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Prevalence and Risk Factors of *Strongyloides Stercoralis* Infection in Selected Tea Garden of Sylhet, Bangladesh

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Abstract

Background: Strongyloidiasis infection is partially an asymptomatic infection and the diagnosis of patent infection is difficult using conventional parasitological methods. The residents of tea garden community of Sylhet, Bangladesh were robustly tested.

Method: The collected stool samples were tested by *Harada mori* culture for the presence of larval stage of Strongyloides stercoralis and to reaffirm the same samples were subjected for conventional PCR, using primer sets deigned to amplify partial ribosomal DNA of S. stercoralis genome. Finally data analysis was performed by Logistic Regression procedure using STATA 13 (College Station, Texas 77845 USA) and Pearson λ^2 test, with consideration of $P < 0.05$ as an indication of significant.

Result: A total of 300 stool samples freshly collected and examined among those 18 (06.00%) samples were found positive for S. stercoralis in *Harada mori* culture. In amplification of DNA extracted from raw samples and culture fluid of positive sample, the conventional PCR detected S. stercoralis 38 (12.67%) positive. There were 6 samples positive in *Harada mori* culture but did not show any response in sophisticated PCR techniques it might be due to low burden of infection. Periodic anthelmintic does not taking OR= 3.946(95% CI 1.369-11.375; P=0.011) and does not wash feet coming from out OR= 5.158(95% CI 1.656-16.068; P=0.005) significantly associated with Strongyloidiases infection.

Conclusion: This study confirmed that S. stercoralis is prevalent in the tea garden community of Sylhet identified by both parasitological and molecular methods. The preventive measures by deworming are warranted. Public health education regarding properly periodic anthelmintic taking, wash feet coming from out and personal hygiene are also additional required elements.

Keywords: *Strongyloides stercoralis*; Prevalence; Risk factors; *Harada mori* culture; PCR

Introduction

The threadworm *Strongyloides stercoralis* is a common intestinal nematode affects 30-100 million people worldwide [1,2]. S. stercoralis is only parasite of soil transmitted helminths (STH) group which can cause auto infection and thus ultimately lead to high parasite intensity specifically in immune-compromised individuals [3-5]. Strongyloidiasis is endemic in areas where sanitation conditions are poor and where the milieu is warm and humid [6] such as Asia, Africa, Southeast Asia, Bangladesh, Central and South America [7-9]. Coprological and recent serological serological studies, in slum areas of Dhaka, ensured its continued existence in Bangladesh [6,10]. Severe complication with clumsy infection of strongyloidiasis may lead to substantial mortality as high as 87% [8]. Paucity of information is available on prevalence of *S. stercoralis* infection on most of these setting [11]. Confirmation of Strongyloides infection by coprological examination is difficult because of irregular excretion of the parasite especially in chronic cases; prevalence of infection thus underestimated [9,12]. Widely used diagnostic procedures, such as direct fecal smear, Baermann technique and Koga agar plate are not satisfactory when used in single stool samples [13,14]. The detection of larvae of Stgongyloides in the stool is an evidence of infection [8]. The diagnostic methods such as direct fecal smear, *Harada mori* culture [15,16] have been used to detect larvae in stool but the exact sensitivity of these diagnostic approaches is debated [17]. Most of the infections may remain asymptomatic [18-20] but diarrhea and abdominal pain are the most common symptoms [21,22]. The common dermatological aspects of chronic strongyloidiasis are itching and rash [23]. There is scarcity of information on *S. stercoralis* infection in rural setting of tea garden community though few work available in Dhaka slum [10, 24]. The objectives of this study conducted in tea garden community of Sylhet to ascertain the prevalence and plausible determinants for strongyloidiasis including socio demographic and household factors based on *Harada mori* culture and molecular techniques.

