



## Original Research Article

# A STUDY ON THE PREVALENCE OF SOIL TRANSMITTED HELMINTHES AND MOLECULAR ANALYSIS OF FECAL SAMPLES AMONG CHILDREN OF AGE GROUP 5-10 YEARS IN THE RURAL AREA OF CHENGALPATTU

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### ABSTRACT

**Background:** Soil-transmitted helminths (STH) infections are a neglected tropical disease affecting two billion people worldwide. **Purpose:** To identify the prevalence of Soil Transmitted helminthes among children living in remote and poverty-stricken areas with poor sanitation and low education.

**Materials and Methods:** we used the wet mount method, saline and iodine, Formol ether concentration technique, Kato-Katz method, and Multiplex PCR to identify STH.

**Results:** In this cross-sectional study, out of 350 stool samples collected from children aged 5-10 years in the villages of Chengalpattu District, 58(16.57%) were positive for STH infections. Out of the total positives, 51.7% of the STH infections were in the age group 6- 7 years. In this study, the male-to-female ratio was 2:1.4. Out of the total STH-affected children, 60.34% were found to be females. The prevalence rate of STH infection is estimated to be 16.57 % by Kato-Katz and Multiplex PCR methods. Among the Individual STH infections, Hookworm was the most common STH (10.87%), followed by Ascaris lumbricoides & Hookworm coinfection (2.57%), A. lumbricoides (1.71%), and Trichuris trichiura (1.4%) co-infection out of 350 samples.

**Conclusions:** On comparison of various methods, the sensitivity of the a) Wet mount method was 62.24% (95% CI 53.66-78.99%). b) Formal-ether concentration method was 82.76% (95% CI 70.57-91.41%), and c) Multiplex PCR assay was 100% (95% CI 98.74-100.0%) in comparison with the Kato-Katz method out of the 350 samples analyzed. Compared with other methods, the Kato-Katz method and the Multiplex PCR assay showed better and equal detection of soil-transmitted Infections with a significant P value (>0.0001).

**Keywords:** Soil Transmitted Helminthes(STH), Hookworm, Kato Katz method, Formol-Ether Concentration Technique, Ascaris lumbricoides.

## INTRODUCTION

Soil-transmitted helminth (STH) infections, a neglected tropical diseases,<sup>[1]</sup> occurs in remote and poverty-stricken areas with poor sanitation and low education.<sup>[1]</sup> The prevalence in India varies from 12% to 91%, with varying prevalence rates for

individual parasites.<sup>[2]</sup> India has a wide range of climatic conditions, providing an ideal environment for the survival of parasite eggs.<sup>[3,4,5]</sup> A recent study in Vellore and Thiruvannamalai districts of Tamil Nadu showed the prevalence of STH infections to be 6.28% for Hookworm (*Ancylostoma duodenale* and *Necator americanus*), 1.21% for *Ascaris*

lumbricoides, and 0.8% for *Trichuris trichiura*, with an overall prevalence of less than 10%.<sup>[6]</sup>

The infection due to STH is acquired by ingestion of contaminated food, water, and vegetables that contain the eggs of STH (*Ascaris lumbricoides*, *Trichuris trichiura*),<sup>[7,8]</sup> or by penetration of the skin by infective stage larvae present in the soil (Hookworm -*Ancylostoma duodenale* and *Necator americanus*).

The diagnosis is by detection of helminth eggs or larvae in human stool using the microscopic observation of parasite eggs in stool, direct fecal microscopic examination by wet mount method, concentration technique, high sensitivity and specificity Kato-Katz method, and molecular analysis. Performing a range of techniques on a single sample may enhance the detection of STH infection since different techniques vary in their sensitivity and specificity. Based on this background, this study estimates the prevalence of STH infections and their associated risk factors among children aged 5-10 years.<sup>[9]</sup> This study also compares the sensitivity of the wet mount method, Formol-Ether concentration method, Kato-Katz method, and molecular analysis to detect STH infections.

#### **Aims and Objectives**

##### **Aim**

- To study and estimate the prevalence of STH.

