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INTESTINAL PARASITIC PLETHORA

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ABSTRACT

In an era where anti-helmithic use is more common, it is rare to see any parasite in the stool sample. But in our patients there is a high prevalence rate of parasitic infestation attending our hospital, as proved by the routine stool examination. Aim: To know the prevalence and distribution of the different parasitic infestations in our patients, to identify the ideal method for demonstration of the parasitic ova/cyst. To demonstrate the association of anemia in relation to parasitic infestation. To identify parasitic ova/egg by using Lactophenol cotton blue (LPCB) wet mount. Methods and Material: A total of 300 stool samples were collected and subjected for macroscopic & microscopic examination. Those samples which were negative by wet mount were further subjected for flotation, formal ether sedimentation techniques. For the patients with severe anemia, blood samples were collected and their serum Ferritin levels (ELISA) were assayed. Results: Out of the 300 samples, 129 (43%) were Positive for parasitic infestations which is very high in the current scenario. Our study showed *Entamoeba histolytica* (30%) *Ancylostoma duodenale*(22%), *Ascaris lumbricoides* (16%), *Giardia(*11%), *Taenia*(5%), Rhabditiform larva(4%), *Trichuris trichiura*(2%), Mixed infections(7%), *Cyclospora(*2%), *Chilomastix*(1%). Among 129 positive samples, serum Ferritin levels were very low in 88 cases. LPCB wet mount showed better appreciation of morphology than LPCB staining. Conclusions: Simple wet mount is still a reliable technique for screening& LPCB mount for identifying the ova/cyst morphology in stool samples. The Coexistence of anemia and parasitic infestation is augmented in our study by detection of reduced hemoglobin and serum Ferritin levels (P< 0.001). Formal ether is superior to flotation.

KEY-WORDS: **Wet mount, stool, Lactophenol cotton blue, Concentration technique, Anemia.**

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INTRODUCTION

Knowledge of common parasites date back to antiquity, worm fitting the description of *Enterobius vermicularis, Ascaris lumbricoides*, *Taenia* have been mentioned in the ancient writings from many countries[1]. Intestinal parasitic infections vary considerably from place to place & are closely related to poor socio-economic status, poor environmental hygiene, overcrowding, contaminated food & water[2]. Under nutrition status especially iron deficiency anaemia is observed in the infected individual[3]. In India like other developing countries intestinal parasitic infections is a major health problem. Keeping these aspects in mind we planned to conduct a study to know the prevalence and distribution of the different parasitic infestations in our patients, to identify the ideal method for demonstration of the parasitic ova/cyst, to demonstrate the association of anaemia in relation to parasitic infestation and to identify parasitic ova/egg by using Lactophenol cotton blue (LPCB) wet mount.

MATERIALS AND METHODS

Study Design: Cross-Sectional Study

Statistical analysis used: SPSS 16.0 statistical software and windows Excel - 2007.

Duration of the study: A total of 300 patients were screened in this six months (February 2011to July 2011) period who visited our hospital. (In & out patient).

Samples Collected: Stool, Blood.
Stool was collected in a sterile container and transported to microbiology Lab immediately & and if there was undue delay, refrigerated. The samples were duly labeled and properly filled requisition form with relevant details pertaining to the patient like age, sex, clinical features was submitted to the microbiology lab. Blood samples were collected with sterile precautions for estimation of serum Ferritin levels by ELISA technique[3].

Inclusion Criteria: Patients with diarrhoea & H/O passing worms
Exclusion Criteria: Vague abdominal pain.

Macroscopic examination:
Direct macroscopic examination of faeces was performed to detect adult worms, segments of tape worm, larvae, blood and mucus. The stool consistency i.e. formed; soft, loose, watery was also recorded[4].

Microscopic examination: The stool specimens were processed and examined microscopically with x10, x 40 objectives by Saline, Iodine and LPCB preparation in a compound binocular microscope with attached camera and connected to system.

