faecal examination (4.52%). The 28S rRNA gene (28S) and mitochondrial cytochrome c oxidase subunit 1 (COI) gene of morphologically identified adult *S. indicum* was amplified with amplicon sizes 590 bp and 372 bp, respectively which could be used as molecular marker for diagnosis *S. indicum* infection. However, based on worm pair detection, the study showed an increasing incidence of schistosomiasis from the month of May onward reached highest peak in July and gradually decline to lowest point in April, while coprological examination showed the highest peak in August and the lowest in March. The risk factors include age, breed and management systems of the cattle. Therefore, this study emphasizes on employing both conventional and molecular techniques to detect the various risk factors of *S. indicum* infection in cattle for successful control measures.

Keywords: Assam, Cattle, Molecular diagnosis, Prevalence, Risk factors, *Schistosoma indicum*

Among the common and debilitating infectious agents of man and domestic animals, the schistosomes are one of the most important snail-borne parasites. Schistosomiasis is a common parasitic disease of animals, mainly of cattle in Asia and Africa infecting about 200 million people, especially children, and 165 million livestock (De Bont and Vercruysse 1998, Chitsulo *et al.* 2000). The schistosomes have a geographic distribution that encompasses much of the world and their charming biology could draw attention of both basic and applied biologists around the globe, 19 species of *Schistosoma* currently recognized around the world are distributed in four groups on the basis of egg morphology, geographical distribution and identity of snail intermediate hosts (Rollinson and Southgate 1987). Those are *S. mansoni* group, *S. haematobium* group, *S. japonicum* group and *S. indicum* group. Among these, *indicum* group comprising of *S. indicum*, *S. spindale*, *S. nasale* and *S. incognitum* are prevalent in the Indian subcontinent causing enormous economic losses to the

farming community of the country (Bulbul 2016, Bulbul *et al.* 2019b). The adult males of *S. indicum* have 5-12 testes and mildly tuberculated cuticle while females many oval shaped eggs with a terminal spine and contain well developed miracidium inside (Soulsby 1982). In livestock, hepato-intestinal or visceral schistosomiasis are the two major clinical syndromes seen (Islam *et al.* 2011, Kerie and Seyoum 2016). Emphasis has been given on economic importance of visceral schistosomiasis caused by *S. indicum* and *S. spindale* in domestic animals in Indian sub-continent (Sumanth 2004, Cherian and D'Souza 2009, Sudhakar *et al.* 2016). Prevalence of schistosomes was also reported from Assam (Borkakoty and Das 1980, Rajkhowa *et al.* 1992, Bulbul *et al.* 2017), but the tangible picture of the visceral schistosomiasis caused by the *S. indicum* is unrecognised.

Various conventional coprological and post-mortem examination has been employed to diagnose hepato-intestinal schistosomiasis in bovine (Ameni *et al.* 2001, Yeneneh *et al.* 2012, Gebru *et al.* 2015, Tsega and Derso 2015). These methods not only consume more time, but require a high level of experienced-based skill and the examination of eggs become difficult. To overcome these limitations, various molecular methods have been devised for their speciation, so that proper control strategies can be evolved (Johnston *et al.* 1993, Littlewood and Johnston

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1995, Barker and Blair 1996, Agatsuma *et al.* 2002). The information about the molecular diagnosis of the *indicum* group is scanty particularly in Assam. Therefore, based on faecal and slaughtered animal examinations along with employing molecular approach, present study was conducted to determine the various risk factors of epidemiology of visceral schistosomosis caused by *S. indicum* in Assam.

MATERIALS AND METHODS

Ethical permission: The study was approved by the Institutional Animal Ethics Committee (Approval No. 770/ac/CPCSEA/FVSc/AAU/IAEC/15-16/337 dated 10.04.2015). The samples were collected as per standard procedure and no animals were harmed.