##### **Objectives**

- To estimate the prevalence of STH among children in the age group 5-10 years in Thirukazhikundram and Salavakkam villages of Chengalpattu, Kancheepuram district, Tamil Nadu.
- To perform and to compare the wet mount method, Formol-Ether concentration method, Kato-Katz method, and molecular analysis of faecal samples for the identification of STH.
- To study the association of risk factors and the occurrence of STH infection.

## **MATERIALS AND METHODS**

#### **Inclusion Criteria**

Children aged 5-10 years in Thirukazhikundram and Salavakkam villages of Chengalpattu, Kancheepuram district, Tamil Nadu.

#### **Exclusion Criteria**

- The children whose age does not fall between 5-10 years.
- The consent was not provided to collect samples.

#### **Ethical Clearance**

Approval was obtained from the Institutional Ethics Committee at Chengalpattu Medical College before commencement of the study. Informed consent was obtained from the parent or guardian of all the children participating in the study.

**Statistical Analysis:** Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) statistical software and Microsoft Excel 2010.

#### **Sample Collection**

Children fulfilling the criteria were included in the study after obtaining informed consent. The data on the history of open defecation, nail biting, lack of appetite, habit of eating mud, and method of washing hands were obtained using a structured questionnaire. The parent of study participants was instructed on how to collect stool samples and were provided with labeled, clean, wide mouthed screw capped; plastic container fitted with spatula, toilet tissue paper and an applicator stick.<sup>[4]</sup>

#### **Examination of stool sample**

The macroscopic physical nature of the sample was examined. The stool sample was checked for consistency (Formed, soft, loose, and watery). The presence of blood and mucus mixed in the stool was noted.<sup>[10]</sup>

Each sample was examined by using the wet mount method, the Kato-Katz method, and the Formol-Ether concentration method following standard procedures. The sample was preserved and stored by adding 10% formalin solution, which adequately preserves protozoan cysts, helminth eggs, and the morphology of larvae.

Three parts of formalin solution are mixed with one part of the stool specimen, and then thoroughly mixed to preserve the stool. Approximately 2g of each sample was also preserved in a 5 ml aliquot container along with buffer solution containing 70% ethanol, for molecular analysis, using PureFast® Stool DNA mini spin purification kit, and Primers: 1) Helini Ready to use *Ancylostoma duodenale* Primer mix - 5µl/reaction, 300bp, 2) *Ascaris lumbricoides* Primer mix - 5µl/reaction, 192bp, 3) *Trichuris trichiura* Primer mix - 5µl/reaction, 498bp, 3) *Necator americanus* Primer mix - 5µl/reaction, 330bp.

## **RESULTS**

Out of 350, STH was positive in 58(16.57%) samples examined by wet mount method, Formol-ether concentration technique, Kato-Katz techniques, and Multiplex Polymerase chain reaction.

Out of the total positives, 51.7% of the STH infections were in the age group 6- 7 years. In this study, the male-to-female ratio was 2:1.4. Out of the total STH-affected children, 60.34% were found to be females. The prevalence rate of STH infection is estimated to be 16.57 % by Kato-Katz and Multiplex PCR methods. Among the Individual STH infections, Hookworm was the most common STH (10.87%), followed by *Ascaris lumbricoides* & Hookworm coinfection (2.57%), *A. lumbricoides* (1.71%), and *Trichuris trichiura* (1.4%) co-infection.

**Table 1: Age Wise Distribution of Soil Transmitted Helminth Infections**

Age In Years	Ascaris lumbricoides	Ascaris lumbricoides & Hookworm Co-Infection	Hookworm	Trichuris trichiura	Total positive for STH
5	0(0%)	0(0%)	3(50%)	3(50%)	6(100%)
6	1(7.6%)	2(15.4%)	10(77%)	0(0%)	13(100%)
7	3(17%)	4(23%)	9(53%)	1(6%)	17(100%)
8	2(22.2%)	2(22.2%)	4(44.4%)	1(11.2%)	9(100%)
9	0(0%)	1(12.5%)	7(87.5%)	0(0%)	8(100%)
10	0(0%)	0(0%)	5(100%)	0(0%)	5(100%)