Materials and Methods

Study area

The study areas of Sylhet district located 315 km south east from capital city Dhaka were selected for this study as it is the poorest area of Bangladesh. Sylhet is located at 24.8917°N and 91.8833°E. It has 86074 units of house hold and total area 323.17 km^2 [25]. This district is occupied by high proportion of ethnic minorities, stingy household condition, and poor road condition, no prohibition for preventive and curative measures.

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Ethical Consideration

Before commencement of the study, ethical clearance was obtained from the SAU Ethical Review Board. A consent form was provided to each study subject together with stool containers a day before the day of data collection. Parents were asked to sign the consent forms if they agreed on their children to be involved in the study.

Data collection

The study was conducted for a period of 12 months starting from June 2014 to May 2015. Before enrollment of the participants the verbal consent of the parents or legal guardians was taken. Data were collected by following structured questionnaire approved by ICDDR'B, Dhaka, Bangladesh.

Collection of stool sample

Supply and collection of stool pot: During each phase of study appropriately labeled plastic stool container for the collection of stool specimen was provided to the parents. The label of the stool pot had the subject's name, date of sample collection, identification number and the name of the areas. They were instructed on how to collect and put stool in the container at the toilet. The next day morning the stool pot collected directly from the participant's guardians with making proper questionnaire. The specimens were packed in a cool box with ice packs and transported by a vehicle to the Parasitology Laboratory, Sylhet Agricultural University, Faculty of Veterinary and Animal Science, Sylhet, Bangladesh and for molecular detection sent to parasitology laboratory of International Center for Diarrhoeal Disease Research Bangladesh (ICDDR'B), Dhaka, Bangladesh.

Analysis of stool specimens

Direct smear method and *Harada mori* culture: The stool samples collected from each participant were examined direct saline smear for the presence of parasitic eggs. The every stool sample were cultured at 25-28°C in incubator for ten days and examined from five days onward up to ten days to evaluate the presence of larvae in culture fluid [26]. Any sample shows positive either in direct smear or *Harada mori* culture was considered as positive sample. The extracted DNA from filariform larvae was used as control DNA for molecular analysis.

Extraction of genomic DNA

For the isolation of DNA from positive culture water, QIAGEN DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) was used. The culture positive water was vortexed and centrifuged then diluted by filtered PBS. 200 µl of sample was taken into 1.5 ml micro-centrifuge tubes and 20 µl Proteinase K was added. The tubes were vortexed by kept for incubation for 10mins at room temperature. 200 µl of buffer ATL was added to the tubes and mixed well. The samples were incubated in a water bath at 56°C for 1 hour. 200 µl of 100% Et-OH was added to the tubes. The samples were then transferred to the spin columns. The spin columns were centrifuged at 8,000 rpm for 1 min. Collection tubes were changed. 500 µl of AW1 buffer was added to the columns. Again centrifuged at 8,000 rpm for 1 min. The liquid was removed from the collection tubes and placed back. 500 µl of AW2 buffer was added to the columns. Centrifuged at 14,000 rpm for 3 min. The columns were placed in a fresh micro-centrifuge tube. 60 µl of AE buffer (elution) was added. Centrifuged at 14,000 rpm for 2 min. The columns were discarded and the DNA was ready.

Conventional PCR

Purified DNA template was used for amplification in a DNA thermal cycler using a species specific primer set as described by [27]. Positive and negative controls were systematically incorporated in each PCR run. Forward (SSF: 5´ATC GTG TCG GTG GAT CAT TC 3´) and reverse (SSR: 5´CTA TTA GCG CCA TTT GCA TTC 3´) primer pair was used and target 114bp gene. PCR reactions were performed using the following reaction mixture. 2 µl of purified DNA template was used for the PCR with 3 µl of each primer, 2.5 µl of 10X Taq buffer (New England Biolabs Inc.), 0.5 µl of 10 mM dNTPs (GENE Mate), 2 µl of 25 µM $MgCl_2$ and 0.2µl of Taq DNA Polymerase (New England Biolabs Inc.) In total volume of 25 µl reaction mixture. The cycle conditions for the PCR (40 cycles) step with an initial denaturation period of 5 min at 95°C were: denaturation at 95°C for 40 sec, annealing at 50°C for 40 sec, extension at 68°C for 20 sec and final extension for 8 min to insure that all product were full-length. The amplified PCR products were analyzed immediately by electrophoresis on a 2.0% agarose gel (Sigma -Aldrich Inc., USA).

