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Screening of Parasites in Stool Sample of Orphanage and Destitute Home Children in Kalaburagi

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A B S T R A C T

Intestinal parasitic infections are prevalent in conditions which have poor hygiene, environment and sanitation. Orphans always lack in basic monetary and sanitary assets in their livelihood. Overcrowded places with lack of cleanliness like orphanages and destitute homes are most dangerous as the infection spreads through them swiftly leading to multiple infections (1). In this tender age group, children are not outspoken about their problems, which can increase the chronicity of the infestation leading to serious impairment in their health. Kalaburagi being a dry and developing city has a great possibility that parasitic infections may be highly prevalent. Aim of the project was to find the most common infestation in the orphanage and destitute home children of age group 5 to 15 years in the city of Kalaburagi. Also the aim was to advise the children about personal hygiene and sanitation. Material methods: The study was performed over 2 months from the month of June 2015 in seven orphanages and destitute homes which constituted of both government and private funded organizations, in the city of Kalaburagi. A total of 232 children were examined. All the procedures were referred from WHO recommended ―Manual of basic techniques for a Health Laboratory. Results: From the 182 samples examined by direct smear and concentration techniques, 89 samples (48.9%) were noted positive for the presence of intestinal parasite. The most common infestation was *Entamoeba coli* in 38 samples (42.69%), followed by *Entamoeba histolytica* with 32 samples (35.95%) showing positivity. The third most common parasite was Giardia lamblia with 18 positive samples (20.2%). *Hymenolepis nana* was prevalent in 15.7 % of positive samples. 1.1% of *Ascaris lumbricoides*, 2.2% of *Strongyloides stecoralis*, 1.1% of *Trichuris trichiura*, 2.2% of *Trichomonas hominis* and 1.1% of *Enterobius vermicularis* were also found. This study re-instates the fact that parasitic infections are still prevalent among children of age group 5-15 years. The onus of parasitic infection mingled with poor sanitary facilities must be considered as an alarming public health issue. This clearly denotes there is requirement of government intervention and programs to improve health status of these individuals.

Introduction

Intestinal parasitic infections are prevalent in conditions which have poor hygiene, environment and sanitation. These conditions are clearly visible in debilitated socio-economic societies of a developing country like India. In addition poverty, low health status and malnourishment improves the growth and spread of intestinal parasitic infections. Orphanages are a classical example of such a scenario. Orphans always lack in basic monetary and sanitary assets in their livelihood. Hence their debilitated lifestyle makes them susceptible to infections. Overcrowded places with lack of cleanliness like orphanages are most dangerous as the infection spreads through them swiftly leading to multiple infections (1). The practice of maintaining self hygiene and sanitation is taught to children by parents. Although this is the job of a caretaker present at the orphanage, yet these infections are evidently present in such institutions.

According to WHO, out of 17 Neglected Tropical Diseases (NTDs), eight diseases are intestinal parasitic infections. These parasitic infections cause immense human morbidities as they play their role in aiding poverty and hampering the development of the country. Development of a country like India is hindered immensely due to the lack of health monitoring, especially in the rural regions of the country. India is the 7^{th} largest country and currently the 2^{nd} largest population in the world. It is a humongous task to provide safe drinking water, hygiene and sanitation to each and every citizen of the country. Stool sample examination which is not routinely done in regular investigations complicates the situation. In spite of widespread incidence, due to loss of acute nature, these infections have rendered intestinal parasitic infection unimportant (2).

According to WHO, there are 2600 million cases of parasitic infections globally and out of these 880 million children are in need of immediate treatment (3). Moreover, these statistics are from the symptomatic cases coming to the hospital and health care centers. A majority of intestinal parasitic infections are asymptomatic and there is lack of authentic studies on such cases. Intestinal parasitic infections lead to malabsorption syndrome proceeding to lack of mental, physical and psychomotor development in children in pediatric age group. In this tender age group, children are not outspoken about their problems, which can increase the chronicity of the infestation leading to serious impairment in their health. Recurrent infections to the body can affect the growth of immune system in the body, as it is in a developing stage. Parasitic infections are more common in children as compared to adults (2).