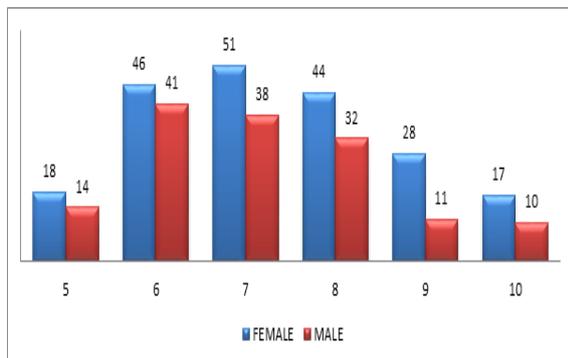
**Table 2: Comparison of various methods to detect STH infections**

Soil-transmitted helminth	Number of positive for STH infections			
	Wet mount method	Formol-ether concentration method	Kato-Katz technique	Multiplex PCR assay
Ascaris	1(3%)	5(10.4%)	6(10%)	6(10%)
Ascaris lumbricoides and Hookworm Coinfection	5(13%)	8(17%)	9(15%)	9(15%)
Hookworm	31(79%)	31(64.6%)	38(66%)	38(66%)
Trichuris trichiura	2(5%)	4(8%)	5(9%)	5(9%)
<b>Total</b>	<b>39(100%)</b>	<b>48(100%)</b>	<b>58(100%)</b>	<b>58(100%)</b>

The detection of the *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm and co-infection with *Ascaris* and hookworm infection in Kato-Katz method and Multiplex PCR method was 6(1.71%), 5(1.42%), 38(10.85%) and 9(2.57%) respectively.

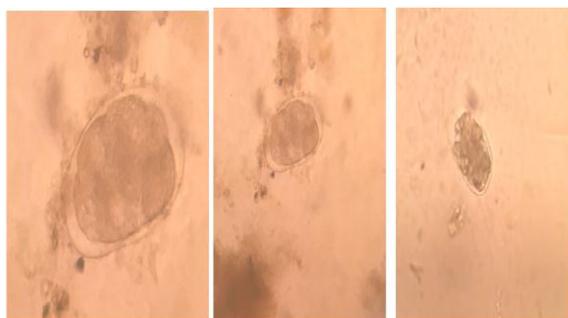
**Table 3: Prevalence of STH infection from various Studies in India**

Place of study (Reference)	Samples		% of samples positive		
	N	positive%	Ascaris	HW	TT
Andhra(Bidinger et al) <sup>(22)</sup>	335	32	15	29	2
Puduchery(Latha Ranganathan) <sup>(3)</sup>	1125	35.56	43.21	28.89	10.87
Vellore(Gandeep Kang et al) <sup>(8)</sup>	993	45.4	6.9	22.8	0.8
Chennai(Veerannan et al) <sup>(10)</sup>	348	16	10.6	6.9	2.6
Chengalpattu (Present study)	350	16.57	4.28	13.42	1.4

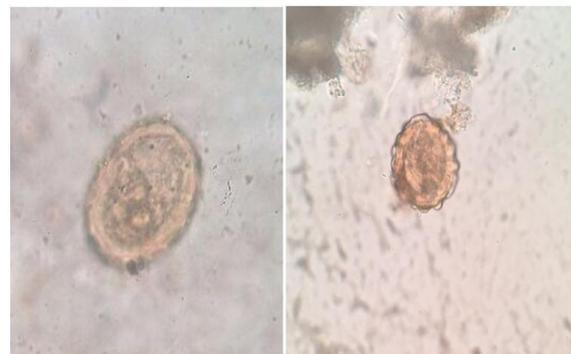


**Figure 1: Age and Sex Distribution of the Children in the Study Group**

Of the 350 samples, 58(16.57%) were positive for STH infections. Of the 58 total 62 positives (51.7%) of the STH infections were detected in the age group of 6- 7 years, as shown in Table 1.



