

SOIL-TRANSMITTED HELMINTHS IN CHILDREN WITH CLINICAL SYMPTOMS OF INFECTION

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ABSTRACT: The study was conducted to know the prevalence of soil-transmitted helminths (STH) in children aged 1-14 years attending outpatients and inpatients from both urban and rural background. Out of the 550 stool samples examined, 450 were from the study group and 100 from the control group. All the 450 children of the study group presented one or more of the classical symptoms like abdominal pain, low grade fever, diarrhoea, dysentery, constipation and pica. Hundred children from the control group did not have any classical symptoms. Thirty five per cent from the study group and 21 per cent from the control group were found positive for one or more of the helminthic infection.

The prevalence of *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale* & *Necator americanus* (hookworm), *Hymenolepis nana* and *Enterobius vermicularis* were 27%, 15%, 0.4%, 2% and 0.22%, respectively in the study group and 15%, 10%, 4%, 2%, and 2%, respectively in the control group. *Strongyloides stercoralis* was not found either in the study or control groups. Helminthic infections were more common in 7-9 years of age group, urban children with low socio-economic strata, and with male preponderance. Majority of children who harboured STH were suffering from abdominal pain and low grade fever.

Total 450 clinical samples (control group) examined by direct wet mount, saturated salt floatation, and formalin-ether sedimentation; and only 391 samples examined by Kato-Katz semiquantitative techniques. Twenty six per cent positive by direct wet mount, 35% by both saturated salt floatation and formalin-ether sedimentation whereas 38% of the samples were found positive by Kato-Katz semiquantitative technique. In conclusion, concentration techniques like saturated salt floatation and formalin-ether sedimentation are the best diagnostic tools to get authentic and reliable results and to rule out fallacious inference caused by relying only on direct wet mount preparation, whereas Kato-Katz, a semiquantitative technique is the best diagnostic method to know the prevalence and intensity of the STH infection. This technique is simple, rapid, reliable, and mild infections can also be detected. No special equipment and expertise are needed and it can be performed in the field survey.

KEYWORDS: Soil-Transmitted Helminths, Paediatric Age Group.

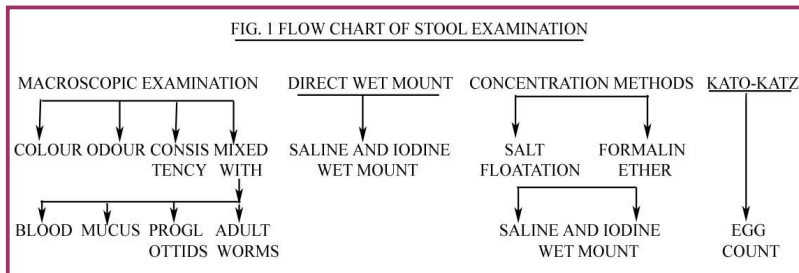
INTRODUCTION: The soil-transmitted helminthic (STH) infections are caused by helminths like *Ascaris lumbricoides*, *Ancylostoma duodenale* & *Necator americanus* (hookworm), *Trichuris trichiura* and *Strongyloides stercoralis*. The global burden of roundworm, hookworm and whipworm is 1000 million, 900 million and 800 million, respectively, whereas in the developing world it is estimated to be 700 million, 800 million and 500 million respectively. At any one moment in the world, more than 2 billion people are infected with STH infections. The sustained infection and disease burden cause effects on the development of the child, educational achievement, reproductive health, adult productivity and ultimately socio-economic development. According to the World Development Report of 1993, intestinal worm infection is the leading cause of morbidity in school children, accounting for 10.6 and 9.2 million of Disability-Adjusted Life Years (DALYs) lost in males and females, respectively.¹

The provision of safe drinking water, sanitary latrines, and sanitary methods of refuse disposal, adequate nutritious diet, proper housing, and health education to the rural and urban dwellers reduce the incidence and prevalence of STH infection. They should also guide to lead a healthy life style with the available limited resources. Regular chemotherapy in school age children is the most cost-effective public health intervention.