Out of the infected 2 billion people in the world with intestinal parasites, more than half are school going children (4). Orphans are the most vulnerable part of this group and they need the maximum support from the government. There is a paucity of information on the prevalence of infections in orphanages of India. In India, the overall prevalence rate of intestinal parasitic infections range from 13-66%, due to varying geographical conditions. Parasitic infections especially protozoan and helminthic are closely associated with tropical climate and low altitude (5). The city of Kalaburagi is placed in subtropical zone of India with a low altitude (1490 feet). Hence, the persistence of parasitic infections is a possibility. It is one of the high temperature zones of Karnataka which can increase the prevalence of protozoan to helminthic infections.

Kalaburagi being a dry and developing city has a great possibility that parasitic infections may be highly prevalent. Aim of the project is

To find the most common infestation in the orphanage children of age group 5 to 15 years in the city of Kalaburagi.

To know about the treatment aspects for each of them.

To advise the children about personal hygiene and sanitation.

The children receiving proper treatment for the infection will clear out on their respective morbidities and become healthy individuals. The work experience gained in the field and also in the diagnostic laboratories will help me as medico to understand about parasitic infection and improve my caliber of rural service in India.

Materials and Methods

Study plan

The study was performed over 2 months from the month of June 2015 in seven orphanages which constituted of both government and private funded organizations, in the city of Kalaburagi. A total of 232 children were examined and their Performa was duly filled.

The inclusion criteria were that the child must be staying in the orphanage and must come under the age group of 5 to 15 years. The children of age less than 5 years and the adults were excluded from the study. A meeting was set up with the institute authority and the warden at each institution.

The project procedure, implications and advantages were thoroughly explained to them. Then, a brief presentation was taken on the implications of parasitic infections on children's health for the orphanage children to sensitize them about the issue. A written consent was taken from the institute authority as a guardian of the children.

Study design

A case file was made for each child and their symptoms were asked privately which were noted down on their Proforma. Followed by that, a general physical examination was done on them. Apart from abdominal complaints other symptoms and signs were noted down and referred to specific specialists.

Each child was specifically instructed about collection and preservation of sample, at the same time to maintain personal hygiene. A sterilized, broad mouthed, labeled, cap with attached spoon plastic container of capacity 30ml (HiMedia laboratories) was provided to every individual.

All individuals were asked to provide a morning fecal sample with no contamination of urine. The holidays and Sundays were earmarked on the calendar. On these days samples were collected from selected candidates and were examined within 4 hrs at the Department of Microbiology in the medical college.

Materials and apparatus

Materials: Glass slides, Cover slips (22mm*22mm), Light Microscope, Analytical funnel (75mm), Sterilized nichrome wire loop, Spirit lamp, surgical Gauze piece, Centrifuge tubes (15ml), Test tubes (15ml), Discarding jar.

Chemicals: Normal saline, Lugol's iodine, Eosin stain (1%), Formalin (10%), Ethyl acetate

Apparatus: Centrifugation machine with capacity of 8 tubes.

Methods

Gross examination: The sample was examined for color, consistency and smell

Saline mount: Using normal saline
Iodine mount
1 % Eosin staining (for suspected protozoa)
Ridley-Allen modification of Formol- Ethyl acetate sedimentation technique.

All the procedures were referred from WHO recommended "Manual of basic techniques for a Health Laboratory"2nd edition. All the slides were initially screened at 10x objective of light microscope. Then high power objective was used to identify the parasite. If negative, the samples were concentrated using the sedimentation technique and re examined under light microscope in both saline and iodine mount. The observations were noted down on the Proforma. The information was filled on a database using Microsoft Access 2007 Software to maintain the record of each child. The criteria set up in the database were same as that of headings in the Proforma. The samples were examined by the student assisted by the guide. One in 20 samples was examined by another colleague.

Results and Discussion

Out of the 232 children examined in the age group of 5 to 15 years, 182 children(78.4%) gave their stool samples.50 children who initially accepted for the study, did not give the samples later. The male to female ratio in our study was 0.9:1 depicting almost equal sex ratio at the institutes.