Characteristics	Frequency	Total no. of sample	Percentage
Participants Schooling		300	
Primary	104		34.67
Secondary	16		05.33
Not applicable	180		60.00
Mother's schooling		300	
Primary	63		21.00
Secondary	03		01.00
Not applicable	234		78.00
Father's schooling		300	
Primary	86		28.67
Secondary	72		24.00
Not applicable	142		47.33

Table 1: Educational status of the participants and parents in tea garden community.

Level of occupation	Frequency	Total no. of sample	Percentage
Father's occupation		300	
Unemployed	11		03.67
Day laborer	182		60.67
Service	80		26.67
Tea garden worker	9		03.00
Other	18		06.00
Mother's occupation		300	
Housewife	167		53.33
Service	1		00.33
Business	132		44.00

Table 2: Occupational status of the parents in tea garden community.

Electrophoresis

The amplified PCR products were analyzed immediately by electrophoresis on a 2.0% agarose gel (Sigma -Aldrich Inc., USA). The gel was stained with ethidium-bromide and visualized under UV trans-illumination (GelDoc®, Biorad, USA). The sizes of the PCR products were estimated using 100 base pairs (bp) DNA ladder marker (Sigma-Aldrich Inc., USA).

Statistical analysis

Statistical analysis was performed by Logistic Regression procedure using STATA 13 (College Station, Texas 77845 USA) and the level of significance was considered as $P<0.05$. In the univariable analysis, λ^2 test done for risk factors associated with infection status. In the multivariable logistic regressions, variables with a P-value of ≤0.20 in

Other demographic information	Frequency	Total no. of sample	Percentage
Age group		300	
0-10	242		80.67
11-20	19		06.33
21-30	13		04.33
31-40	12		04.00
41-up	14		04.67
Sex group		300	
Male	177		59.00
Female	123		41.00
Name of the areas		300	
Khadim tea estate	47		15.67
Burjan tea estate	79		26.33
Lakkatora tea estate	65		21.00
Malnichara tea esstate	63		21.67
Daldali tea estate	46		15.33
Category of weight (kg)		300	
0-20	186		62.00
21-40	65		21.67
41-up	49		16.33
Income of house		300	
≤5000	103		34.33
>5000	197		65.67
No of People living in house		300	
≤4	145		48.33
≥5	155		51.67
Name of the season		300	
Rainy	65		21.67
Winter	116		38.67
Summer	119		39.67

Table 3: Other demographic information of tea garden community.

the univariable analysis were included as predictors. ORs and 95% CI were reported. P-values <0.05 were regarded as significant.

Results

Study participants

Of 300 participants 59.00% male and 41.00% female were enrolled in this study out of five tea garden areas. Only 40.00% of the participants had primary education whereas majority of the participants 60.00% had not received primary education. The primary education completed possesses 10.56% (95% CI 06.476-15.992) infection and illiterate participant's 15.83% (95% CI 09.8102-23.616) infection.

Prevalence of *S. stercoralis* infection

Of 300 tested sample only 38 cases found positive with S. stercoralis infection. The prevalence of strongyloidiasis is decreasing with the increase of ages though there is increase in the age group 21-30 years. From all ages group prevalence was higher in female 13.82% (95% CI 08.262-21.204) than male 11.86% (95% CI 07.496-17.562) with S. stercoralis infection.

Foot ware is a factor for S. stercoralis infection because having no foot ware showing higher prevalence than having foot ware. The prevalence is higher in winter season 18.97% (95% CI 12.283-27.293) than rainy 15.38% (95% CI 07.632-26.478) and summer 05.04% (95% CI 01.872-10.651). The crowd house have higher rate of infection than small family (Tables 1- 4).