The impact of STH on health and economy has been largely underestimated. STH infection is a major public health problem in developing countries and less significance has been given to it, because its clinical presentations are not acute. The worm infection is prevalent in school-going children. Directly or indirectly it causes malnutrition, growth retardation, anaemia and impaired cognition. The morbidity of these parasitic infections can be reduced remarkably by proper diagnosis and treatment. Therefore, this study was undertaken with the following objectives.

- To study the STH in clinically suspected cases in the paediatric age group of 1-14 years.
- To study the association of STH with age, sex and economic status of the patients.
- To evaluate the relative merits of diagnostic methods like direct wet mount with concentration methods, such as saturated salt floatation, formalin-ether sedimentation and Kato-Katz semiquantitative techniques.

METHODS: Four hundred and fifty stool specimens from children of 1 to 14 years of age, attending the outpatient and inpatient departments, suffering from one or more of the classical symptoms like abdominal pain, diarrhoea, dysentery, constipation, low grade fever and pica and 100 control group without any classical symptoms of STH were collected. The study was undertaken from May 2003 to May 2004 in the Department of Microbiology, Bangalore Medical College, Bangalore, Karnataka, India. Out of 450 samples 270 were males and 180 were females, and 327 patients were from urban area and 123 were from rural area with different socio-economic strata.



Fresh faecal specimens were collected in a clean wide-mouthed, leak-proof plastic container. The majority of specimens were submitted to the laboratory within 30

minutes of passage. Patients were advised not to contaminate the specimen with urine or water. The specimens thus collected were examined in the following methods within two hours as per the recommended procedures. The samples were subjected to macroscopic and microscopic examinations for eggs, larva, or whole adult worm, saturated salt floatation, formalin-ether sedimentation and Kato-Katz techniques. Kato-Katz, a semiquantitative technique is used for STH and schistosomiasis epidemiology and control work and is recommended by WHO (Figure 1 and Figure 2).³

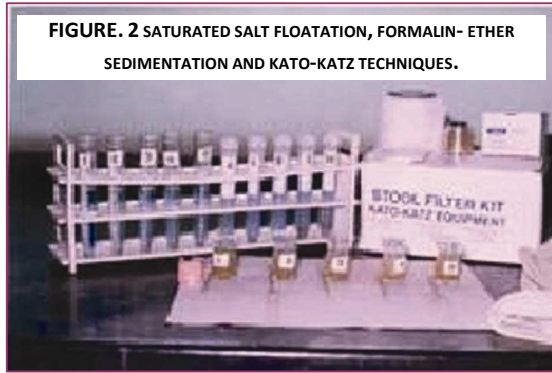


FIGURE. 2 SATURATED SALT FLOATATION, FORMALIN- ETHER SEDIMENTATION AND KATO-KATZ TECHNIQUES.

PROCEDURE OF KATO-KATZ METHOD (CELLOPHANE FAECAL THICK-SMEAR):

Pieces of nylon screen measuring 30-35 mm were cut. The hydrophilic cellophane of 30-35 mm were cut and placed in a jar containing glycerol and left for at least 24 hours. To increase the visibility of the eggs, 1 ml of 3% aqueous methylene blue was added to the glycerol solution. The cellophane strips were soaked in 50% glycerol-methylene blue solution for 24 hours before use. A small

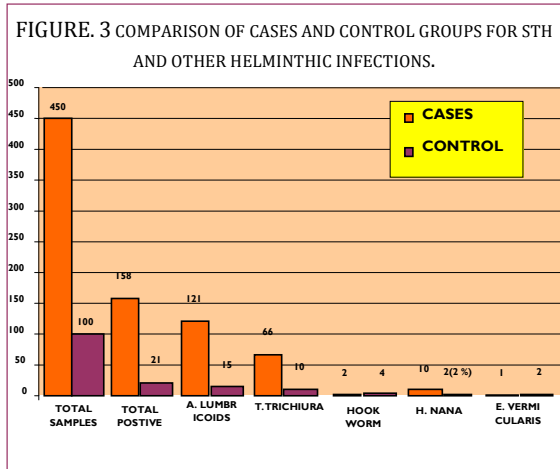
amount of faeces was transferred onto a piece of filter paper. The mesh was pressed on the top of the faecal sample. By using the flat-sided spatula the stool sample was collected from the nylon mesh by scraping the upper surface of the screen. The template was placed on a clean microscopic slide. Scraped faecal material was filled in the hole of the template with an applicator, which holds 41.7 mg of faecal material. The template was removed carefully so that all the faecal material was left on the slide and none was sticking to the template. With the help of fine forceps, the cellophane strip was soaked in glycerol and applied on the faecal material. With the help of another microscopic slide, the preparation was pressed enabling smooth and even spread of material. When the excess glycerol was present on the surface of the cellophane, it was wiped with a piece of filter paper. The microscopic slide was gently removed sideways from the cellophane in such a way that the cellophane was not separated.^{2,3}