From the 182 samples examined by direct smear and concentration techniques, 89 samples (48.9%) were noted positive for the presence of intestinal parasite. Out of 89 samples 71 (79.7%) were tested positive for a single parasitic infestation and 18 children (20.2%) had infestation with minimum of 2 intestinal parasites.

The most common infestation was *Entamoeba coli* in 38 samples (42.69%), followed by *Entamoeba histolytica* with 32 samples (35.95%) showing positivity. The third most common parasite was *Giardia lamblia* with 18 positive samples (20.2%). *Hymenolepis nana* was prevalent in 15.7 % of positive samples. *Entamoeba* species were most commonly found parasite in multiple infections out of which *Entamoeba coli* was in 15 samples (16.85%) and *Entamoeba histolytica* in 12 samples (13.48%). 1.1% of *Ascaris lumbricoides*, 2.2% of *Strongyloides stecoralis*, 1.1% of *Trichuris trichiura*, 2.2% of *Trichomonas hominis* and 1.1% of *Enterobius vermicularis* were also found. *Enterobius vermicularis* adult male worm was also found on gross and microscopic examination (Fig. 1–6).

Gender (refer to Table 1)

The chi square statistic (x^2 = 0.187), our predetermined alpha level of significance (0.05), and our degrees of freedom (df = 1). The Chi square distribution table with 1 degree of freedom and reading along the row we find our value of x^2 (0.187) less than 0.5. The corresponding probability is greater than 0.5, i.e. 0.66. This means that the p-value is above 0.05. Since a p-value of 0.66 is greater than the conventionally accepted significance level of 0.05 (i.e. $p > 0.05$) we fail to reject the null hypothesis. In other words, there is no statistically significant difference in the proportion of male and female who were detected with parasites.

Age (refer to Table 2)

The chi square statistic (x^2 = 2.287), our predetermined alpha level of significance (0.05), and our degrees of freedom (df = 3). The Chi square distribution table with 3 degree of freedom and reading along the row we find our value of x^2 (2.287) more than 0.5. The corresponding probability is greater than 0.5, i.e. 0.515. This means that the p-value is

above 0.05. Since a p-value of 0.515 is greater than the conventionally accepted significance level of 0.05 (i.e. $p > 0.05$) we fail to reject the null hypothesis. In other words, there is no statistically significant difference in the percentage detection of parasites with respect to age group.

The study indicated a prevalence of 48.9% intestinal parasitic infections in the children of orphanages of age group 5-15 years. This is comparable to overall prevalence rate in India which is 11-90% across all geographic conditions (30). Interestingly the numbers of protozoan infections were more than that of helminthic, similar to study performed in Thailand (28) (31). The overall prevalence rate obtained is approximately same as the study conducted in school children at Srinagar city (46.7%). This may be due to semi-urban and developing nature of both Srinagar and Kalaburagi. A relatively less prevalence (19.8%) was seen in another study done at Chikballapur district, Karnataka. This is probably due to the influence of clean water and sanitation levels provided by Bangalore organizations which is a metropolitan city.

Other studies performed in Turkish orphanage infers prevalence of very high amount of intestinal parasites (94%) (32). This can be attributed to change in geographical location and also due to timeline taken up for conducting the field work. The month of June-October is rainy season in Kalaburagi which is a low transmission season for intestinal parasitic infections (33).

The commonest infestation in our study was *Entamoeba* species, similar to the study performed by Parameshwarappa *et al.*, at Kalaburagi, *Bisht et al.*, (34)(35) The prevalence was high in the age group of 5-10 years (39.2%) which is comparable with results of other studies (*Bisht et al.*, 68%). This may be due to increased contact of these children with soil and unhygienic conditions. The prevalence of *Entamoeba* has been observed as a common finding in tropical and subtropical countries like India (34).

