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## Research Article

### INCIDENCE OF INTESTINAL PARASITIC INFESTATION AND ANEMIA AMONG SCHOOL CHILDREN IN AMMAPETTAI

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#### ABSTRACT

**Background:** Soil-transmitted helminthiases (STHs) are the most common intestinal worm infections affecting disproportionately the most deprived communities throughout the tropics and subtropics. In many parts of the developing world, these parasitic worms and anaemia are of considerable public health and economic importance. **Objectives:** To determine the relative distribution of the incidence of different intestinal parasitic infestations among school children in rural the study area and also to find its association with anaemia and to prove the efficiency of different methods of parasite detection viz., Lacto phenol cotton blue wet mount and Normal saline wet mount. **Methods:** Stool samples from a total of 335 children were collected and subjected for routine macroscopic as well as microscopic examination. For microscopic examination of stool, various methods such as saline, iodine, LPCB wet mount preparations were performed. From each subject, blood was collected to estimate the total haemoglobin levels. **Results:** overall parasitic infestation was detected in 228 of 335(68.05%) children. This study revealed mixed parasite infections in 7.1% of children. The overall incidence of anaemia in parasitized children was high (55.70%). **Conclusion:** This study revealed a concrete evidence of parasitisation and anaemia which demands the need for improved sanitation and better living conditions for the school going children in rural areas. Thus a global strategy for the control of STHs, based on regular anthelmintic treatment, health education and improved sanitation standards, is warranted. The use Lacto phenol cotton blue stain along with saline wet mounts for routine microscopic examination of stools for ova& cyst.

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## INTRODUCTION

Parasitic disease in human beings has worldwide distribution but in developing countries like India the incidence is very high. Intestinal Parasitic infestations are endemic worldwide and have been described as the greatest cause of illness and disease [1]. It is a major health problem in children of developing countries because of poor-socioeconomic conditions and lack of good hygienic living. It causes not only nutritional deficiencies and anemia but also leads to intestinal obstruction in the infested children [2]. Intestinal helminthes infections are main causes of disease burden in children in developing countries [3].

Iron deficiency is a significant global problem. It is one of the major public health problems in school children and according to the World Health organization 43% of the world's children

are parasitized [4]. The relationship between parasitic infestation with adverse effect on hemoglobin and indirectly on the nutritional status of the human host is well established. This will cause ill health along with disturbance in mental acumen of the diseased children [5]. Therefore it has utmost importance in public health and preventive medicine in knowing the incidence of the parasitic infested children in the given area.

Helminth infestation leads to anemia, nutritional deficiency and impaired physical developments which will have negative consequences on cognitive function and learning ability. The prevalence rate of intestinal parasitic infestation ranges from 14.6% to maximum of 91% in different studies, conducted in various parts of the country [2]. In spite of health education and hygienic habits being improved the parasites continue to infect the people in rural area more often than people in urban area. This parasitic infestation is more commonly seen in children who have the habit of playing in mud, sand and eating without

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washing their soiled hands which leads to more morbidity and mortality in severe conditions. Keeping these aspects in mind we undertook a study on the Incidence of intestinal parasitic infestation and anemia among school children around our medical college which is situated in a rural area of Ammapettai, Kancheepuram District, Tamil Nadu.

### Objectives

To determine the incidence of intestinal parasitic infestations among school children in rural kanchipuram with Hemoglobin estimation and to prove the efficiency of different methods of parasite detection viz., Lacto phenol cotton blue wet mount and Normal saline wet mount.

## MATERIAL AND METHODS

This surveillance study was carried out for one year in Kancheepuram District, Tamil Nadu, after getting prior permission from the Institutional Ethical Committee (IEC).

**Study population:** Consist of students from: 2 Government schools and 1 private school going children aged 6-12 years in rural Ammapettai village, Kancheepuram District, Tamil Nadu.

### Total number of samples:

A total of 335 samples were collected. Informed consent was taken from the parents of all children. Each participant was provided with a sterile standard collection container labeled properly with details of name, age, sex, name of school and clinical features. After getting sample, it was transported to the microbiology laboratory immediately and if there was any delay, it was refrigerated [6].

### Processing of samples and detection of Parasites

#### Stool examination

##### Macroscopic examination

Consistency, Presence of blood and mucus, presence of round worms, thread worms or tapeworm proglottids, color and smell of the stool.