Overall, in examination by conventional PCR, 38 out of 300 stool samples were found positive for S. stercoralis where by *Harada mori* culture for the same samples the detected positive cases was 18 (Table 5).

Points	Total no. of tested	Total no. of positive	Prevalence (95% confidence Intervals)
Season			
Rainy	65	10	15.38 (07.632-26.478)
Winter	116	22	18.97 (12.283-27.293)
Summer	119	06	05.04 (01.872-10.651)
Age			
≤10	242	32	13.22 (09.223-18.151)
11-20	19	01	05.26 (00.133-26.028)
21-30	13	02	15.38 (01.920-45.447)
31-40	12	01	08.33 (00.210-38.479)
≥41	14	02	14.29 (01.779-42.812)
Areas			
Khadim tea estate	47	12	25.53 (13.944-40.349)
Burjan tea estate	79	13	16.46 (09.063-26.494)
Lakkatora tea estate	65	05	07.69 (02.544-17.045)
Malnichara tea estate	63	08	12.69 (05.645-23.496)
Daldali tea estate	46	-	-
Sex			
Male	177	21	11.86 (07.496-17.562)
Female	123	17	13.82 (08.262-21.204)
People in house			
≤4	127	18	14.17 (08.621-21.471)
≥5	135	20	14.81 (09.290-21.948)
Have foot ware			
No	05	02	40.00 (05.274-85.336)
Yes	257	36	14.01 (10.007-18.862)
Work in bare foot			
No	48	07	14.58 (06.070-27.764)
Yes	214	31	14.49 (10.059-19.927)

Table 4: Prevalence of Strongyloides stercoralis infection in tea garden community.

Risk factor assessment for *S. stercoralis* infection

The study participants corresponding data were analyzed by λ^2 test for univariable analysis and found several factors significantly associated with strongyloidiasis infection. The climatic factors such as season (P=0.004) and areas (P=0.003) were significantly associated.

Other factors such as periodic anthelmintic therapy, monthly family income, rubbing hand after toilet, toilet floor and using shoes in toilet are contributing tools for strongyloidiasis infection (Table 6). On the other hand sex, fathers occupation, household floor, disposal of stool, use of disinfectant cleaning toilet, possession of foot ware and working in bare foot are not significantly associated with Strongyloidiasis infection (Table 7).

Discussion

Of three hundred stool samples of tea garden community of

Name of the test		*Harada mori* Culture		Total
		Positive	Negative	
Conventional PCR	Positive	12	26	38
	Negative	6	256	262
Total		18	282	300

Pearson chi (λ^2) test done and P<0.001

Table 5: Comparison of the results of conventional PCR and *Harada mori* culture examinations for detection of Strongyloides stercoralis infection in single stool samples.