In many orphanages, children were observed bare footed, working and cleaning their surroundings, but still soil transmitted infections like *Ancylostoma* were not noted. This may be due to deworming. Albendazole treatment was given in these orphanages at least 1 year back. Other concentration methods like saturated salt solution technique in the methodology could have been of more help along with Ridley Allen modification of Formol-ethyl acetate sedimentation technique.

*Giardia*sis was the third most common infestation (20.1%) supplementing the fact that *Giardia*sis is commonest intestinal infestation in the world. There is chronicity of these infections as they are asymptomatic causing maximum morbidities in children. *Giardia*sis was consistently found in all orphanages unlike *Entamoeba*. This is because of usage of chlorinated water to which *Giardia* cysts are tolerant due to presence of strong outer shell.

Table.1 Contingency table for the Data acquired with respect to gender

Gender	Men	Women	Total	Chi test value	DOF	Probability
Positive	44	45	89	0.187	1	0.66
Negative	43	50	93			
Total	87	95	182			

Table.2 Contingency table for the data acquired with respect to the age group

Age	**5-7**			**8-10**		
	Observed	Expected	(O-E)2/E	Observed	Expected	(O-E)2/E
Positive	13	12.225	0.049	27.000	24.451	0.266
Negative	12	12.775	0.047	23.000	25.549	0.254
Total	25			50.000		
Age	5-7			8-10		
	Observed	Expected	(O-E)2/E	Observed	Expected	(O-E)2/E
Positive	13	12.225	0.049	27.000	24.451	0.266
Negative	12	12.775	0.047	23.000	25.549	0.254
Total	25			50.000		

Age	11-13			14-16			Total
	Observed	Expected	(O-E)2/E	Observed	Expected	(O-E)2/E	
Positive	37.000	36.676	0.003	12.000	15.648	0.851	89.000
Negative	38.000	38.324	0.003	20.000	16.352	0.814	93.000
Total	75.000			32.000			182.000

Chi-test	DOF	Probability
2.287	3.000	0.515

Fig.1 Graphical representation of different intestinal infestation in the orphanage children

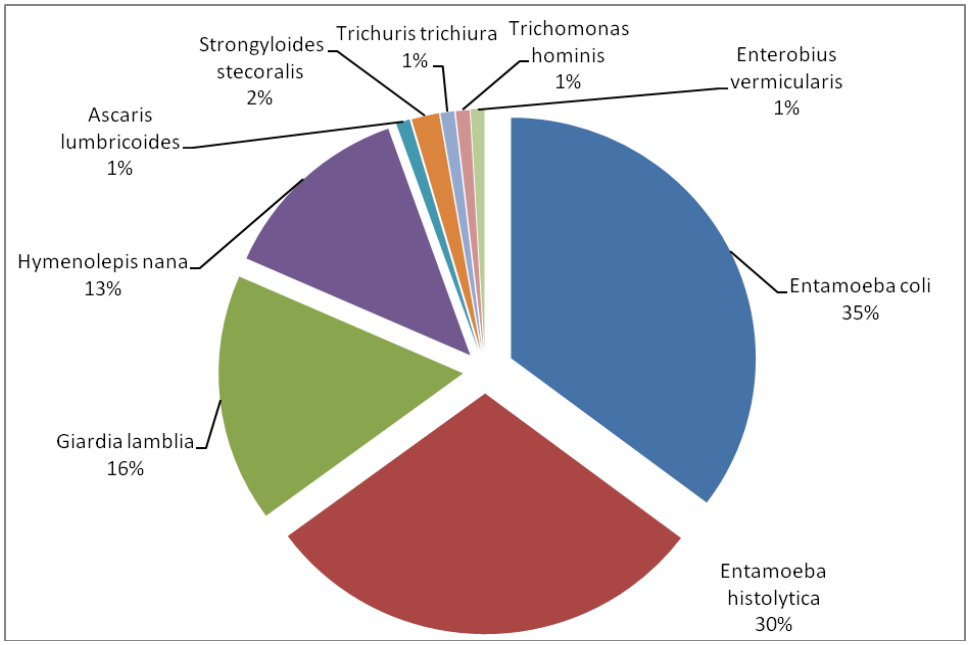


Fig.2 Comparison of intestinal parasitic prevalence in male and female.