LPCB wet mount: LPCB wet mounts were prepared by mixing a drop of LPCB stain with a small volume of stool on a glass microscope slide and placing a cover slip on the mixture. LPCB contains 20 g of phenol crystals, 20 ml of lactic acid, 40 ml of glycerol, 0.05 g of cotton blue stain, and 20 ml of distilled water [8]. The saline mount was screened for trophozoites and Iodine mount for cysts. If there was the presence of the ova, cyst or trophozoite, it was identified and reported as per standard protocols. Then the sample was subjected for staining with LPCB. The smear was made from mucous portion of the stool sample, on a clean grease free glass slide and was allowed to air dry. The smear was flooded with LPCB, allowed to wait for 3-5 minutes. Then the excess LPCB was decanted and air dried. Then the smear was examined in x100. A comparative LPCB wet mount of the stool sample was made from the same patient [2]. Those samples which were found to be negative in Saline, Iodine and LPCB mount were subjected for flotation, formal ether sedimentation techniques as per lab standards. If it was negative by this procedure it was reported as negative[5]. For those samples which showed the presence

of *Cyclospora*, *Taenia*, Acid fast staining was done. For the patients with severe anaemia, patient's blood samples were collected and their serum Ferritin levels (ELISA) were assayed[5].

RESULTS

The study was done over a period of six months from February 2011to July 2011. We collected 300 samples in this period. Out of the 300 patients, 98 were inpatients, 202 out patients. Male: Female ratio 11:14.

Table 1
Gender wise distribution of samples

Gender	No of samples	Percentage
Males	133	44
Females	167	56
Total	300	100

They were 98 children and 202were adults. The youngest patient was 1year old and oldest was 70years old. Our study showed that out of the 300 samples screened 129 were positive for parasitic infestations which gives 43% positivity which is very high in the current scenario[5,6,7]. The analysis showed the following distribution.

Table 2
Distribution of parasites in the total no. of positive samples

Parasites	*No. of isolates*	*Percentage*
Entamoeba histolytica	*39*	*30*
Ancylostoma duodenale	*29*	*22*
Ascaris lumbricoides	*20*	*16*
Giardia lamblia	*14*	*11*
Taenia spp	*7*	*5*
Rhabiditiform larva	*5*	*4*
Trichuris trichiura	*3*	*2*
Mixed infection	*9*	*7*
Cyclospora	*2*	*2*
Chilomastix	*1*	*1*
Total	***129***	***100***

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Mixed infections 7% (Ankylostoma vwith Ascaris & Ankylostoma with Entamoeba).We also had Entameba coli identified in 15 samples (11.6%) but we did not include in the positive parasitic data as it was a commensal. The direct Saline, Iodine and LPCB wet mount preparations were positive in 77 samples and remaining 223 samples were subjected to concentration techniques. Of which 20 became positive by flotation technique & 37 were positive by sedimentation method.

Table 3
Comparison of three methods for the identification of parasites

Method	No.of samples tested	No. of positives	Percentage
Wet mount(Saline, Iodine & LPCB)	300	77	26
Floatation technique	300	97	32
Formal ether sedimentation method	300	129	43

Images showing the comparison between Normal Saline & Lactophenol Cotton Blue Wet mount Preparation in demonstrating the parasitic cyst / ova.

Entamoeba histolytica - (40X)

Normal Saline Wetmount Preparation

Lactophenol Cotton Blue Preparation

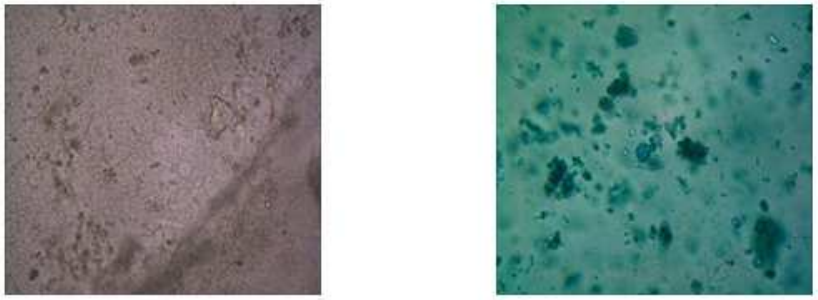


Figure 1
Entamoeba histolytica Cyst in LPCB Mount: - The cytoplasm is stained deep blue. The cyst wall is stained lightly but is clearly defined. It is surrounded by a clear halo. Nuclei are clearly visible when compared to Normal Saline Wet mount Preparation.