READING OF KATO-KATZ PREPARED SLIDES (CELLOPHANE FAECAL THICK SMEAR): After preparation the slide was kept in an incubator at 40 °C for 30 minutes. Then the slides were wiped with a blotting paper and observed under microscope. The smears (cellophane faecal thick-smear) were examined in a systematic manner and number of eggs of each species in every slide was counted and noted. Later a multiplication factor, 24 was used to estimate the number of eggs per gram of faeces. The result was noted down, eggs per slide, eggs per milligrams of faeces for each child separately. From the observations of the eggs, precise estimation of intensity of infection and prevalence of the species in the study population was interpreted.³

CULTIVATION OF ASCARIS LUMBRICOIDES AND TRICHURIS TRICHIURA EGGS IN THE SOIL.

The positive stool samples of *A. lumbricoides* and *T. trichiura* were taken into a plastic container with small amount of soil and water, and lid of the container was just covered, but not completely closed. Everyday moisture was maintained by adding a little amount of water for about 4 to 7 weeks. The development of embryo within the eggs was observed everyday under the low and high power microscope.

RESULTS: A total of 550 stool samples were examined, of which 450 were from the study group and 100 were from the control group. Thirty five per cent from the study group and 21 per cent from the control group were found positive for one or more helminthic infection (Figure 2). Fifty four per cent of males and 46 per cent of females were positive for one or more STH in the study group, whereas in the control group, the positivity was 13% and 8% in males and females respectively. However, it was not statistically significant in both the study and the control groups (P - 0.07 and P - 0.57 respectively).



A. lumbricoides was the commonest worm followed by T. trichiura, H. nana, Hookworms and E. vermicularis in the study group (Figure 3). On the other hand, in the control group, the prevalence of Hookworms, H. nana and E. vermicularis are significantly more than the case group (Figure 3). Mixed infections were seen in 38 children (24%), of which double infections found in 35 (22%), triple in 4 (3%) and quadruple infections in 1 (0.22%) in the study group. S. stercoralis was not found either in the study or the control groups. The present study correlates relatively

with the study of Bora et al. (2001) who also used WHO sampling design methodologies and Kato-Katz technique in school children (Table 1).

TABLE 1. COMPARATIVE STUDY OF DISTRIBUTION OF STH

TYPE OF WORMS	Bora et al ⁴ (2001)	PRESENT STUDY (2004)
A. LUMBRICOIDES	26%	27%
T. TRICHIURA	25%	15%
HOOKWORM	0.74%	0.44%

The predominant age group for STH infection was found in 7-9 years which was statistically highly significant (P - 0.0019) (Table 2). By contrast, in the control group it was not statistically significant (P - 0.13). This finding is in agreement with Paul et al. (1999) study who also found maximum prevalence in this group.

The prevalence of helminthic infection was seen from 1 year onwards, but decreased in the 5-7 years and reached a peak in 7-9 years age. A. lumbricoides and T. trichiura infection was seen in all age groups. A. lumbricoides was more common in 7-9 years, whereas T. trichiura infection was more in 5-7 years (Table 2). Hookworm infection was seen only in six children, of which 2 were in the study group and 4 in the control group.

Male preponderance was found compared to females in the present study. However, it was not statistically significant (P - 0.076) in the study as well as in the control groups (P - 0.57). Among this, 54% were male and 46% were female children. A. lumbricoides and T. trichiura infections were more common in male children. By contrast, H. nana was more common in female children. However, Sex preponderance was not seen with hookworm infection.