##### Microscopic examination (wet mounts)

The stool specimens collected were processed and examined microscopically under low and high power objective by saline, iodine, LPCB wet mount preparation.

##### Saline wet mount

It is the simplest and easiest technique. A wet mount can be prepared directly from faecal material or from the concentrated specimens. Saline wet film is made by mixing small volume of stool with a drop of Normal saline (0.85%) on a glass slide and placing a cover slip over the mixture. It was screened to demonstrate motile trophozoites, ova/cyst and larva.

##### Iodine wet mount

Iodine mounts were prepared by adding a small amount of stool to a drop of Lugol's iodine diluted about five times with distilled water on a glass slide and placing a cover slip on the mixture. Iodine mount was screened for cyst.

##### Lacto phenol cotton blue (LPCB) wet mount

It is the simplest and easiest technique. LPCB is a common reagent used in mycology, but it is also very effective in

finding ova/cyst and the internal structures of the parasitic pathogen.

LPCB mounts were prepared by mixing a drop of LPCB stain with a small amount of stool on a glass microscope slide and placing a cover slip on the mixture. If there was the presence of the trophozoites, cyst and ova by Saline, Iodine & LPCB wet mounts. It was identified and reported. Those samples which were found to be negative in saline, Iodine and LPCB wet mounts were subjected for concentration techniques like flotation and formal ether techniques [8].

**Concentration Methods:** - If the number of parasites in the stool specimens is low, examination of a direct wet mount may not detect them, and hence the stool should be concentrated. Eggs, cysts and larvae are recovered after concentration procedures.

##### Flotation technique: Saturated salt solution method

**Sedimentation technique: Formal-ether concentration:** Then after subjecting samples for concentration techniques if it was found negative by this procedure it was reported as negative.

##### Estimation of Hemoglobin in blood samples

Trained laboratory technicians collected the blood samples by finger prick method with sterile precautions in their respective schools. From each subject, 20 micro-liter of blood was collected through a hemoglobin pipette and was preserved in a vial containing 5 ml of hemoglobin reagent which had been prepared and bottled. Then all samples were brought to the laboratory of the Department of Microbiology, Hemoglobin was measured by the Cyanmethemoglobin method using a colorimeter where exact wavelength of 540 nm [10]. (HEMOCOR-D, Lot: HBD1552, Mfd: Nov-2011, Exp: Oct-2013).

##### Calculations on colorimeters

$$\text{Hemoglobin in g/dl} = \frac{\text{Abs.T} \times 251 \times 60}{\text{Abs.S} \times 1000}$$

Where, 251 is the Dilution Factor i.e Total reaction Vol. (5.02ml) / Sample Vol. (0.02ml).

1000 is the Multiplication Factor to convert mgs to grams.

60 is the Concentration of the HEMOCOR Hemoglobin Standard in mg%.

## RESULTS

Out of 335 participants of school children, 228(68.05%) were Positive and 107(32%) were Negative for parasitic infestation (Table-1). Among them 177(53%) were males and females were 158(47%). Compared to males 109(32.53%), females 119(35.52%) had higher incidence of parasitic infection.

**Table 1** Parasitic infestation among children (n=335)

Parasitic infestation	Males	Females	Total
Positive	109	119	228
Negative	68	39	107
Total	177	158	335

The study showed 109 males and 119 females were positive for parasitic infestation, making total positivity of 68.05%. The

most common parasites in both males and female children are *Ascaris lumbricoides* and followed by other parasites (Table-2).

The youngest group showed the highest parasitic incidence 90%. The parasitic infestation was more in young children in the age group of 6-7 years where the incidence was as 90% and it had a low infestation 33.3% in the age group of 11-12 years, thus in our study as the age increased in years incidence of parasitic diseases decreased (Table-3).