Study area: The study area is located in 26°28'48.1" - 26°02'21.2" North latitude and 91°40'10.2" - 90°48'17.2" East longitude subtropical Assam, India. The study was conducted for a period of one year from March 2015 to February 2016. The calendar year was divided into four seasons as per India Meteorology Department, Borjhar, Guwahati viz. winter (December, January and February), Pre-monsoon (March, April and May), Monsoon (June, July, August and September) and Post-monsoon (October and November) to evaluate the seasonal prevalence. Most of the areas were featured by the presence of marshy lands, ponds, ditches, paddy fields, grazing lands, rivers and water pools (Bulbul *et al.* 2020a,b). The macrophytes and suitable snail intermediate hosts, *Indoplanorbis exustus* were prevalent in the water bodies of study areas. Domesticated animals, mostly the local cattle frequently graze in the areas which are the natural habitats of snails.

Animal management: The indigenous local cattle and crossbred cattle (Jersey × local cattle and Holstein Friesian × local cattle) were reared under both traditional and modern (semi-intensive) livestock farming system. The traditional management systems, cattle are often kept outdoors and grazed all day near the vicinity of the marshy lands, rivers, ponds, pools, ditches etc. These grazing lands were potential source of *Schistosoma* infection due to the frequent contact of animals to the water bodies. In semi-intensive management system, cattle are kept in-doors and partly out-door. While indoors, they are supplemented with adequate qualities of foods and fodders collected from low laying areas of the study areas and clean water.

Sample collection and parasitological procedure: A total of 420 faecal samples and intestinal mesenteries and liver before slaughtering and after slaughtering the same animals, respectively were collected and brought to the laboratory of Department of Parasitology. The collected faecal samples were examined by the Acid-Ether technique for presence of eggs as per the method of Soulsby (1982). Simultaneously, the intestinal mesenteries and portal veins were examined for the presence of flukes as described by Sumanth (2004). The collected schistosomes were first directly examined under microscope to see the oval shaped eggs with terminal spine at one end in the uterus

of female without staining and photographs were taken for identification. The effect of seasons, age and sexes of animals on prevalence of schistosomosis was also recorded. About 93, 159, 66 and 102 samples were collected in pre-monsoon, monsoon, post-monsoon and winter seasons, respectively. Similarly, 140 samples from each breed and 210 samples from each managemental system were considered to see their effect. Moreover, out of 420 faecal samples, 101, 117, 109 and 93 samples were collected from 2-<4 years, 4-<6 years, 6-<8 years and 8->8 years of age groups, respectively.

Molecular investigation: Morphologically identified adult *S. indicum* were validated through amplification of 28S RNA gene and mitochondrial COI gene by application of PCR as described by Agatsuma *et al.* (2002).

DNA extraction and PCR: The DNA was extracted from morphologically identified *S. indicum* adults collected randomly from 50 different slaughtered cattle using DNeasy Blood and Tissue Kit (Qiagen®, Germany) according to manufacturer's protocol. The 28S RNA gene (28S) and mitochondrial COI gene were amplified using PCR in a Techne-500 thermal cycler (Bibby Scientific). The PCR reaction mix (25 µl) contained 12.5 µl PCR master mix (Qiagen, Germany), 6 µl nuclease free water, 0.5 µl (1.5 unit) of taq DNA polymerase, 4 µl (150-250 ng) DNA template and 1 µl (10 pmol/µl) of each forward and reverse primer. The PCR conditions were as follows: 94°C for 1 min, 50°C for 2 min, 72°C for 3 min, for 30 cycles. Primers used for the 28S were 5'- GTA CCG TGA GGG AAA GTT G -3' (TSD2, forward direction), and 5'- GTC CGT GTT TCA AGA CGG G -3' (D4AR, reverse direction) (Littlewood and Johnston 1995), and for the COI region were 5'-TTT TTT GGG CAT CCT GAG GTT TA-3' (FH3, forward direction) and 5'-TAA AGA AAG AAC ATA ATG AAA ATA ATC-3' (FH5, reverse direction) (Bowles *et al.* 1993). The PCR products obtained after DNA amplification were subjected to gel electrophoresis in 1.5% agarose gel in TAE (1×) buffer containing Ethidium Bromide (0.5 µg/ml) for 90 min at constant voltage - (60 V) and subsequently visualized and photographed under gel documentation system (DNR Mini Lumi, Applied Bioimaging).