TABLE 2. AGE AND SEX DISTRIBUTION OF HELMINTHIC INFECTION

AGE IN YEARS	TOTAL SAMPLES	POSITIVE CASES							
		TOTAL POSITIVE (%)	SEX DISTRIBUTION		A. LUMBRICOIDES (%)	T. TRICHOURA (%)	HOOK WORM (%)	H. NANA (%)	E. VERMICULARIS (%)
			MALE (%)	FEMALE (%)					
1-3	112	23 (19%)	12 (50%)	11 (50%)	19 (81%)	07 (38%)	00 (00%)	00 (00%)	00 (00%)
3-5	84	31 (36%)	14 (47%)	17 (53%)	23 (74%)	16 (50%)	01 (03%)	04 (8%)	01 (03%)
5-7	54	18 (28%)	08 (47%)	10 (53%)	12 (63%)	09 (53%)	00 (00%)	03 (16%)	00 (00%)
7-9	65	31 (48%)	17 (53%)	14 (47%)	25 (82%)	13 (38%)	00 (00%)	02 (06%)	00 (00%)
9-14	135	55 (37%)	35 (66%)	20 (34%)	42 (76%)	21 (39%)	01 (06%)	01 (06%)	00 (00%)
TOTAL	450	158 (35%)	86 (54%)	72 (46%)	121 (75%)	66 (41%)	02 (03%)	10 (02%)	01 (0.22%)

There were more positives among boys when compared to girls. However the prevalence was higher among girls (40%) when compared to boys (36%). But it was statistically not significant (P - 0.076).

The prevalence of STH was more in urban (36%) compared to rural population (32%), however statistically it was not significant (P - 0.35). This finding was in contrast with Usha C. Parekh et al. (1972) in urban (39%) and

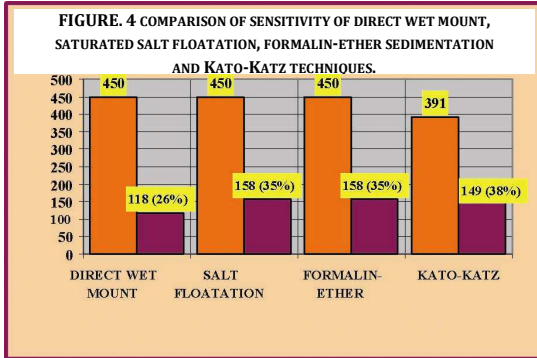
rural (43%). In the control group, 6 and 15 children were positive for one or more STH form rural and urban background, respectively. As in the cases, it was also not statistically significant (P - 0.82). The prevalence of hookworm was seen only in 0.44% of the children.

TABLE 3. DISTRIBUTION OF HELMINTHIC INFECTION IN DIFFERENT SOCIO-ECONOMIC STRATA

SL. NO	SOCIAL CLASS	Aggarwal et al ⁷ (1972)	PRESENT STUDY (2004)
1	LOWER CLASS	137 (36%)	65 (38%)
2	MIDDLE CLASS	63 (21%)	93 (34%)
3	HIGHER CLASS	1 (3%)	NIL

The prevalence of helminthic infection was highest in the lower socio-economic status followed by the middle socio-economic status. However, none of the children in high socio-economic status was positive (Table 3). In the control group 4% and 17% of children were found positive in the lower socio-economic status and the middle socio-economic status respectively. While the difference among

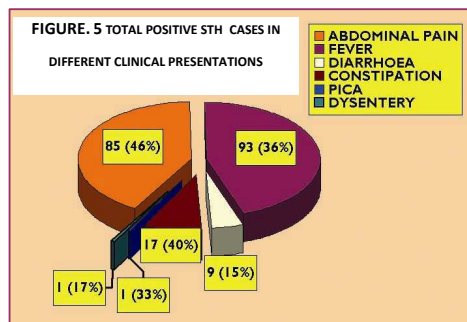
the socio-economic groups was statistically not significant in cases (P - 0.208), in the control group, it was statistically significant (P - 0.012). The same finding, also observed by Agarwal et al. (1972) showed the highest prevalence in the low socio-economic status followed by the middle socio-economic status and least in the high socio-economic status (Table 3).