Giardia lamblia- (40X)

Normal Saline Wet mount Preparation

Lactophenol Cotton Blue Preparation

Figure 2
Giardia lamblia cyst in LPCB Mount: The cytoplasm is stained light blue. The axostyle is stained deep blue, clearly visible when compared to Normal Saline Wet mount Preparation

Trichuris trichiura (40X)

Normal Saline Wet mount Preparation

Lactophenol Cotton Blue Preparation

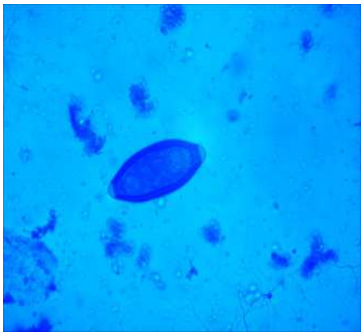
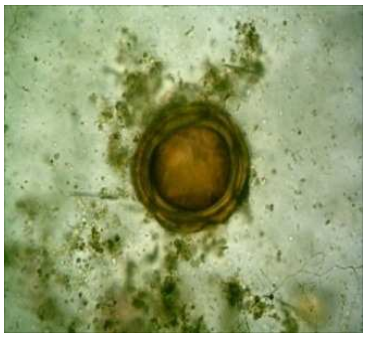


Figure 3
Trichuris trichiura egg in LPCB Mount: The double eggshell is stained deep blue. Mucus plugs are not stained but well-defined. Clearly visible when compared to Normal Saline Wet mount Preparation

Ascaris lumbricoides (40X)

Normal Saline Wet mount Preparation



Lactophenol Cotton Blue Preparation

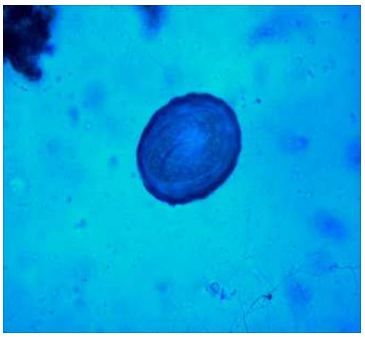
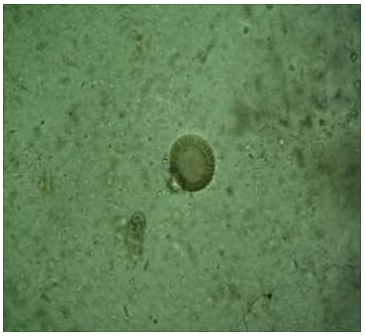


Figure 4
Ascaris lumbricoides egg in LPCB Mount: The outer thick wall is stained deep blue. The ovum is stained deep blue. Clearly visible when compared to Normal Saline Wet mount Preparation.

Taenia spp (40X)

Normal Saline Wet mount Preparation



Lactophenol Cotton Blue Preparation

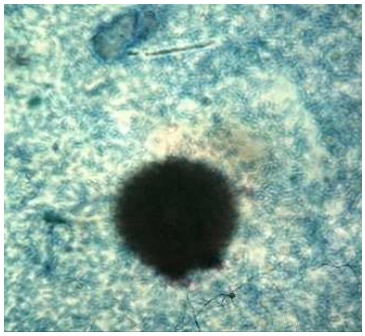


Figure 5
Taenia spp egg in LPCB Mount: The eggshell is not stained. Hook lets are faintly visible. Clearly visible when compared to Normal Saline Wet mount Preparation.

Ancylostoma duodenale (40X)

Normal Saline Wet mount Preparation

Lactophenol Cotton Blue Preparation

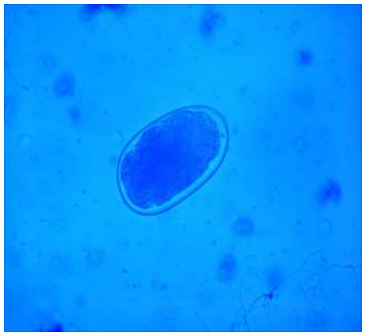
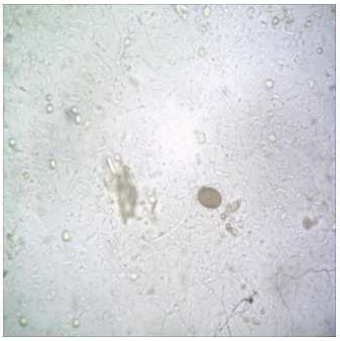


Figure 6
Ancylostoma duodenale egg in LPCB Mount: Blastomeres are stained deep blue. The eggshell is not stained but clearly discernible. Clearly visible when compared to Normal Saline Wet mount Preparation.