**Figure 2 Egg of Hookworm**



**Figure 3: Eggs of Ascaris lumbricoides**



**Figure 4: Eggs of Trichuris trichiura**

## DISCUSSION

The present study revealed that the prevalence of STH infections was 16.57% among children of age group 5- 10 years, similar to the study at Chennai.<sup>[11]</sup>

Out of 58 children with STH infection, 30 were in the age group 6-7 years. A similar pattern in the prevalence of STH among children of 6-14 years was observed in a study conducted by Deepthi Kattula et al.<sup>[8]</sup> In the present study, the prevalence rate of Hookworm was 10.87% followed by co-infection of *Ascaris lumbricoides* and Hookworm, 2.57%, *Ascaris lumbricoides*, 1.71%, and *Trichuris trichiura*, 1.42%. A study conducted by Latha Ranganathan et al.<sup>[3]</sup> in Puduchery, the overall prevalence of STH was 34.56%, *Ascaris lumbricoides* 43.21%, Hookworm 28.89%, *Trichuris trichiura* 10.87%. Similarly, in a study conducted at Vellore and Thiruvannamalai district by Deepthi Kattula et al.<sup>[6,8]</sup> 3706 children aged 6-14 years were screened to estimate the prevalence of STH, which showed 7.8% positivity. In the present study, the prevalence of Hookworm was estimated to be higher when compared to roundworm infections, as also seen in studies by Vipin Alexander and Gandee Kang et al.<sup>[8]</sup> in Vellore and Thiruvannamalai districts. The total prevalence rate of STH in the present study was on the lower side of the range,<sup>[4]</sup> of the global estimated burden and among the other studies in India. This could be explained by the administration of Anthelmintic treatment once every six months in the communities by the Public Health Department, which will reduce the intensity of STH infection as recommended by WHO.<sup>[8]</sup> The risk of acquiring STH infection and its high prevalence cannot be attributed to one factor, but due to coexistence of various factors such as biological, social, behavioral, and environmental factors.<sup>[8]</sup>

In the present study, more than one method was employed to detect STH infections to increase the diagnostic yield.

The wet mount method showed 39 (11.14%) samples positive for STH among 350 samples with Sensitivity of 67.24%, 95% CI (53.66-78.99%) and specificity 100% (95% CI 98.74-100%) as compared to the Kato-Katz method.

In a study conducted by Mengistu Endris et al.<sup>[12]</sup> with comparison of various methods showed a prevalence of intestinal parasites using the single wet mount, Formol Ether Concentration, and Kato-Katz techniques were 38.4%, 57.1%, and 59%, respectively.

A study,<sup>[4]</sup> Showed that the traditional methods such as direct wet mount suffers lower sensitivity of 34%, compared to concentration techniques and other methods to detect STH infections in case of light infection and post treatment scenarios.

In the present study, out of 48 (13.71) samples positive for STH, by Formol-Ether concentration method showed Sensitivity of 82.76%, (95%CI 98.74-100%) and specificity of 100%, (95% CI 98.74-100%).

A study conducted by D. S. Ridley And B. C. Hawgood et al.<sup>[13]</sup> David Goodman, Saravanakumar P et al.<sup>[14,15]</sup> showed that Formol-Ether concentration

method showed better detection of STH with greater sensitivity of 89.6% compared to wet mount method. In the present study Kato-Katz method showed 58 samples positive for STH out of 350 samples, with significant P value (<0.0001), A study,<sup>[15]</sup> showed that Kato-Katz was more sensitive 95% that of other methods. A study Showed Kato-Katz method to have a sensitivity of 99%. In a study conducted by Mengistu Endris et al.<sup>[12]</sup> revealed that Kato-Katz method and Formol-Ether concentration methods showed a better sensitivity (wet mount, FEC, and Kato-Katz techniques were 38.4%, 57.1%, and 59%, respectively )when compared to direct wet mount method.