The stool specimens were processed by direct wet mount and concentration methods like saturated salt floatation and formalin-ether sedimentation methods and by Kato-Katz semiquantitative method eggs in the preparation were counted to determine the intensity of infection. In the saturated salt floatation and formalin-ether sedimentation techniques, 35% children were found positive (both sensitivity and specificity was 100%) when compared to the direct wet mount method, where only 26% were positive, the sensitivity was only 74.49% and specificity was 100%. These concentration techniques helped to detect overall 9% more positives which were not detected by direct wet mount preparation (Figure 4). The positive predictive value was 100% in all the three techniques.

Similarly, in the control group, the sensitivity of direct wet mount, saturated salt floatation and formalin-ether sedimentation techniques were 63.15%, 100% and 100% respectively and the specificity was 97.46%, 100% and 100% respectively. The positive predictive value was 85.71% in the wet mount technique whereas it was 100% in both the concentration techniques.

Out of 450 clinical samples, only 391 specimens were processed by Kato-Katz semiquantitative technique. Fifty nine stool specimens were loose or watery in consistency and hence could not be processed by this method, as the template hole could not hold loose or watery stool specimens. Out of 391 samples examined 38% were found positive (Figure 4). This technique helped to detect overall 12% more positives which were not detected by direct wet mount preparation



Out of 450 specimens from the study group maximum children presented with abdominal pain, (Figure 5 and Table 4). The present study correlates with Daniel, S. Blumenthal. & Myron, G. Schultz (1975), who also showed that 48% of children had abdominal pain out of 21 children screened, who harboured helminths. Fever is the second most common symptom. Low-grade fever is the classical presentation in STH and other intestinal helminths.

Daniel, S. Blumenthal. & Myron, G. Schultz (1975) showed fever in 38% of 21 children examined suffering from ascariasis in their study. This is followed by diarrhoeic presentation; Louw (1966) conducted a study on abdominal findings like watery stools in 28% out of 68 cases. Only about 8% children had constipation, Daniel, S. Blumenthal. & Myron, G. Schultz. (1975), reported prevalence of intestinal obstruction in children infected with *Ascaris* from Southeastern United States and obstipation (absolute constipation) for 24 hours was found in 43% patients out of 21 children screened. Out of 3 children presented with pica one child was found positive for *A. lumbricoides* (Figure 5 and Table 4).

TABLE 4. DIFFERENT CLINICAL PRESENTATION

SL. NO	CLINICAL PRESENTATION	NUMBER OF CASES	POSITIVE	SENSITIVITY	SPECIFICITY
1	ABDOMINAL PAIN	185 (41%)	85 (46%)	53.79%	65.75%
2	FEVER	261 (47%)	93 (36%)	53.86%	42.46%
3	DIARRHOEA	59 (11%)	9 (15%)	5.69%	82.87%
4	CONSTIPATION	43 (8%)	17 (40%)	10.75%	91.09%
5	PICA	3 (0.66%)	1 (0.22%)	---	---
6	DYSENTERY	6 (1%)	1 (17%)	0.63%	98.63%

A total of 6 children had dysentery, of which one male child was positive for a mixed infection with *A. lumbricoides* and *T. Trichiura* (Figure 5 and Table 4). Callender et al. (1993) studied the developmental levels of young children with *T. trichiura* dysentery syndrome (TDS) in 19 children and the most severe form of *T. trichiura* infection is characterised by chronic, mucoid bloody diarrhoea. These children had

serious lag in the development of 16 Developmental Quotient Points, stunted growth and were anaemic.

TABLE 5. INTENSITY OF STH INFECTION IN THE STUDY GROUP (WHO METHOD)

TYPE OF STH	POSITIVE CASES	LIGHT INFECTION	MODERATE INFECTION	HEAVY INFECTION
A. LUMBRICOIDES	115	98 (85%)	17 (15%)	00 (00%)
T. TRICHIURA	62	12 (19%)	26 (42%)	24 (39%)
HOOKWORM	02	00 (00%)	01 (50%)	01 (50%)

The intensity of infection was calculated by Kato-Katz method in 149 positive cases of the total 391 children screened (Table 5). The intensity of infection was calculated based on the WHO methodology.¹¹