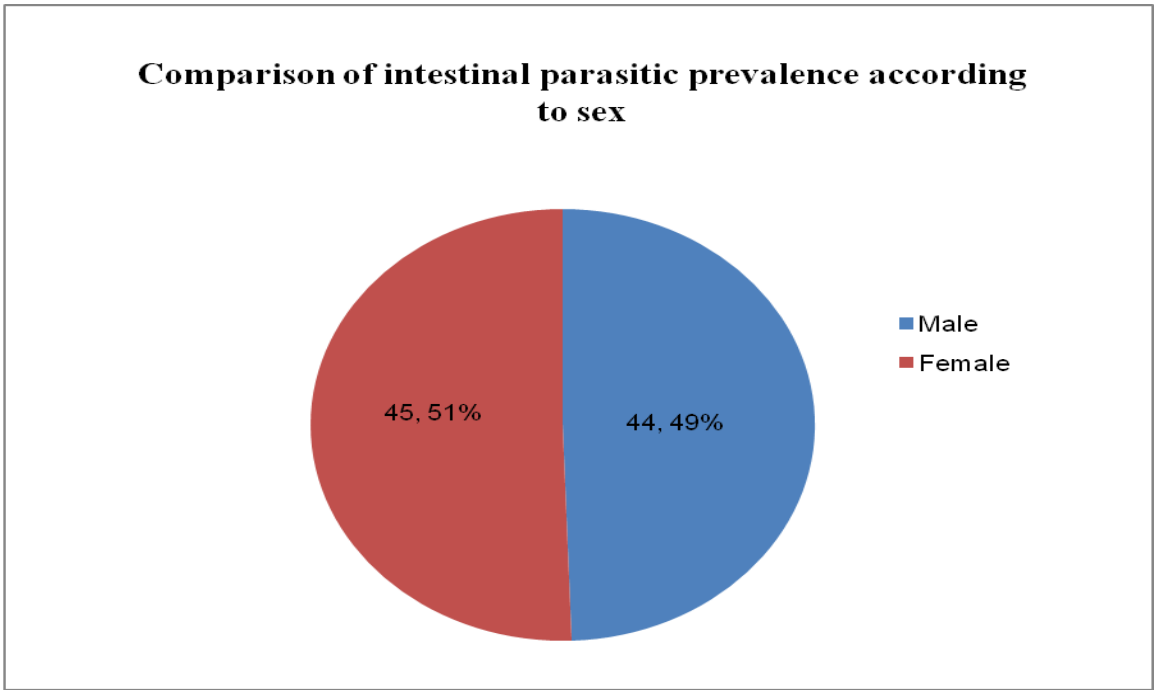


Fig.3 Percentage parasitic prevalence in male on the basis of different age groups