Statistical analysis: All the data collected throughout the investigation period were stored on Microsoft (MS) excel spread sheet program and analysis was done by SPSS Version 16 of SPSS software program. The prevalence per cent was calculated by dividing number of positive animals by total number of animals tested and multiplied by 100. The data generated for prevalence study were statistically analyzed using chi square (χ^2) test as per method of Snedecor and Cochran (1994) to determine whether risk factors were associated with disease significance or not.

RESULTS AND DISCUSSION

Prevalence of hepato-intestinal or visceral schistosomosis: The prevalence of hepato-intestinal or visceral schistosomosis was 4.52% (19/420) and 12.38%

Table 1. Prevalence of hepato-intestinal schistosomosis in cattle of Assam with respect to season, age, breed and management system

Risk factors		No. of animal examined	Positive for worm pairs	χ^2 value	No. of faecal sample examined	Positive for eggs	χ^2 value
Season	Pre-monsoon	93	6 (6.45)	8.94*	93	3 (3.23)	0.65 ^{NS}
	Monsoon	159	29 (18.24)		159	7 (4.40)	
	Post-monsoon	66	6 (9.09)		66	3 (4.55)	
	Winter	102	11 (10.78)		102	6 (5.58)	
	Total	420	52 (12.38)		420	19 (4.52)	
Age	2-<4 years	101	6 (5.94)	6.12 ^{NS}	101	2 (1.98)	5.31 ^{NS}
	4-<6 years	117	14 (11.97)		117	3 (2.56)	
	6-<8 years	109	17 (15.60)		109	8 (7.34)	
	8->8 years	93	15 (16.13)		93	6 (6.45)	
	Total	420	52 (12.38)		420	19 (4.52)	
Breed	Local	140	36 (25.71)	34.54**	140	14 (10.00)	14.58**
	Jersey $\times$ local	140	9 (6.42)		140	3 (2.14)	
	HF $\times$ local	140	7 (5.00)		140	2 (1.43)	
	Total	420	52 (12.38)		420	19 (4.52)	
Management System	Traditional	210	45 (21.43)	31.69**	210	16 (7.62)	9.32**
	Semi-intensive	210	7 (3.33)		210	3 (1.43)	
	Total	420	52 (12.38)		420	19 (4.52)	

Figures in the parenthesis indicate percentage, *Significant at $p < 0.05$, **Significant at $p < 0.01$, NS- Non-significant.

(52/420) by faecal and liver/intestine of slaughtered animals examination (Table 1). The schistosomes were identified as *S. indicum* by observing the rough cuticle in male and oval shaped eggs with terminal spine in one end in the uterus of female. The ventral sucker was also observed. The infection rate of the diseases was found to be more in mesenteric and hepatic portal vein examination than the faecal examination methods.

The mesenteric worm count along with liver pressed methods revealed more infection rate as compared to faecal examination which was also observed by Lakshmanan *et al.* (2011) in Kerala and Sudhakar *et al.* (2016) in Hyderabad, India. In Ethiopia, Solomon (2008) recorded bovine schistosomosis caused by *Schistosoma bovis* in 10% copro and 27% post mortem examination and Melkamu (2016) reported 12% in coproscopic and 22% in post mortem findings like our findings. Hence, the present study established the fact that the animals harbouring parasites may not show positivity in coprological examination. This can be attributed to the fact that the eggs might get trapped in granulomas in different organs and not getting avenues to come out in the excreta. On the other hand, old cattle may have developed stronger acquired immunity which suppresses worm fecundity and the release of parasitic eggs in faeces (Bushara *et al.* 1982). A number of workers recorded *S. indicum* infection based on mesenteric vein examination as well as faecal examination in India and abroad viz. Agrawal *et al.* (2003) in Odisha (63.30%), Cherian & D' Souza (2009) in Karnataka (schistosomosis 6.8%; where majority of the cases was of *S. indicum* infection with prevalence rate of 93.1%), Sudhakar *et al.* (2016) in Telangana (visceral schistosomosis 28.70%). Moreover, Islam *et al.* (2011) recorded 42.50% infection rate of visceral schistosomosis in cattle of Bangladesh