TABLE 6. MEAN INTENSITY AND STANDARD DEVIATION BY EGGS PER GRAM (EPG) OF DIFFERENT WORMS

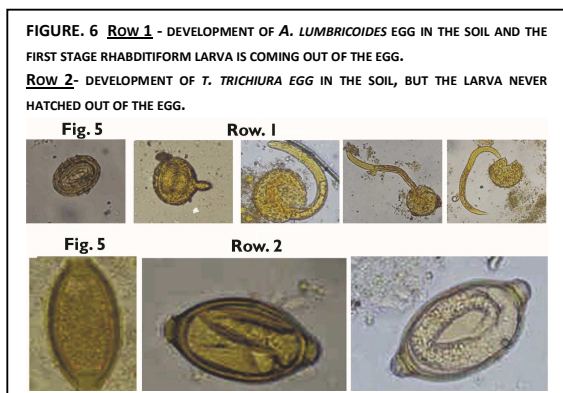
INFECTION	CASES MEAN/ STANDARD DEVIATION	CONTROLS MEAN/ STANDARD DEVIATION
A. lumbricoides	7359.58/ 5034.99	6214.38/ 5223.97
T. trichiura	7164.42/ 5510.90	1640.71/ 2524.84
Hookworm	3722.5/ 1358.35	1498.25/ 404.71

Table 6 presents the Mean and Standard Deviation of intensity of *A. lumbricoides*, *T. trichiura* and hookworm infections for cases and control groups. In the study group, the mean ova count for *A. lumbricoides* was slightly more than *T. trichiura* and least in hookworm infection. On the contrary, in the control group, the mean eggs count of *A. lumbricoides* was 5 times more than *T. trichiura*, and similar to cases, the hookworm mean ova count was lowest in number in the control group also. A notable difference in mean ova count was seen with *T. trichiura* where it was 6 times more in the cases than the control children. Similarly, the difference was also found with

hookworm infection. However, between the two groups, there was no such wide variation as for as *A. lumbricoides* concerned. The mean ova count was almost equal between cases and control groups for *A. lumbricoides*. On the contrary, there was a wide variation between the two groups for *T. trichiura* and hookworm. This indicates that *A. lumbricoides* has a high tendency to shed eggs in large numbers in spite of being asymptomatic in the present study. The high standard deviation values indicate wide variation in the intensity of STH infection. The highest standard

deviation was found in *A. lumbricoides*, followed by *T. trichiura* in both study and control groups.

CULTIVATION OF *A. LUMBRICOIDES* AND *T. TRICHIURA* EGGS IN THE SOIL: Initially, the unsegmented ova started growing to the blastomeric stage in about one to two weeks in the soil. In the 3rd or 4th week the larvae showed movement within the eggs. By 5th or 6th week, the first stage Rhabditiform larvae of *A. lumbricoides* hatched out of the ova (Figure 6, Row 1).



Similarly, the *T. trichiura* larvae also were grown in the soil. However, the larvae never hatched out of the eggs, though larvae were showing movement within the eggs (Figure 6, Row 2).

The development of these ova were observed under the 10X and 40X objectives everyday by wet mount preparation such as saline and iodine mount. The hatching of *A. lumbricoides* larvae were seen especially in iodine mount than in saline mount. This may

be probably due to an inducing stimulus of iodine on the embryonated eggs to hatch. Surprisingly, the embryonated ova of *T. trichiura* did not hatch either in the saline or iodine preparation although the larvae were moving vigorously within the ova (Figure 6, Row 2).

DISCUSSION: Out of the 550 stool samples examined 450 were from the study group and 100 were from the control group. All the 450 children of the study group were presented with the classical symptoms of soil-transmitted and other intestinal helminthes. One hundred children were selected in the control group did not have any classical symptoms. Thirty-five per cent from the study group and 21% from the control group were found positive for any one of the parasites.

Ascaris was more common in the age group of 7-9 year old children, whereas *Trichuris* was common in 5-7 years. Hookworm eggs were found in only two children from 3-5 and 9-14 years of age group. This finding suggests that ascariasis might be the disease of the urban and hookworm infection is the disease of rural area. Apart from this, most of the children who come to government hospitals are mostly from urban slums, where the people live in overcrowded and unsanitary conditions. On the hand, hookworm infection is more common in rural children, where people have the habits of open-air defaecation and walk barefoot. The high prevalence of STH in school age group could be due the fact that children of that age play outdoors and probably tend to put everything into their mouths, which might be contaminated with faeces. In addition, there is lack of awareness regarding personal cleanliness and hygiene.

