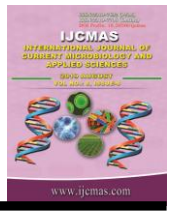
Int.J.Curr.Microbiol.App.Sci (2019) *8*(8): 2133-2143

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 8 Number 08 (2019)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2019.808.249

Comparison of Direct Stool Microscopy with Formol Ether Concentration in the Isolation of Soil Transmitted Helminths in Adult Population

A.S. Liji[1], Nonika Rajkumari[1*], Subhash Chandra Parija[1] and K.T. Harichandrakumar[2]

[1]Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry – 605006, India
[2]Department of Medical Biometrics and Informatics (Biostatistics), Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry – 605006, India

**Corresponding author*

Keywords

Microscopy, Formol ether concentration, Soil transmitted helminths, Adults, Ascaris, Isolation

Article Info

Accepted:
15 July 2019
Available Online:
10 August 2019

A B S T R A C T

Soil transmitted helminths or geohelminths still contribute a major burden to many countries including India. It's of utmost importance to detect them accurately and timely. Studies on this group of intestinal parasites in adults are rare. Hence, the study explored the usefulness of stool concentration technique and compared them with the direct microscopy for better isolation of the geohelminths in adults. In this study, 254 consecutive stool samples were taken from adult patients of the hospital. Direct and formol ether concentration techniques was performed for each sample and the positive sample smears were stained with suitable stains for proper identification of the parasites. Positivity of egg/ova or adult worm/larva by either method was noted and results were compared and analysed. Of the total 254 samples, 39 samples were positive for an intestinal parasite by concentration method and 22 samples by direct wet mount microscopy. Cohen's Kappa is 0.686. P value < 0.001. Geohelminths were identified in 23 positive samples. Mixed infection was seen in only 1 sample. Overall, *A. lumbricoides* (45.23%) was the predominant parasite followed by *Blastocystis* (21.43%). Comparing these helminths to the other intestinal parasites, its isolation was more (55%) than the other intestinal helminths (45%) thereby indicating its common occurrence in the study population. Female predominance (56%) was seen among the enrolled patients. Better recovery was seen by the concentration technique and hence its detection. Adding this technique over to the direct microscopy will be a useful adjunct to detect and treat patients with low parasite count especially in the immunocompromised population.

Introduction

Intestinal helminths are a major problem worldwide especially in the tropics and subtropics, of which soil transmitted helminths (STHs) or geohelminths forms a major burden. It is estimated that in India, 241 million children need deworming to avert the negative consequences STH infections can have on child health and development (Greenland *et al.*, 2015). They are responsible for much morbidity in school going children (Al-Mekhlafi *et al.*, 2007; Adams *et al.*, 2005). Of the STHs, the most common one to cause infections are roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworms (*Necator americanus* and *Ancyclostoma duodenale*)

(Hotez *et al.,* 2006) and less commonly *Strongyloides stercoralis*. These helminths are transmitted either through faeco-oral route (*A. lumbricoides, T. trichiura*) or through intact skin (hookworms) (Greenland *et al.,* 2015). Various factors like poverty, biophysical environment and cultural practices in the tropical countries favor the transmission of intestinal helminthiasis (Terefe *et al.,* 2011; Nkengazong *et al.,* 2010; Tadesse, 2005). Certain immunomodulatory products are secreted by STHs which enable them to survive in the mammalian host for many years (Akinseye *et al.,* 2015). STH infection is a major health care problem in many developing countries. Diseases conditions like malnutrition, anemia, hypoalbuminemia, vitamin A deficiency and impaired cognition are generally attributed to soil transmitted helminths (Angraini *et al.,* 2005; Ananthakrishnan and Das, 2001). For effective control of soil transmitted helminthic infections, good environmental sanitation, health education and chemotherapy are the main three measures which are needed (Ananthakrishnan and Das, 2001). STH infections are a major cause of morbidity and mortality in children and immunocompromised in the poor and developing countries (Shivekar *et al.,* 2011).

Proper diagnosis is required for the recovery of soil transmitted helminths. As STHs cause serious morbidity to humans, early and accurate diagnosis is extremely important for timely therapy and prevention of infection. Direct stool microscopy is routinely used for screening of STHs and also for other helminths. With this method, we tend to miss out positive cases if the helminthic load is low. Studies done on adults regarding STHs infections are rare with most studies being done in school going children. With this in view, this study tried to analyse the detection capacity between direct stool microscopy and concentration method of stool and at the same time tried to observe the STH infections in adult population.