Characteristics	Positive n=38 (%)	Negative n=262 (%)	P-value
Season			
Rainy	10 (15.38)	55 (84.62)	
Winter	22 (18.97)	94 (81.03)	
Summer	06 (05.04)	113 (94.96)	0.004
Areas			
Khadim tea estate	12 (25.53)	35 (74.47)	
Burjan tea estate	13 (16.46)	66 (83.54)	
Lakkatora tea estate	05 (07.69)	60 (92.31)	
Malnichara tea esstate	08 (12.70)	55 (87.30)	
Daldali tea estate	0.00	46 (100.0)	0.003
Sex			
Male	21 (11.86)	156 (88.14)	
Female	17 (13.82)	106 (86.18)	0.616 (NS)
Weight			
<10	21 (11.29)	165 (88.71)	
11-20	11 (16.92)	54 (83.08)	
>21	06 (12.24)	43 (87.76)	0.449 (NS)
Participant's schooling			
Not applicable	19 (15.83)	101 (84.17)	
Primary	19 (10.56)	161 (89.44)	0.050
Father's occupation			
Unemployed	02 (18.18)	09 (81.82)	
Day laborer	24 (13.19)	158 (86.81)	
Service	05 (06.25)	75 (93.75)	
Tea garden worker	02 (22.22)	07 (77.78)	
Other	05 (27.78)	13 (72.22)	0.100 (NS)
How many time pass stool			
Two	22 (26.83)	60 (73.17)	
One	16 (07.34)	202 (92.66)	<0.001***
Monthly family income			
≤5000	19 (18.45)	84 (81.55)	
>5000	19 (09.64)	178 (90.36)	0.030
Receive treatment			
No	26 (20.80)	99 (79.20)	
Yes	12 (06.86)	163 (93.14)	<0.001***
Treatment 4 month interval			
No	34 (19.43)	141 (80.57)	
Yes	04 (03.20)	121 (96.80)	<0.001***
Household floor			
Mud	38 (13.82)	237 (86.18)	
Semi-cemented	0.00	24 (100.0)	
Cemented	0.00	01 (100.0)	0.138 (NS)
Toilet floor			
Mud	20 (09.95)	181 (90.05)	
Bamboo	18 (18.18)	81 (81.82)	0.044
Where dispose stool			
Around house	24 (11.37)	187 (88.63)	
Jungle/Tea garden	14 (15.73)	75 (84.27)	0.300 (NS)
Rub hand after toilet			
No	28 (10.94)	228 (89.06)	
Yes	10 (22.73)	34 (77.27)	0.030
Material for rubbing hand			
Soap	01 (25.00)	03 (75.00)	
Ash	12 (19.35)	50 (80.65)	
Soil	15 (07.89)	175 (92.11)	
other	10 (22.73)	34 (77.27)	0.012
Use disinfectant toilet cleaning			
No	13 (56.76)	61 (43.24)	
Yes	29 (36.73)	197 (63.27)	0.308 (NS)

Type of material washing hand			
Soil	13 (22.41)	35 (74.47)	
Ash	13 (06.67)	182 (93.33)	
Soap	12 (25.53)	45 (77.59)	<0.001***
Wash feet using toilet			
Never	30 (14.25)	182 (85.85)	
Not always	04 (05.13)	74 (94.87)	
Always	04 (40.00)	06 (60.00)	0.004
Have foot ware			
No	02 (28.57)	05 (71.43)	
Yes	36 (12.29)	257 (87.71)	0.200 (NS)
Use shoe going to school			
Never	22 (10.58)	186 (89.42)	
Rarely	12 (27.27)	32 (72.73)	
Most time	04 (08.33)	44 (91.67)	0.005
Work in bare foot			
No	07 (12.73)	48 (87.27)	
yes	31 (12.65)	214 (87.35)	0.988 (NS)
Wash feet coming from out			
Not always	22 (17.19)	106 (82.81)	
Always	16 (09.30)	156 (90.70)	0.042

*** Highly significant (P<0.05), NS= Not significant

Table 6: Invariable analysis of the factors associated with Strongyloides stercoralis infection in tea garden community of Sylhet.

Name of the variable	Odds ratio (95% Confidence Intervals)	P-value
Season		
Rainy	3.424 (1.183-9.905)	0.023
Winter	4.407 (1.716-11.320)	
Summer	1	
Participants Schooling		
Not applicable	2.923 (1.096-7.793)	0.032
Primary	1	
Income		
≤5000	2.540 (0.970-6.649)	0.051
≥5000	1	
Receive anthelmintics		
No	3.946 (1.369-11.375)	0.011
Yes	1	
Receive anthelmintic 4 month interval		
No	3.812 (1.079-13.464)	0.038
Yes	1	
Toilet floor		
Mud	2.710 (1.035-7.101)	0.042
Bamboo	1	
How many time pass stool a day		
Two	3.645 (1.385-9.593)	0.009
One	1	
Wash feet using toilet		
Never	12.33 (2.449-62.100)	0.043
Not always	3.049 (1.038-8.959)	
Always	1	
Type of material washing hand		

Soil	3.431 (1.422-8.276)	0.006
Ash	02.80 (1.231-6.367)	
Soap	1	
Going to School		
Never	5.556 (1.959-15.753)	<0.001***
Rarely	5.000 (0.937-26.683)	
Most time	1	
Wash feet coming from out		
Not always	5.158 (1.656-16.068)	0.005
Always	1	

*** Highly significant (P<0.05), OR= Odds Ratio, CI= Confidence Interval

Table 7: Multivariable analysis of Strongyloides stercoralis infection in Tea garden community of Sylhet.

