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 designed 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 $\chi^2$ 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 Strongyloidiasis 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 Strongyloidiasis 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 Strongyloides 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 km² [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 filiform 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 MgCl₂ 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 2 min 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).

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, λ² test done for risk factors associated with infection status. In the multivariable logistic regressions, variables with a P-value of ≤0.20 in statistical analysis were included in the final logistic model.

Table 1:

| Characteristics                  | Frequency | Total no. of sample | Percentage |
|----------------------------------|-----------|---------------------|------------|
| Participants Schooling           |           |                     |            |
| Primary                          | 104       | 300                 | 34.67      |
| Secondary                        | 16        | 300                 | 05.33      |
| Not applicable                   | 180       | 300                 | 60.00      |
| Mother’s schooling               |           |                     |            |
| Primary                          | 63        | 300                 | 21.00      |
| Secondary                        | 66        | 300                 | 01.00      |
| Not applicable                   | 234       | 300                 | 78.00      |
| Father’s schooling               |           |                     |            |
| Primary                          | 86        | 300                 | 28.67      |
| Secondary                        | 72        | 300                 | 24.00      |
| Not applicable                   | 142       | 300                 | 47.33      |

Table 2:

| Level of occupation             | Frequency | Total no. of sample | Percentage |
|---------------------------------|-----------|---------------------|------------|
| Father’s occupation             |           | 300                 |            |
| Unemployed                      | 11        | 300                 | 03.67      |
| Day laborer                     | 182       | 300                 | 60.67      |
| Service                         | 80        | 300                 | 26.67      |
| Tea garden worker               | 9         | 300                 | 03.00      |
| Other                           | 18        | 300                 | 06.00      |
| Mother’s occupation             |           | 300                 |            |
| Housewife                       | 167       | 300                 | 53.33      |
| Service                         | 1         | 300                 | 00.33      |
| Business                        | 132       | 300                 | 44.00      |
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 possessed 10.56% (95% CI 06.476-15.992) infection and illiterate had not received primary education. The primary education completed had primary education whereas majority of the participants 60.00% infection. The prevalence of strongyloidiasis is decreasing with the increase of ages though there is increase in the age group 21-30 years. Of three hundred stool samples of tea garden community of 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 5).

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).

Risk factor assessment for *S. stercoralis* infection

The study participants corresponding data were analyzed by λ² 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 | 12                  | 26     | 38    |
|                  | 6                   | 256    | 262   |
| Total            | 18                  | 282    | 300   |

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   | Negative   | P-value |
|---------------------------------|------------|------------|---------|
|                                 | n=38 (%)   | n=262 (%)  |         |
| **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) |         |
| Mahichara tea estate            | 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) |
### 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 (1.038-8.059)                  |         |
| Always               | 1                                    |         |
| **Type of material washing hand** |                                  |         |

**Highly significant (P<0.05), NS= Not significant**
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 Strongyloidiasis 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 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 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, 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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### Table 7: Multivariable analysis of *Strongyloides stercoralis* infection in Tea garden community of Sylhet.

|                | Odds Ratio (95% CI) | P-value |
|----------------|--------------------|---------|
| Soil           | 3.431 (1.422-8.276) | 0.006   |
| Ash            | 0.80 (1.231-6.367)  |         |
| Soap           | 3.431 (1.422-8.276) | 0.006   |

** Going to School **

|                | Odds Ratio (95% CI) | P-value |
|----------------|--------------------|---------|
| Never          | 5.566 (1.959-15.753) | <0.001 ** |
| Rarely         | 5.000 (0.937-26.683) |         |
| Most time      | 3.424 (1.096-7.793)  | 0.032   |

** Wash feet coming from out **

|                | Odds Ratio (95% CI) | P-value |
|----------------|--------------------|---------|
| Not always     | 5.158 (1.656-16.068) | 0.005   |
| Always         | 1                  |         |

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

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