In a study conducted by Mengistu Endris et al.<sup>[12]</sup> Men-Bao Qian, Peiling Yap et al.<sup>[16]</sup> for comparison of the Kato-Katz, wet mount, and Formol-Ether concentration techniques showed Kato-Katz techniques to be more sensitive and were considered a gold standard in detection of STH compared to all other methods.

In the present study Multiplex PCR Method showed 58 samples positive for STH infection out of 350 samples, which was equal to the results obtained by KatoKatz method. In a study conducted by Romano Ngui, Lee Soo Ching, Tan Tiong Kai et al.<sup>[17]</sup> showed that 81% sensitivity by PCR method for the detection of STH infections. In a study conducted by Orawan Phuphisut et al.<sup>[18]</sup> used PCR to detect STH in fecal samples and compared the results with Kato-Katz method and showed that sensitivity and specificity of multiplex PCR was 87% and 83% respectively.

In a study conducted by Oliver BaÈrenbold, Giovanna Raso et al.<sup>[19]</sup> Kato-Katz method showed a better performance in detection of STH compared to PCR assay and in a study conducted by Orawan Phuphisut et al.<sup>[18]</sup> showed 13% of samples positive by Kato-Katz were negative with Multiplex PCR. This might be explained in terms of presence of natural DNA polymerase inhibitors in the fecal samples, DNA degradation during storage, and inefficient disruption of the helminth eggs.

Another study conducted by Jaco.J Verweij et al.<sup>[20]</sup> which compared KatoKatz method , wet mount method and multiplex PCR reveled that Multiplex PCR having Good sensitivity of 100% compared to KatoKatz method.<sup>[21]</sup> Stefanie Knopp, Nahya Salim et al.<sup>[21]</sup> in their study in which Diagnostic Accuracy of Kato–Katz and PCR Methods for the detection of Light-Intensity Hookworm showed that the sensitivities of PCR and the Kato–Katz method were equal. The reagent costs associated with performing molecular diagnostic testing are higher than costs associated with conducting traditional microscopy-based diagnostics.<sup>[2]</sup> The total cost associated with performing a Kato-Katz method under field conditions has been estimated to be five times lower than a PCR.<sup>[2]</sup>

On comparison of various methods to detect STH infections, in the present study Kato-Katz method showed a greater detection of STH infections.

According to a systematic review for Prevalence of STH infection in India by Sudhir Kumar Jain and Awanindra Dwivedi et al,<sup>[2]</sup> showed similar findings. This study also pointed out that highest prevalence was recorded using Kato-Katz method. and considered it to be a most sensitive diagnostic method. Apart from Kato-Katz method among the other available methods to detect STH infections Formol-Ether concentration method was considered better.<sup>[2]</sup>

In the current study, among the 58 STH positive samples there was significant association of STH infection with habit of nail biting, habit of open defecation, history of Lack of appetite and habit of Hand washing with the prevalence of 41 (11.71%),51 (14.57%),50 (14.12%) and 46 (13.14%) respectively. This was comparable to a study conducted by Gandeep et al,<sup>[8]</sup> which showed a significant association between occurrence of STH infection and unhygienic practices.

## CONCLUSION

We conclude that the prevalence of STH infection among children aged 5-10 years in Thirukazhikundram, Salavakkam, Chengalpattu, Kanchipuram District was 16.57%. The total prevalence rate of STH in this study was on the lower side of the range of global estimated burden, and also on the lower side of the range reported by the other studies in India. This could be explained by the administration of Antihelminthic treatment once every six months in the communities, which will reduce the intensity of STH. To increase the sensitivity of detection of STH infections, this study employs more than one method.

In the present study Wet mount method, Formol-ether concentration method, Kato-Katz method, and Multiplex PCR were performed on each sample, and the results were compared. This study also concludes that the Kato-Katz method and Formol-Ether concentration methods showed better sensitivity when compared to the direct wet mount method.

The Multiplex PCR assay showed equal results to those of Kato-Katz. The performance of the Kato-Katz method was found to be better compared to all the other methods. The main advantage of the Kato-Katz technique is its minimal need for supplies and equipment to perform, as well as its capacity to identify soil-transmitted helminthes.

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### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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