Materials and Methods

This was a prospective study conducted in a tertiary care teaching institute of south India from February 2017 to January 2018. All the stool samples of adult patients which were sent from both the OPDs and wards of the hospital for screening to the parasitology laboratory of the Department of Microbiology were taken for the study. Institute ethical clearance was obtained prior to the start of the study. A total of 254 stool samples of adults were collected and included during the said period. Stool specimens were collected in a clean wide mouthed container and care was taken not to contaminate with water or urine. All these specimens were properly labeled with name, hospital number, date and time of collection. They were examined immediately after collection and processed immediately for screening using microscopy by both concentrated as well as direct methods.

Various concentration techniques are available (Bineshlal *et al.,* 2015). For the purpose of this study, formol ether concentration technique was used for all the samples (Chatterjee, 2009; Garcia, 2007) and the sediment formed after the technique was used for screening. Both saline and iodine wet mounts were made for both the direct and concentrated methods. The wet mounts were microscopically screened, initially by using a low power objective (10x) and then by using a high power (40x) objective of a compound light microscope. Stool smears of any positive samples was then stained with trichrome stain or with modified acid fast staining based on the parasite seen under the wet mount microscopy. Positivity of egg/ova or adult worm/larva of STHs by either method was noted. Comparison of the result of each single method and the combination of both the methods were analyzed.

Statistical analysis

Sample size was estimated using the statistical formula for estimating a proportion. The sample size was calculated as 254 with 12% prevalence and 4% precision. In this study, the distribution of categorical data such as gender, socio-demographical status, clinical characteristics, microbiological profile etc were expressed as frequency and percentages. The comparison of microbiological profile between concentrated and unconcentrated stools were done using χ^2 or Fisher exact test. The independent factors associated with STH and microbiological profile of STHs was explored by using logistic regression analysis. The level of agreement in the diagnosis of STH between concentrated and unconcentrated samples was done using kappa statistics. All statistical analysis was carried out at 5% level of significance and p-value <0.05 was considered as significant.

Results and Discussion

Of the 254 stool samples, 215 samples were found to be negative by both the methods and intestinal parasites were positive in 39 samples. All these 39 samples were positive by formol ether concentration technique. However, only 22 stool samples were positive by direct wet mount microscopy. Single parasite was identified in 38 (14.96%) samples whereas only one sample (0.39%) showed mixed intestinal parasite infection. Among the positive samples, soil transmitted helminths were identified in 23 samples. From these 23 samples, 24 STHs were recovered. It was seen that 111 were adult males (43.58%) and 143 were adult females (56%) thereby showing a female predominance among the enrolled patients. In this study, 72% of the patients belong to the lower socioeconomic group with most of them being farmers and manual labours. It was observed that intestinal parasites were recovered mainly from age group between 21to 60 years and specifically more in the 31 to 40 years age group which is the economically productive group in the community (Figure 1).

Regarding its clinical profile, the most common condition of these patients was immunocompromised (64%) due to a disease or its therapy. Majority of these patents had rheumatoid arthritis, ulcerative colitis, systemic lupus erythrematosis or Crohn's disease on treatment. One patient from whom multiple intestinal parasitic infections were detected was a patient living with HIV/AIDS. The other major burden was contributed by patients who had anaemia, diarrhoea or similar condition.

Of the 254 samples processed during the study period, 39 samples were positive for intestinal parasitic infections. All the thirty nine samples came positive by concentration technique, in which only 22 samples were positive by direct microscopic examination method (Table 1). Hence, Cohen's Kappa is 0.686. P value < 0.001. The strength of agreement is considered to be moderate. Considering direct microscopy as gold standard and comparing direct microscopy with formol ether sedimentation, the sensitivity of formol ether sedimentation technique was 100% (85.13% - 100%) and the specificity was 92.64% (88.53% - 95.13%). The positive predictive value is 56.41% (40.98% - 70.7%) and the negative predictive value is 100% (98.24% - 100%). It was seen that after performing both tests on all the samples, isolation of intestinal parasite were more with formol ether concentration technique compared to direct wet mount.