**Table 2** Gender wise incidence of individual parasite in children (n=335)

Parasitic infestation	No. of males (%)	No. of females (%)	Total (%)
<i>Ascaris lumbricoides</i>	30 (13.15)	33 (27.7)	63 (18)
<i>Entamoeba histolytica</i>	20 (8.77)	16 (13.4)	36 (11)
<i>Ancylostoma duodenale</i>	12 (5.26)	18 (15.1)	30 (9.0)
<i>Giardia lamblia</i>	11 (4.82)	17 (14.2)	28 (8.4)
<i>Taenia species</i>	8 (3.50)	16 (13.4)	24 (7.2)
<i>Entamoeba coli</i>	1 (0.43)	6 (5.0)	07 (2.1)
<i>Trichuris trichiura</i>	2 (0.87)	4 (3.3)	06 (2.0)
<i>Enterobius vermicularis</i>	2 (0.87)	4 (3.3)	06 (2.0)
<i>Strongyloides larva</i>	3 (1.31)	1 (0.8)	04 (1.2)
Mixed infection	16 (7.01)	8 (6.7)	24 (7.1)
Total	109 (32.53)	119(35.52)	228(68.05)

**Table 3** Incidence of parasitism with respect to age and gender

Age group	Total number of gender screened	Male			Female		
		No.screened	+ve	%	No.screened	+ve	%
6 – 7	123	63	42	66.6	60	54	90
8 – 9	115	65	40	61.5	50	34	68
10 – 11	80	43	25	58.1	37	26	70.27
11 – 12	17	6	2	33.3	11	5	45.45
Overall	335	177	109	61.58	158	119	75.31

Incidence of anemia among the children are 167(49.85) and normal children without anemia are 168(50.14) making a total of 335 children. The overall incidence of anemia in parasitized children was males: **57** and females: **70** making total number of **127**, high degree of associated anemia among the female children who are infected with parasites when compared with males. After applying chi-square test, **P=0.002**, significant association between parasitic infestation and anemia (Table-4).

**Table 4** Distribution of parasitic infestation and haemoglobin

Parasitic status	Haemoglobin	Males	Females	Total
Parasitic infestation	Anemic (<11g/dl)*	57	70	127
	Normal haemoglobin(>11g/dl)*	55	46	101
No infestation	Anemic	28	12	40
	Normal haemoglobin	37	30	67
Total		177	158	335

\* Parasitic infestation with anemia, after applying chi-square test  $X^2 = 9.775$  and  $p=0.002$ , so it shows there is a significant association between parasitic infestation and anemia.<sup>16</sup>

The correlation between parasitic infestations with hemoglobin showed: Out of the total 228 positive parasitized children, 127(55.70%) showed anemia, while 101(44.29%) had normal hemoglobin. The *Trichuris trichiura* (83.33%), followed by *Ancylostoma duodenale* (76.66%), *Ascaris* (65.07%), *Mixed infestation* (62.5%), *Taenia species* (54.16%), *Strongyloides*

*Larva* (50%), *Entamoeba histolytica* (47.22%), *Giardia spp* (32.14%) and *Entamoeba Coli* (28.57%) making positivity of 127(55.70%) (Table-5).

**Table 5** Correlation of different parasitic infestation with hemoglobin status

Parasitic infestation	Anemic (%) < 11 g/ml	Normal haemoglobin(%) 11 g/ml	Total (%)
<i>Ascaris lumbricoides</i>	41 (65.07)	22 (34.92)	63 (27.63)
<i>Entamoeba histolytica</i>	17 (47.22)	19 (52.77)	36 (15.78)
<i>Ancylostoma duodenale</i>	23 (76.66)	7 (23.33)	30 (13.15)
<i>Giardia lamblia</i>	9 (32.14)	19 (67.85)	28 (12.28)
<i>Taenia species</i>	13 (54.16)	11 (45.83)	24 (10.52)
<i>Entamoeba coli</i>	2 (28.57)	5 (71.42)	07 (3.07)
<i>Trichuris trichiura</i>	5 (83.33)	1 (16.66)	06 (2.63)
<i>Enterobius vermicularis</i>	0 (0)	6 (100)	06 (2.63)
<i>Strongyloides larva</i>	2 (50)	2 (50)	04 (1.75)
Mixed infection	15 (62.5)	9 (37.5)	24 (10.52)
Total	127 (55.70)	101(44.29)	228

The parasitic microscopic identification was also compared by using Lacto-phenol cottonblue and routine saline wet mount. The results with LPCB 135(40.29%) proved to be better than the Saline mount 93(27.76%). (Table-6).