(42.50%) while Aylate *et al.* (2017) in Ethiopia recorded 18.5% bovine schistosomosis. According to Bulbul *et al.* (2019a) the variations in prevalence of schistosomosis in various geographical locations throughout the world may be attributed to sample sizes, sampling periods, techniques used to diagnose the diseases, climatic condition exists in diverse geographical region, epidemiological factors, availability of the stagnant water body, marshy land, drainage system for irrigation practice etc., availability of the snail intermediate hosts and macrophytes and limnological properties in snail habitats and genetic variability of intermediate hosts and management system of the study areas. Thus worm pair detection in mesenteric worm count methods along with liver pressed methods is more specific as compared to faecal examination.

Molecular prevalence: The study revealed that out of 50 morphologically identified *S. indicum* isolates, all the isolates (100%) were amplified using COI and 28S regions of mitochondrial and rDNA respectively. The COI and 28S gene sequences of *S. indicum* were found to be amplified at 372 bp and 590 bp, respectively but no amplicon was amplified in control negative samples. The present findings resembled to that of Agatsuma *et al.* (2002), Morgan *et al.* (2002) and Devkota *et al.* (2015). Our molecular investigation supported the finding that the prevalence recorded based on faecal and slaughtered animal examination was due to *S. indicum* only.

Season-wise prevalence: The seasonal prevalence of *S. indicum* infections was found to be higher in monsoon season as compared to other seasons. The highest prevalence of the disease was recorded in the monsoon season (18.24%) based on adult worm pair detection while winter season showed the highest prevalence (5.88%) based copro examination. The χ^2 test revealed significant difference

($p < 0.05$) between adult parasite prevalence and seasons only (Table 1). The hepato-intestinal schistosomosis based on worm pairs detecting showed an increasing trend from the month of May onward reached highest peak in July and gradually declined to lowest point in April, while coprological examination showed the highest peak of it in August and the lowest in March. The highest prevalence of schistosomosis was recorded in the month of July (24.32%) and November (9.38%) based on worm pair detection and faecal examination, respectively.

The recorded higher prevalence of visceral schistosomosis in monsoon season is comparable with Jeyathilakan *et al.* (2008) who found highest prevalence in monsoon season (33.00%) and lowest in summer season (27.70%) in cattle of Chennai; Bedarkar *et al.* (2000) and Islam *et al.* (2011) who observed highest and lowest prevalence in monsoon and summer season, respectively. The highest infection rate observed in monsoon season might be due to abundance of *I. exustus* snails and their rapid multiplication and dispersion. The rainy season is suitable for the survival *I. exustus* snails (Bulbul *et al.* 2020a, Bulbul *et al.* 2020b) and the parasite itself (Bedarkar *et al.* 2000). Furthermore, rain splashes might help in dispersion of faecal matter containing eggs and miracidia to the snail habitats. These factors enhance the infection of snails by miracidia and cercarial contamination to adjacent areas through water (Bulbul *et al.* 2019a). Moreover, lands of the respective areas favour the propagation and multiplication of the snails and these snails become heavily infected with larval stages of schistosomes. The higher prevalence rate of schistosomosis caused by *S. spindale* in cattle of Assam were recorded in the month of monsoon season by Bulbul *et al.* (2019a); Khajuria and Kapoor (2003) in goats of Punjab. The recorded lower prevalence in pre-monsoon season might be attributed to harsh dry conditions and less chances of infection due to unavailability of snail intermediate hosts as the water sources were scarce in winter months.