Sylhet were screened by *Harada mori* Culture and Conventional PCR found prevalence of *S. stercoralis* infection by copro culture 06.00% (95% CI 03.83-09.28) and conventional PCR 12.67% (95% CI 09.37-16.91) which is almost double than copro culture. These reports have coherence with [28, 29] but contracted with recent study in Dhaka City [10]. The difference of the infection rate is also statistically significant. The previous reports from Thailand revealed that the prevalence of S. stercoralis infection varied widely and ranging between 07.60% and 30.30% [10,30,31]. This is the first time study on Strongyloidiases in tea garden community of Sylhet and there is paucity of information about it. The present study showed female (13.82%) participants have higher prevalence of *S. stercoralis* infection than male (11.86%) which is also supported by [32]. The winter season disclosed highest percentage of infection whereas rainy stood second and summer stood lowest infection. This variation of infection might be due to the environmental factors stimulating development of the parasitic larvae. The Khadimnagar Tea garden's hygienic condition was very poor compared to other tea garden and its parasitic load were highest but one novel findings of our study is Daladali tea garden is free from *S. stercoralis* infection.

The elderly persons had higher prevalence than the young participants in our study which is supported by another study in Cambodia [11].

The risk factors of strongyloidiasis increased with illiteracy OR= 2.923(95% CI 1.096-7.793, P= 0.032) which is similar to the finding of [32] in Ethiopia. The climatic factor such as rainy season OR= 3.424(95% CI 1.183-9.905, P= 0.023) associated with infection than other season. Periodic anthelmintic taking reduces the infection rate but when break in the chemotherapy of four month interval occurs then high rate of infection OR= 3.812(95% CI 1.079-13.464, P= 0.038) revealed. The family income significantly associated with Strongyloidiases infection because the low income family gets more infection owing to their poor family status and unable to keep them in hygienic condition. One study in elderly of Brazil Showed significantly association of family income with Strongyloidises infection [33].

Personal hygiene wash feet using toilet, coming from out significantly associated with Strongyloidiasis infection. Types of material for washing hand significantly contribute to the infection, use of soil 3.431 fold (1.422-8.276), use of ash 2.8 fold (1.231-6.367) risk for S. stercoralis infection. Muddy toilet floor OR= 2.710(95% CI 1.035-7.101, P= 0.042) contribute times higher strongyloidiases than Bamboo floor because the muddy floor provides suitable condition for the development of the infective larvae. When these infective larvae get contact with the intact skin then penetrate the skin initiate its infection in the host body. Washing practices of feet coming from out was important factors for Strongyloidases infection, the participants who did not washed feet get infection OR= 5.158(95% CI 1.656-16.068, P=0.005.

The lack of correlation between copro-culture and Molecular techniques for detection of S. stercoralis infection in our study population might indicate that the two methods are detecting different percentages of infected individuals. Those who were not detected positive by copro-culture showed positive in molecular techniques. There were 18 samples positive in copro-culture and 38 samples were positive in molecular techniques. Nevertheless, our study shows that S. stercoralis infection remains prevalent in Bangladesh particularly in tea garden community.

Conclusion

We can conclude that S. stercoralis infection was highly prevalent in the tea garden community of Sylhet and it does not depend on whether the individual was institutionalized or not. Therefore early diagnosis through specific methods is warranted in asymptomatic elderly in order to prevent the risk of hyper-infection or disseminated infection, thus avoiding high mortality.

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