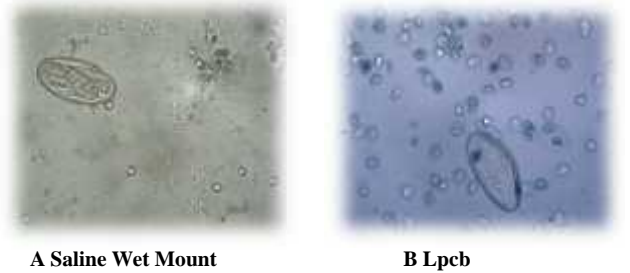
**Table 6** Total no of positives in comparison with lacto phenol cotton blue and normal saline wet mount (n=335)

Methods for detection of parasites	Positives	Negatives	Total
Lacto phenol cotton blue Wet mount	135(40.29)	200(59.70)	335
Wet mount with normal saline	93(27.76)	242(72.23)	335

Comparative microscopic pictures of cyst/ ova and with normal saline and Lactophenol cottonblue (LPCB) mount are depicted as follows (Figure-1).

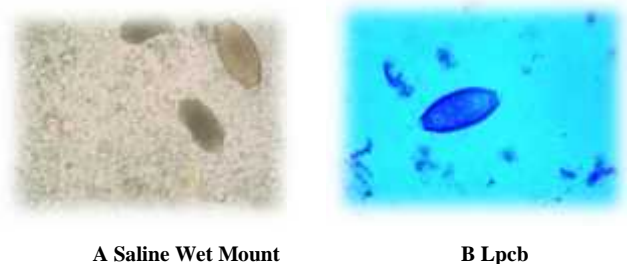
**Figure 1:** Comparative microscopic pictures of eggs with normal saline and lacto phenol cotton blue (LPCB) wet mount.

**Enterobius Vermicularis**



In LPCB wet mounts of *Enterobius vermicularis* egg, the outer shell is stained deep blue and light blue inside.

**Trichuris Trichiura**





In LPCB wet mounts of *Trichuris trichiura* egg the outer wall is stained deep blue. Mucus plugs are not stained but well defined.

## DISCUSSION

In the present study, the incidence of intestinal parasites among children (6-12) was found to be high 68.05% when compared with one of the study carried out in India have reported a prevalence of intestinal parasites from 30-50% among school going children. Atul Aher et al also found prevalence of 30.4% in school children. Our study showed the presence of *Ascaris lumbricoides* to be commonest parasitic disease. The incidence of *Ascaris lumbricoides* were seen in 63(18%), as recent studies done through out India our study showed highest incidence of parasitic infestation in school children<sup>[9]</sup>. Mixed infestation 24(7.1%) are also high when compared to other studies.

On the whole the distribution of parasitic infestation was more prevalent among females 119(35.52%) when compared with males 109(32.53%) making total positivity of 228(68.05%).

Our study shows the high incidence of parasitic infestation among the female children when compared to the males of the same age group. The incidence is high in girl children at a low age group than at a later age group. This can be attributed to the unhygienic habits which are seen among the children of lesser age group as they play in contaminated soil, lack of drinking water and consume food with soiled hands. As they grow, the awareness increases towards health and hygiene; therefore there is reduction in the associated parasitic infestation at a later age group. Psychosocial importance given to boys further improves the care given to boy children when compared to girls who are neglected without proper care and hygiene habit education which makes them vulnerable for parasitic infestation<sup>[9]</sup>.

The study also reveals the link between parasitic harboring with anemia which is 127/335, anemia being more common in female children when compared to boys (70 girls and 57 boys). The use of LPCB is better than the saline mount for the parasitic detection in the microscopic stool examination as proved in our study<sup>[8]</sup>.

Anbumani.n, from Chennai 2011, studied for prevalence of parasitic infection among school going children in 358 in the age group of 5-10 years. 114 were tested positive for various intestinal infections like *Ascaris lumbricoides*, *trichuris trichura* and *taenia species*. At least one helminthic infestation was detected in 25.13% (90/358) children and multiple helminth infestation was recorded in 15.08% (54/358). The most common parasitic helminth was *Ascaris lumbricoides* 60% (84/114), followed by *trichuris trichura* 4.17% (6/114). In this study *Ascaris lumbricoides* was found as single type infection as well as in association with other helminthes in mixed type infection<sup>[11]</sup>.

Ikram Ullah, Ghulam Sarwar, Sabina Aziz, and Muhammad Hussain Khan, performed a cross sectional study in 200 school children, among them 133(66%) were positive for helmenthic infections showing highest frequency of 45.5% (91 cases) was noted for *Ascaris lumbricoides*. Other helminthes found were *Enterobius vermicularis* 4% (16 cases), *Ancylostoma*

*duodenale* 3.5% (7 cases), and whip worm 1.5% (3 cases). In this study a higher percentage of primary school children from rural Peshawar, Pakistan had intestinal worm infestation & majority of them had *Ascaris lumbricoides*<sup>[7]</sup>.