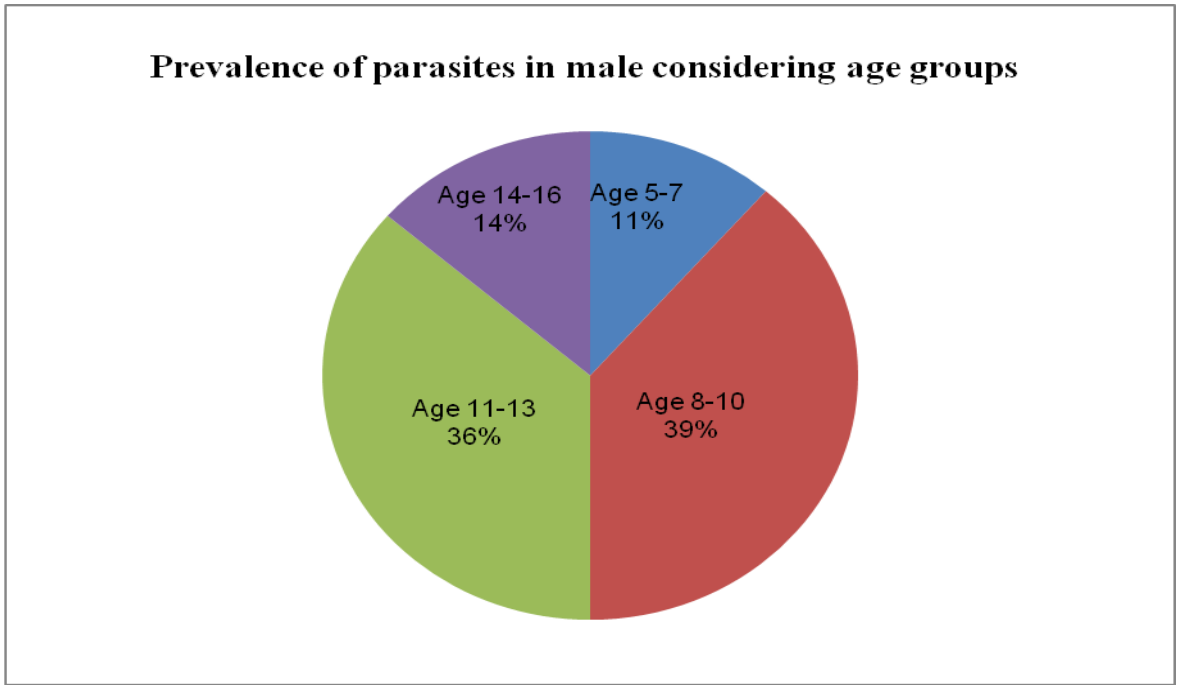


Fig.4 Percentage parasitic prevalence in female on the basis of different age groups