Overall, *A. lumbricoides* (45.23%) was the predominant parasite followed by *Blastocystis* (21.43%), *Entamoeba* species (11.9%) and hookworm (9.5%). Details are shown in Table 2. Of the 39 samples, 38 showed only single parasite but only 1 sample showed mixed

infection. The parasites recovered from the sample showing mixed infection were *A. lumbricoides, Trichura trichiuris* and *Cystoisospora belli* and *Blastocystis* (Figures 2a, b, c, d, e). So, these lead to the total intestinal parasites isolated from 39 samples to 42.

Among the 23 stool samples positive for STH, *A. lumbricoides* (45.23%) was the most common, followed by hook worm (16.66%) and *T. trichiura* (4.16%). Comparing the STHs to the other intestinal parasites, it was seen that STHs was isolated more (55%) than the other intestinal helminths (45%) thereby indicating that STHs were more common in the study population.

This study showed that 39 samples (42 parasites) were positive by formol ether concentration technique whereas only 22 samples were positive by direct wet mount microscopy. Another study also showed repeated sampling and concentration technique increases the recovery of intestinal parasites (Rajkumari and Jayaseelan, 2016) which otherwise would have been missed if the load of the intestinal parasite is low or if they show intermittent shedding of the ova and cysts.

This finding was similar to what was observed in our study. A study conducted in south India showed that both routine direct microscopic examination and formol ether concentration technique increases the sensitivity in detecting the intestinal parasite in stool specimen and observed only 23.25% recovery rate by direct microscopic examination. Formol ether, formol petrol, formol ethyl acetate were having 34.0%, 33.5%, 28.7% recovery rates respectively (Bineshlal *et al.,* 2015). A previous study conducted in Nigeria also showed recovery rate of intestinal parasites by direct smear, formol-ether concentration and nigrosine methylene blue as 23.10%, 29.10% and 47.77% (Sheyin *et al.,* 2013). This is also supported by a study conducted in children where modified formol ether concentration techniques showed a recovery of 64.85% of parasitic infestations (Parameshwarappa *et al.,* 2012).

Direct microscopic examination was negative for the sample showing mixed parasitic infection but was positive for *A. lumbricoides*, *Blastocystis* spp, *T. trichiura* and *Cystoisospora belli* by formol ether concentration technique. This highlights the importance of formol ether concentration technique which otherwise the sample would have been reported as negative by direct wet mount microscopy. A study reported multiple parasitic infections where the parasites detected were *Schistosoma mansoni*, *T. trichiura*, *A. lumbricoides* and hook worm (Terefe *et al.,* 2011). The total parasites isolated were 59.7% and a combination of 2 or > 2 of the above mentioned parasites were isolated. Another report by Someshwaran *et al.,* found *H. nana* and *A. lumbricoides* as mixed infection from a patient's sample (Someshwaran *et al.,* 2015).

Our study also isolated similar parasites like *A. lumbricoides* and *T. trichiura* where mixed infection was seen but other intestinal parasites which were also detected from the same sample includes *Blastocystis* spp and *Cystoisospora belli* unlike those reported from other studies.

A. lumbricoides (45.23%) was overall the predominant parasite in our study. Other studies documented similar findings with 52% and 36.4% isolation rates respectively (Greenland *et al.,* 2015; Ojurongbe *et al.,* 2014). Our study showed a relatively higher percentage of *A. lumbricoides* compared to other studies where the isolation rates were only 2.79% and 1.5% respectively (Shivekar *et al.,* 2011; Kaliappan *et al.,* 2013).

Table.1 Comparison of direct stool microscopy with formol ether sedimentation technique

	Direct Microscopy			
		Positive	Negative	Total
Formol ether concentration	Positive	22 (8.7%)	17 (6.7%)	39
	Negative	0	215 (84.6%)	215
	Total	22	232	254

(P value < 0.001)

Table.2 Distribution of various intestinal parasites isolated from positive stool samples

Intestinal parasites	Number	Percentage (%)
Ascaris lumbricoides	19	45.23
Blastocystis	9	21.42
***Entamoeba* spp**	5	11.9
Hookworm	4	9.5
***Strongyloides* spp**	2	4.8
Cystoisospora belli	1	2.3
Giardia lamblia	1	2.3
Trichuris trichuira	1	2.4
Total	42	100