Male preponderance was seen with *A. lumbricoides* and *T. trichiura*, whereas equal distribution was seen with hookworm. On the contrary females showed preponderance as far as *H. nana* infection is concerned. The higher prevalence of helminthic infections was perhaps due to more outdoor activity in male than female children.

Maximum stool samples were from urban population compared to rural background. This may be due to the fact that in a developing country like India, urbanisation has resulted from unplanned, uncontrolled and constant migration of people from rural areas in search of job

living in urban slums without proper sanitary latrines, meagre resources of water supply, garbage disposal, poor health care and hygiene.

The prevalence of hookworm was seen only in 0.44% of the children because majority of the patients were from the urban setting and usually high prevalence of hookworm infection is seen in rural agricultural population rather than urban dwellers. Hence, the prevalence of hookworm was low in the present study.

The prevalence of helminthic infection was highest in the lower socio-economic status followed by the middle socio-economic status. However, none of the children in high socio-economic status was positive. This is mainly because of poverty, illiteracy, bad hygiene, poor housing conditions, bigger family sizes with small places to live, and women marrying at an early age with bearing more children. Hence, there will be more chances of infection spreading within the families due to unhygienic practices.

Routine examination of faecal samples using direct wet mount of saline and iodine preparations has obvious limitations. In direct wet mount preparation, there is a chance of missing light infections, where helminthic load is low and the relative intensity of eggs cannot be determined. The advantage of concentration methods over the direct wet mount is that light and intensity of infections can be better appreciated. In Kato-Katz semiquantitative technique we can detect very light infections and also intensity of infection. Consequently, we can treat light and asymptomatic children and women of child bearing age. Thus the concentration techniques are better than the direct wet mount technique for authentic and reliable results. The application of either saturated salt floatation or formalin-ether sedimentation technique in conjunction with direct wet mount preparation is proved to be the best.

The clinical manifestations of STH and other intestinal helminths are protean in nature. The child may have many worms in the intestine but still asymptomatic. Among the six classical symptoms of STH, abdominal pain and fever were the best predictors of STH infection, sensitivity and specificity (Abdominal pain-53.79% & 65.75% and Fever 58.86% & 42.46% respectively). On the other hand, constipation and diarrhoea were moderate predictors of STH (sensitivity 10.75% & 5.69% and specificity 91.09% & 82.87% respectively). However, pica and dysentery were poor predictors of STH (sensitivity 0.63% & 0.63% and specificity 99.31% and 98.63% respectively).

It was surprising to see that among 100 children in the control group, 21% were found positive for one or more STH infection in spite of being asymptomatic (Figure 2). Hence, it may be prudent to treat all children with anthelmintics whether symptomatic or asymptomatic. If not treated, the asymptomatic children, in due course might develop the complications associated with STH infections like anaemia, malnutrition, growth retardation etc. Moreover, these children are a dangerous silent source of infection to others. Apart from the soil-transmitted and other intestinal helminths 9% of children showed protozoal infections like *Entamoeba histolytica*, *Giardia lamblia*, and *Balantidium coli*.

CONCLUSION: Concentration techniques like salt floatation and formalin-ether are the best diagnostic tool to get authentic and reliable results and to rule out fallacious inference caused by relying only on direct wet mount preparation. Kato-Katz, a semi quantitative technique is the best diagnostic method to know the prevalence and intensity of the soil-transmitted helminthic infection. The technique is simple, rapid, reliable and mild infections can also be detected. No special equipment is needed other than the microscope, so this can be performed in the field survey as well. This technique does not need the expertise anyone can easily be trained by this

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technique. Thereby prevent the transmission of parasitic infections in the community by giving appropriate treatment and providing sanitary facilities with health education.

The availability of single dose broad spectrum oral anthelmintic drug has simplified treatment and made control programmes logistically feasible. Hence, the corner stone of WHO comprehensive package for control of morbidity due to STH infections is mass anthelmintic therapy for high risk groups of children and women of child bearing age with health education as short term strategies while long term control measures include adequate sanitation, proper drinking water facility, proper defecation sites, wastage disposal and personal hygiene and community participation is suggested as an effective and appropriate preventive programs in primary health care and thus aimed towards achieving Health for all by 2000 AD.

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