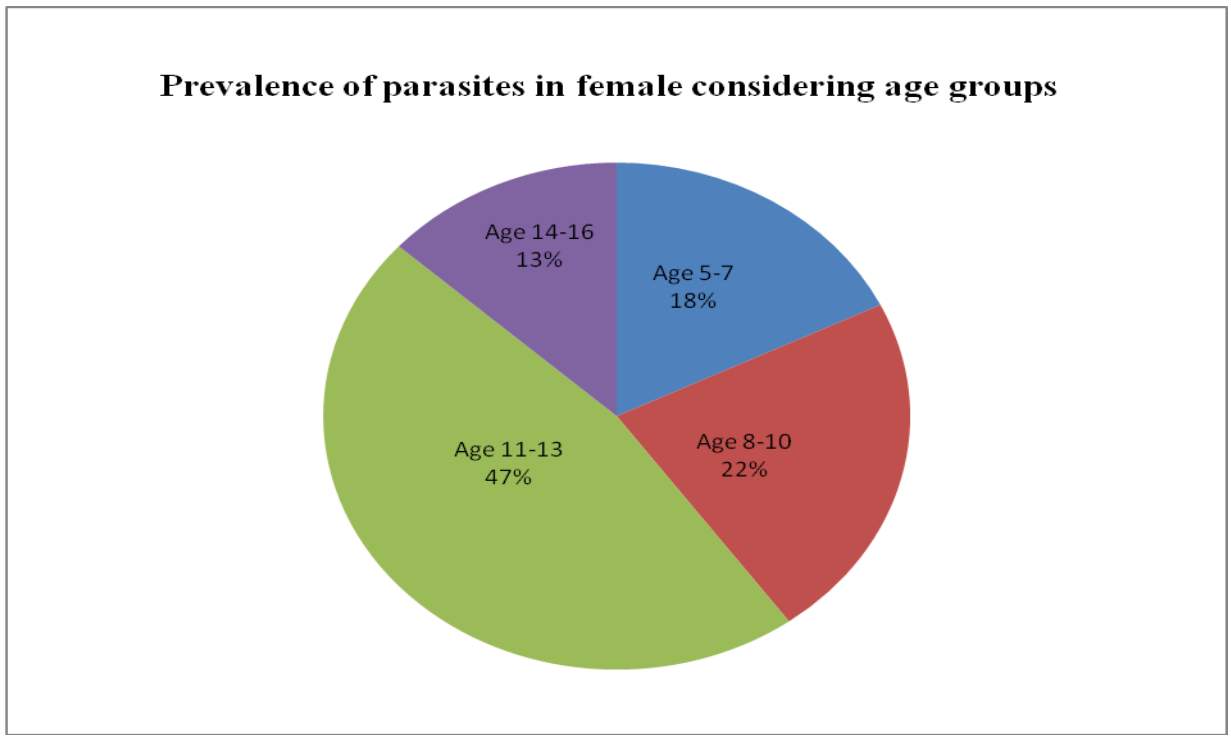


Fig.5 Comparison of affected/non-affected samples with respect to different age groups

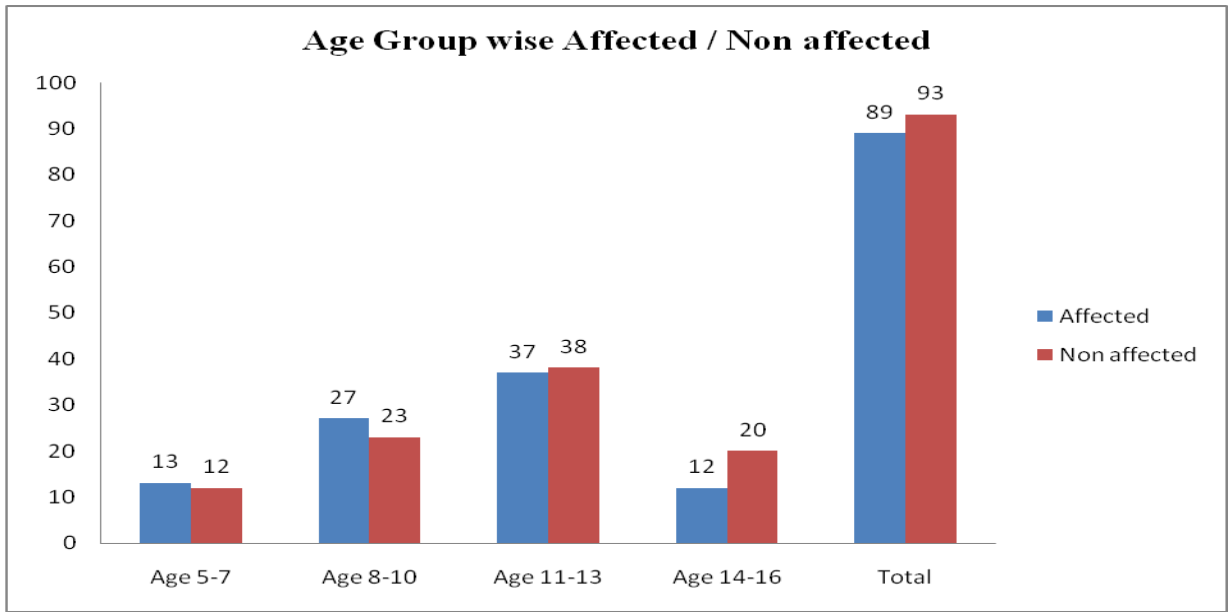
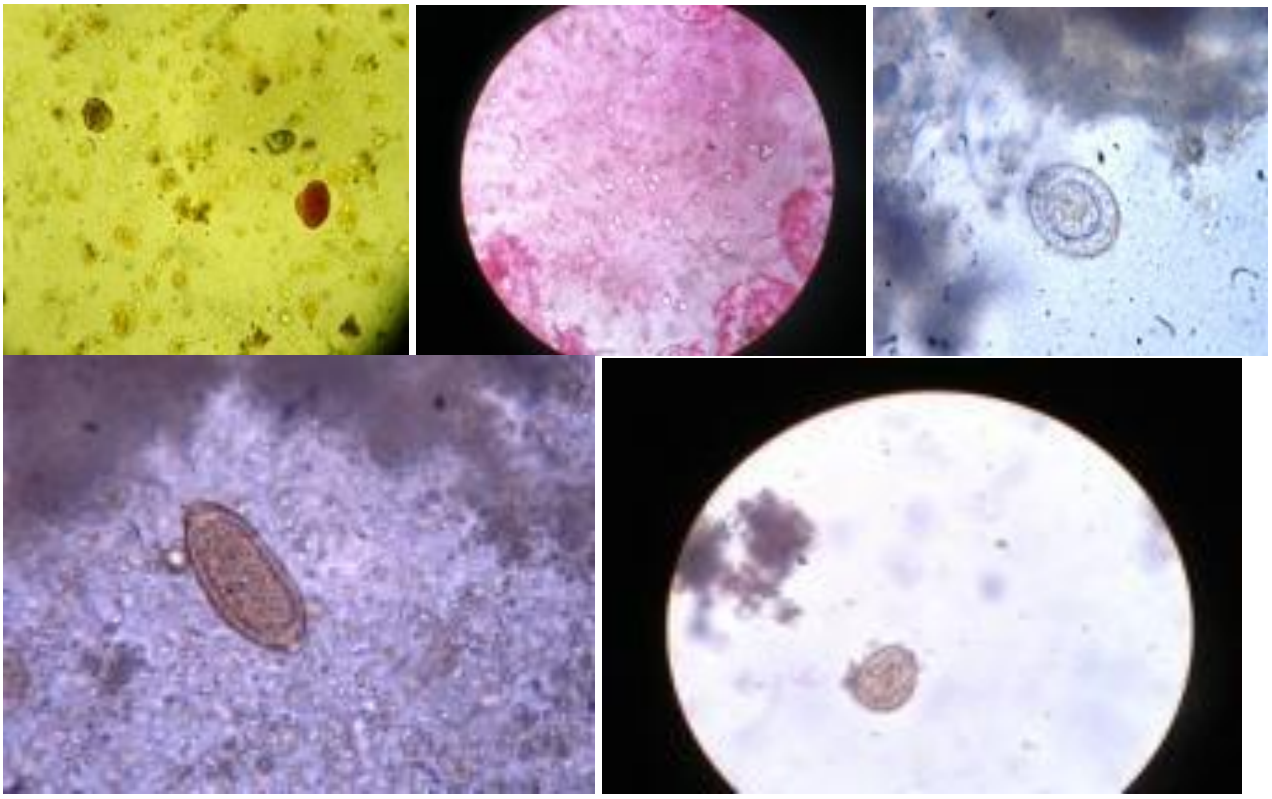