Fig.1 Distribution of age in the positive cases

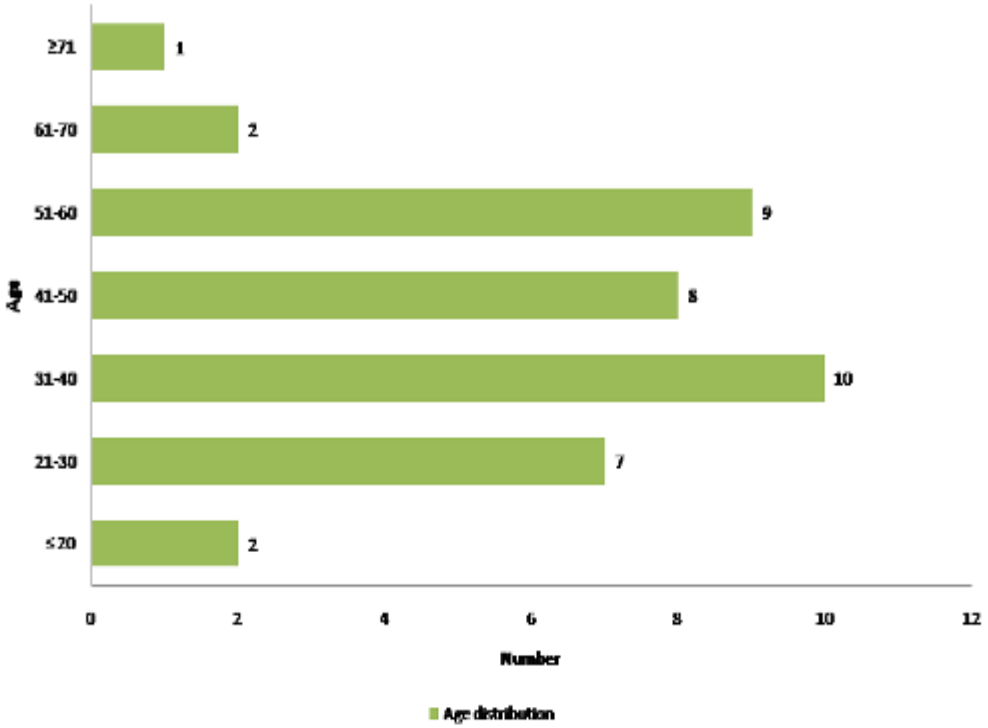


Fig.2a Decorticated egg of *Ascaris lumbricoides* (saline wet mount, 40x)