Age-wise prevalence: The age-wise prevalence was found to be highest in cattle of 8 and above years of age (16.13%) followed by 6 to <8 years (15.60%), 4 to <6 years (11.97%) and 2 to <4 years (5.94%) while examining mesenteric and portal veins. Similarly it was 1.98, 2.56, 7.34 and 6.45% in 2-<4 years of age, 4-<6 years of age, 6-<8 years of age and 8 to > 8 years of age, respectively by faecal examination. These were followed by the different age groups ($p > 0.05$, NS). Faecal examination as well as mesenteric vein examination by Islam *et al.* (2011), Tsega & Derso (2015), Melkamu (2016) and Yihunie *et al.* (2019) also reported higher infection rate in older animals as compare to younger animals. Higher infection in aged animals might be accredited to exposure to cercariae for a longer duration of time and the parasites get accumulated for years as because older animals move long distances in search of scarce pastures and water thereby increasing their chances of getting infection (Bulbul *et al.* (2019a)). Moreover, the young animals do not graze for prolonged

period, so they are likely to acquire a less infection. In abattoir survey, cattle aged 4 years and older showed the higher risk for schistosomosis (Kouadio *et al.* 2020). Lower risk of visceral schistosomosis in younger animals could be accredited to husbandry practices in operation which reduce risk of environmental exposures.

Sex-wise prevalence: It could not be evaluated due to non slaughtering of female animals at slaughtered houses. In farm animals, male cattle had higher infection with *Schistosoma* (Kouadio *et al.* 2020) than females which was not found in the present study. The productive cows were not allowed to be slaughtered hence data were not found for females to compare with males.

Breed-wise prevalence: The prevalence of hepato-intestinal schistosomosis was found to be higher in local cattle as compare to crossbred cattle both in slaughter animals and faecal samples examination (Table 1). There was a significant difference in worm pair detection ($p < 0.01$) and faecal sample examination ($p < 0.01$) among the breeds.

Our findings are in the tone with Yeneneh *et al.* (2012), Gebru *et al.* (2015), Melkamu (2016) and Yihunie *et al.* (2019) who recorded higher prevalence in local cattle as compare to crossbred cattle. But contradictory to the findings of Ameni *et al.* (2001), Solomon (2008), Tsega & Derso (2015) who reported prevalence of bovine schistosomosis higher in crossbred cattle than that of local cattle in Ethiopia. Variations of prevalence rate among local cattle and Jersey-Local and Holstein Frisian-Local crossbred cattle are possibly due to differences in animal husbandry practices as cross breed are kept under semi-intensive or intensive management system and supplemented with good feed and clean water. Therefore, further investigations are required to rule out potential genetic basis of predisposition to hepato-intestinal schistosomosis in Assam.

Management system-wise prevalence: On the basis of worm pair detection the prevalence of the disease was found higher in the traditional free-range management system (21.43%) than semi-intensive management system (3.33%). However there was significant difference ($\chi^2 = 31.54$; $p < 0.01$) between management systems. On the other hand faecal samples examination revealed the significantly ($\chi^2 = 9.32$; $p < 0.01$) higher prevalence rate in cattle reared in traditional system as compare to semi-intensive system. Similar findings were also reported by Melkamu (2016) from Ethiopia. The reasons for high prevalence in extensive cattle may be due to these animals were kept out door. Schistosomes are basically transmitted by skin penetration, hence in semi-intensive cattle rearing system less chance of contact with furcocercous cercariae of *S. indicum* as compare to animals rearing in traditional system.

Based on the study, it can be concluded that the hepato-intestinal schistosomosis in cattle in north-eastern sub-tropical region of the country is under diagnosed. Hence, both conventional and molecular techniques should be employed to screen the disease for its effective control. The prevalence rate was significantly associated with the

season, age, breed and management system of animals. The study also suggests visceral schistosomiasis due to *S. indicum* is one of the important endemic diseases of cattle that deserve serious attention. The findings of the present study will be helpful in making a strategy for the control of visceral schistosomiasis in cattle of the studied region of the country to prevent economic loss. Hence a well-planned deworming schedule shall be prepared based on this epidemiological data to decrease the exposure rate of animals to *Schistosoma* and the impact of schistosome infection in the area. Moreover, further studies on the epizootiology of *S. indicum* in organized and non-organized farm animals based on ecology, biology, bionomics of *I. exustus* is warranted. In addition, limnology of aquatic environment and cercarial bionomics in snail intermediate hosts, molecular detection of genetic variability in snails also need to be demeanour for better thoughtful of *S. indicum* and disease transmission dynamic in Assam.

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