Anah MU, Ikpeme OE, Etuk IS, Yong KE, Ibanga I and Asuquo BE. Performed a study shows 49.7% intestinal helminthes with *Ascaris lumbricoides* 64.4%, hook worms 10.9% and *Trichuris trichura* in 1.1% cases studied. There were 41(23.6%) children with polyparasitism, 33 of them were positive both for *Ascaris lumbricoides* and hook worm<sup>[12]</sup>.

The overall incidence was higher in our study (68.05%) compared to the studies in Uganda 55.9%<sup>[7]</sup>. Nigeria 49.7%<sup>[12]</sup>, Chennai 40%<sup>[11]</sup>, Oman 38.7%<sup>[3]</sup>, Turkey 31.8%<sup>[13]</sup>. But our study showed a less incidence of intestinal parasites 68.05% when compared with a study in Chennai 91%<sup>[14]</sup>.

*Ascaris lumbricoides* infection in our study is 18% which was lower than Nigeria (64.4%)<sup>[12]</sup>, Chennai 60%<sup>[11]</sup> and (52.8%)<sup>[14]</sup>. But was higher when compared to the studies Nepal (3.3%)<sup>[6]</sup> and Chennai (1.1%)<sup>[15]</sup>. The *Entamoeba histolytica* was 11% higher in our study when compared with Nepal 4.4%<sup>[6]</sup> and Chennai (4%)<sup>[14]</sup>. The *Ancylostoma duodenale* infection is low 9.0% in our study when compared with a study in Chennai (37.6%). *Giardia lamblia* is low in our study 8.4% when compared to a study in Chennai (16%)<sup>[14]</sup>. *Trichuris trichiura* is very low 2.0% in our study when compared with a study in Chennai 13.96%. *Taenia species* infection is higher in our study 7.2% compared to a study in Chennai 4.7%<sup>[11]</sup>. Compared to Thailand and Egypt study (0.9%) the *Enterobius vermicularis* infection by routine stool examination was higher (2%) in our study and it was reported as low as 0.4% in other studies<sup>[3]</sup>. *Strongyloides larval* infection is low 0.4% in our study when compared with a study in Chennai 3.2%<sup>[14]</sup>. Our study showed a low mixed parasitic infection 7.1% when compared with a study in Chennai (15.08%)<sup>[11]</sup>.

In this study the rate of infestation is comparatively higher among females 119(35.52%) than in males 109 (32.53%) in contrast it was notably high in a study conducted in Chennai among boys (66.6%) than in girls (33.33%)<sup>[11]</sup>. In our study we observed 228(68.08%) higher incidence of parasites with 37.91% anemic children. The anemic percentage in our study is higher 37.91% when compared with a study conducted in Vietnam i.e. 25% with a prevalence of 90% parasitic infestation<sup>[7]</sup>.

## CONCLUSION

In the present study the incidence of intestinal parasitic infestation of school children was high in present scenario of rural population. Our study also reveals the concrete evidence of parasitization and anemia, which again common in female children when compared to males. It was predominantly seen in children who were positive for *Ancylostoma duodenale*, *Trichuris trichiura*, *Ascaris lumbricoides*, mixed infections, *Taenia species*, and *Entamoeba histolytica*.

The use of Lacto phenol cotton blue as a mounting fluid proved to be more effective than the conventional lugol's iodine for studying the ova and cyst as the intracellular structures well appreciated as depicted in the micro photographs.

This study emphasizes the need for improved sanitation and better living conditions for the school-going children in rural areas. This study shows the incidence of intestinal helminthes in high magnitude in school children of the study area. This indicates the necessity for the institutions to implement control measures, including regular administration of anti-helminthic drugs and periodical examination of stool samples of all school children., coupled with health education program, with special reference to hand hygiene.

The impact of each measure would be maximized through a health education programme directed at school children in particular and at community in general. It should be re-emphasized that regular de-worming is necessary for children to reduce and control parasitic infestations. Efforts should be made to improve the condition through enhancing the existing health education components of the development programmers. Adequate focus should be given to sanitation and excreta disposal system to control the spread of intestinal parasites.

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