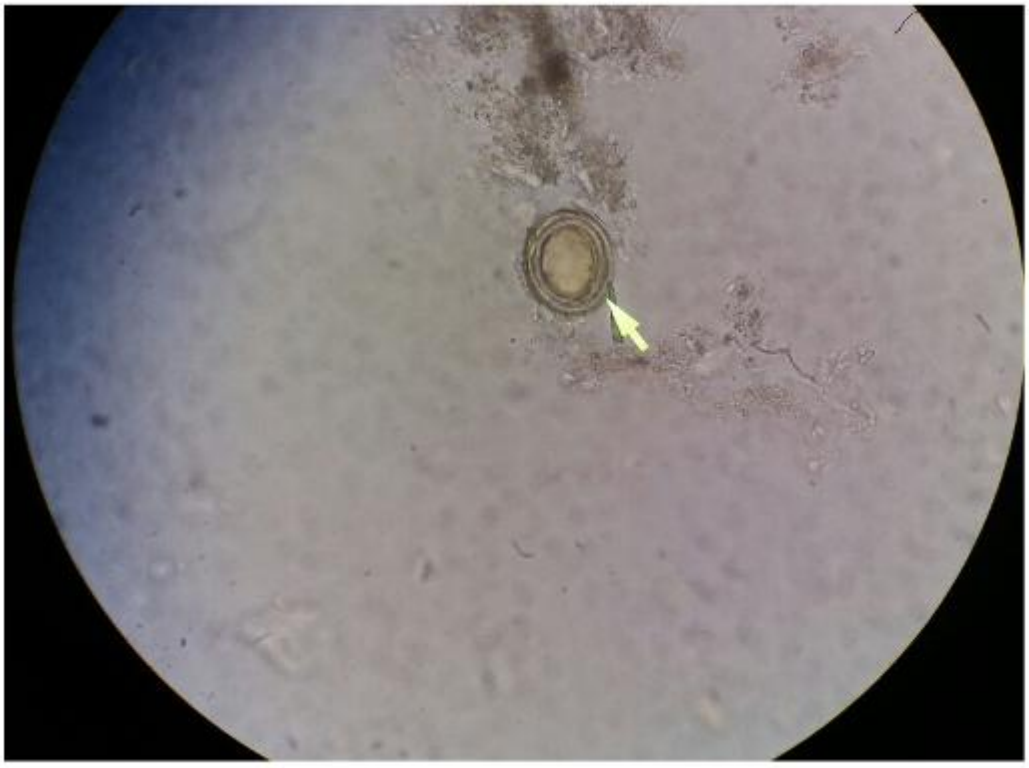


Fig.2b Egg of hookworms (iodine wet mount, 10x; saline wet mount, 40x)

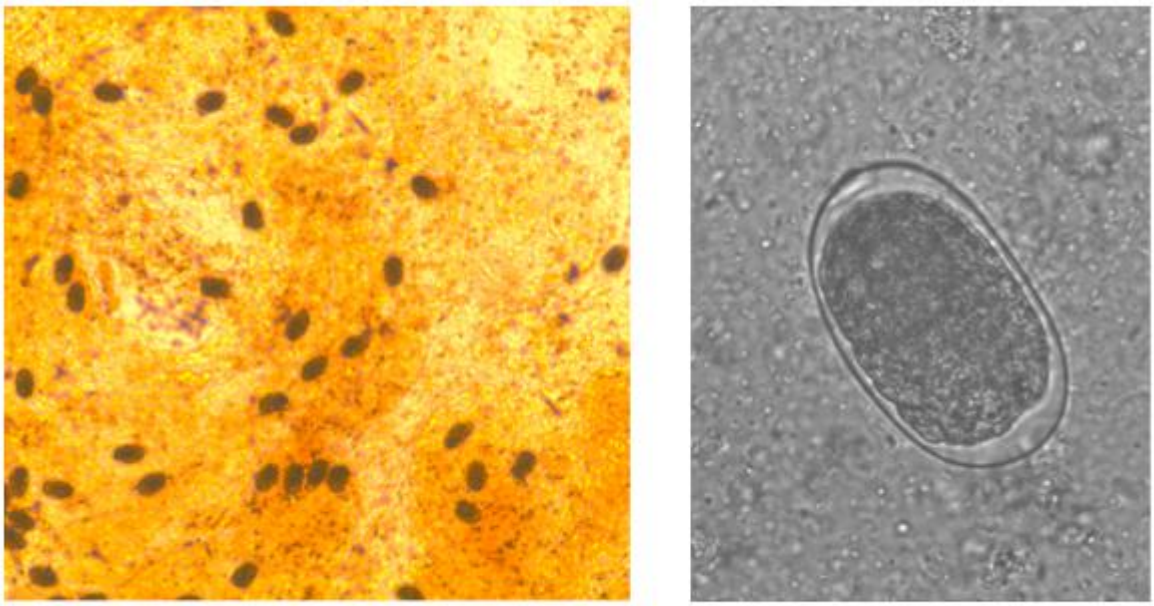


Fig.2c Eggs of *Trichuris trichuira* (trichrome stained, 100x)