Fig.6 Clockwise from top left *Entamoeba histolytica* trophozite in Iodine mount, *Giardia lamblia* cysts in 1% Eosin mount, *Hymenolepis nana* egg in Saline mount, *Trichuris trichiura* egg in Iodine mount, *Ascaris lumbricoides* egg in Saline mount



Hymenolepis was the most common helminthic infection in our study (15.7%), which is similar to other studies (36). Hymenolepiasis is the most common helminthic infection at countries in which sanitation and hygiene are inadequate, like India (CDC). The transmission of *Hymenolepis nana* is mostly person to person. While talking and counseling children, many reported of sharing the same plate for having food. In other orphanages common bathrooms were used which could play an important role in transmission of helminthic infection.

The pattern of prevalence of intestinal parasites varied at each orphanage. Initially *Giardia*sis was very prevalent. It was also observed with many co-infections. Consequently, the pattern changed in other orphanages where cestodic and amoebic infection prevailed.

Environment factors like soil, water and personal hygiene were not considered statistically. Out of 7 orphanages cots were not observed in 6 orphanages inferring that the children sleep on floor, which may be a factor in transmission of helminthic infection. The technique of hand washing was also poor in the orphanages where in children reported of using dishwashing bar, for cleaning hands. These observations clearly suggest the presence of highly unhygienic condition which favours transmission of parasitic infections.

In conclusion, this study re-instates the fact that parasitic infections are still prevalent among children of age group 5-15 years in the study area. The onus of parasitic infection mingled with poor sanitary facilities must be considered as an alarming public health issue. This clearly denotes there is requirement of

government intervention and programs to improve health status of these debilitated individuals.

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