Cyclospora (40X)

Normal Saline Wet mount Preparation



Lactophenol Cotton Blue Preparation

Figure 7
Cyclospora egg in LPCB Mount: stained light blue. Clearly visible when compared to Normal Saline Wet mount Preparation.

We had 2 cyclospora & 7 taenia eggs in the stool sample, all were acid fast. The patients had such high rate of parasitic infestation that a 7 year old female child coughed out a female Ascaris worm when she came for outpatient treatment!!!

Child vomited - Ascaris worm

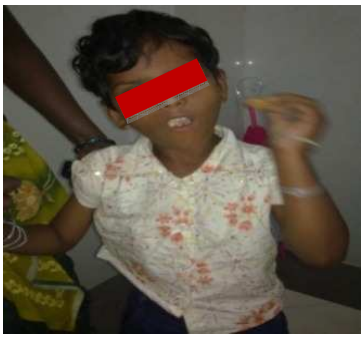


Figure 8
Child vomited - Ascaris worm

Serum ferritin levels were assayed by ferritin ELISA kit manufactured by diagnostics biochem cenada. inc cat no: CAN-F-428, lot no:091790 with an expiry date of 2011-12. . The assay works with monoclonal antibody specific for ferritin[5]. Among 129 positive samples, Serum Ferritin levels were very low in 88 cases out of which 60 were children & the remaining 28 were adults. Note: $p < 0.001$[10].

Table 4
Serum Ferritin levels in parasitic Infested & non infested cases

Types of cases	No. of cases
Parasitic infestation with anaemia	88
Parasitic infestation without anaemia	41
Non Parasitic infestation with anaemia	22
Non Parasitic infestation without anaemia	149

DISCUSSION

Our study shows a higher incidence of parasitic infestation of 43% when compared to the incidence observed in urban population which is 33%[5]. Our study showed the presence of *Entamoeba histolytica* to be the commonest parasite. The incidence of *Entamoeba* were seen in 39 patients (24 - children and 15 adults,) *Ancylostoma duodenale* were seen in 29 cases (20 were children & 9 adults), *Ascaris* –children 8, adult 12, *Giardia* had a distribution of 10 in children 4 in adult , Taenia – children 3, adult 4 Rhabidit iform larva children 1, adults 4 , *Trichuris trichiura* 1 child, adults 2*, Mixed infections*-children 6, adult *3,Cyclospora*-1 child, adult 1*, Chilomastix* –adult 1**.** On the whole the distribution of parasitic infestation is more prevalent among the children than the adults as we had a distribution of 74:55 making a positive total of 129 patients[9]. Among 129 positive samples, Serum Ferritin levels were very low in 88 cases out of which 60 were children & the remaining 28 were adults. The chances of Anaemia are 4 times more in patients having parasitic infestation than in non-parasitic infestations. In 129 positive cases, the wet mount preparation was positive in 77 (60%), Flotation was positive in 20(15%) and Formal ether technique was positive in 32(25%) cases. Our study showed that the careful examination of wet mount in 10X & 40X objective was fruitful in more than 50% of samples. Our study stresses the necessity of going back to the routine stool examination as an important screening technique for the detection of parasitic ova & egg in the stool sample, which has become obsolete in many of the labs including those of teaching medical college departments. If negative by the wet mount, a further confirmation test can be done with flotation & sedimentation techniques as per standard protocol. The comparative analysis of Saline, LPCB wet mount & LPCB staining preparation, LPCB wet mount showed a better morphological characteristics.

CONCLUSION

The simple wet mount is still a reliable technique for confirming parasitic infestation in patients. The formal ether technique is superior to the flotation method. The nutritional deficiency anaemia can be further augmented by the presence of parasitic ova/egg especially in children. The LPCB wet mount can be used for studying the morphology of the parasitic ova& eggs instead of routine saline, iodine wet mount and LPCB staining methods. The high incidence of parasitic infestation is due to the poor sanitary condition, lack of clean drinking water supply and education that is prevalent in the rural India. Use of de worming agents and health education with regards to hygienic habits, bare foot walking& safe drinking water will reduce the incidence of parasitic infestations.

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