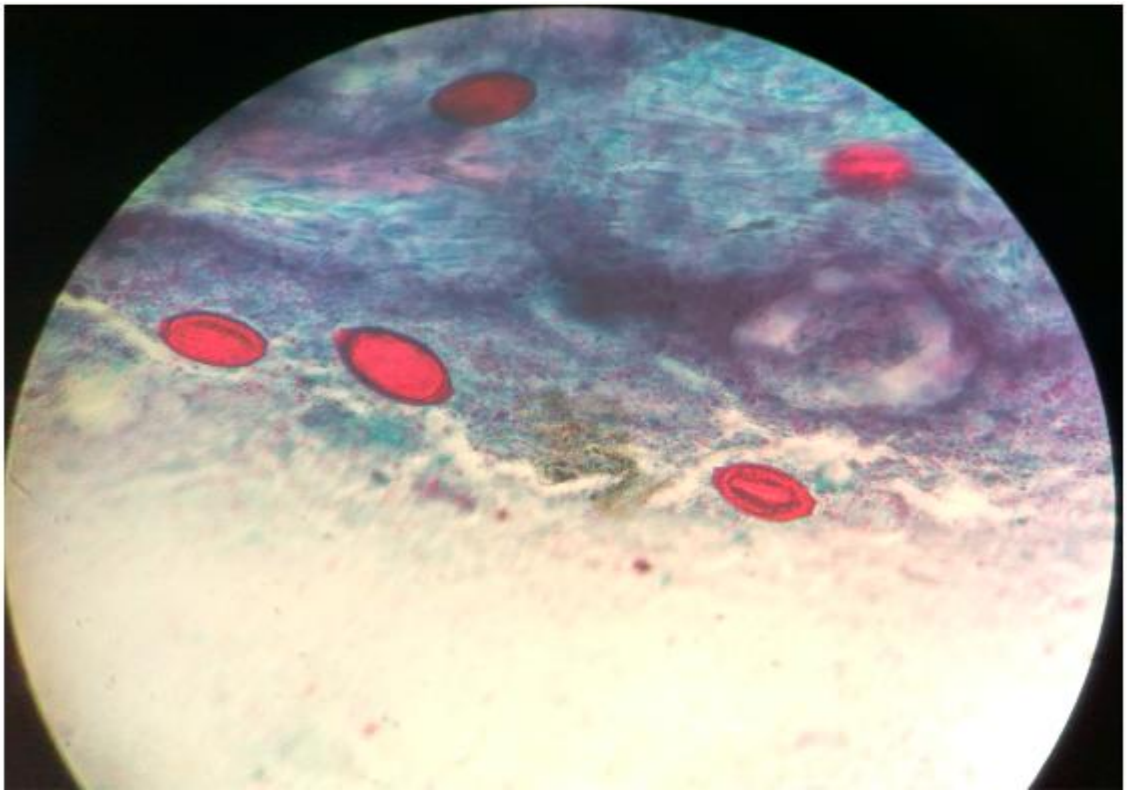


Fig.2d Rhabdtiform larva of Strongyloides stercoralis (saline wet mount, 40x)

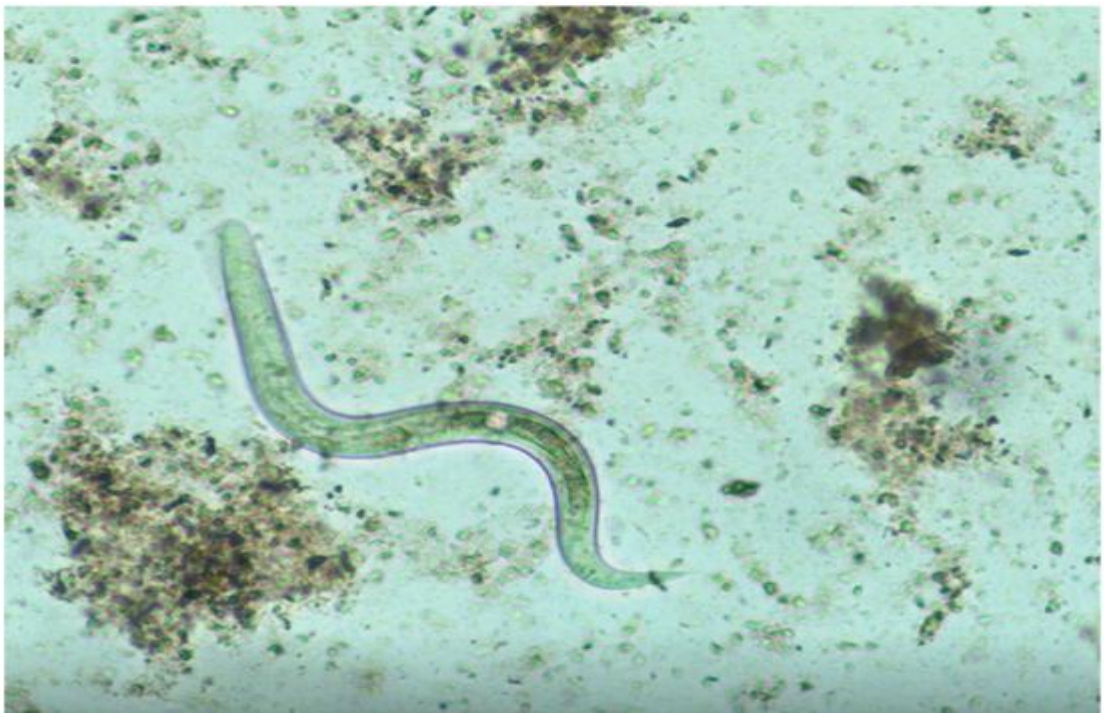
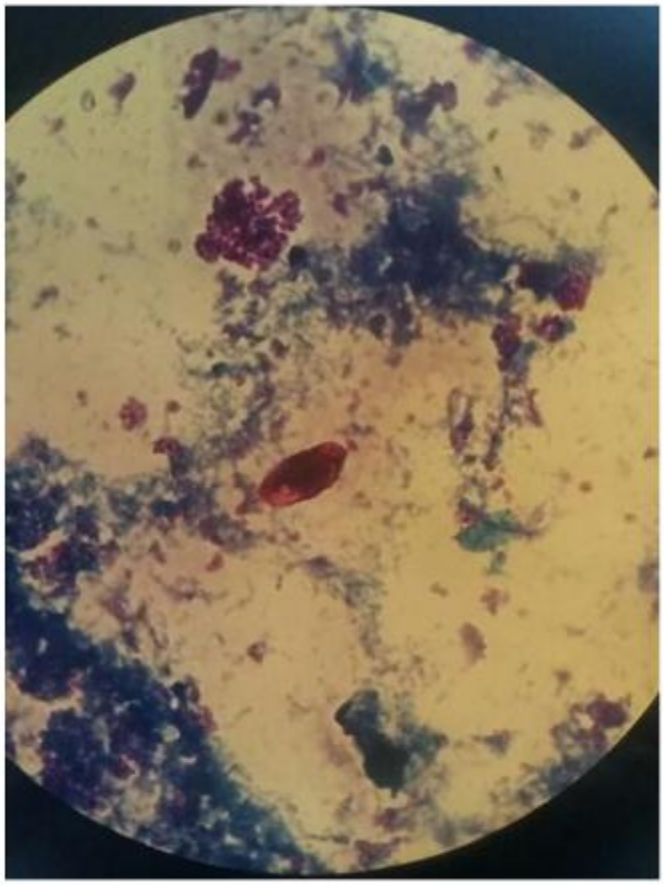


Fig.2e Oocyst of *Cystoisospora belli* (modified acid fast stain, 100x)



The second most common parasite in our study was *Blastocystis* spp. accounting for 21.43% of the total parasites isolated and the third most common parasite was *Entamoeba* species (11.9%). A previous study done in south India showed a prevalence of 6% *E. coli* and *E. histolytica* accounting for 2.5% (Bineshlal *et al.,* 2015). Other isolates seen in our study were *Isospora belli* (2.4%) (*Cystoisospora belli*), *G. lamblia* (2.3%), *Strongyloides* spp (4.8%) and *T. trichiura* (2.4%). Geographical location also has a bearing on the distribution of the different intestinal parasites and hence the difference in the pattern of distribution.

A. lumbricoides was the major parasite detected among the geohelminths, accounting for 79.1%. A study conducted in Nigeria also showed *A. lumbricoides* as the predominant isolate (36.4%) among the STHs (Ojurongbe *et al.,* 2014). Hookworm was the second most common STH accounting for 16.6% (4/24). This finding is relatively low compared to other studies where the isolation rates were 86.36%, and 42% respectively (Shivekar *et al.,* 2011; Greendland *et al.,* 2015). In the current study, *T. trichiura* was the least common among STH accounting for 4.5% (1/24) This study showed almost similar percentage of *T. trichiura* compared to previous studies where the detection rates were 1.04% and 5% respectively (Shivekar *et al.,* 2011; Greendland *et al.,* 2015). Such similar finding was seen in another study (Sam-Wobo *et al.,* 2013).

In the gender distribution of the study population, female predominance of 56.41% was seen compared to male which showed only 43.58%. A previous study conducted in the same institute, showed a female predominance pattern (56%) over the males (43.9%) (Kaliappan *et al.*, 2013). Similar results were also seen in another related study conducted in same department (Rajkumari and Jeeyaseelam, 2016). Another study conducted in Nigeria showed more prevalence in males (28.4%) compared to females (19.8%), (Ojurongbe *et al.*, 2014) which was reverse to what was seen in our study. Such reverse finding was also observed in another study with male predominance pattern 42.9% over females 40. 7 % (Greenland *et al.*, 2015). This reverse finding may be due to the fact that females are more actively involved in agricultural practices and other outdoor livelihood activities in this region which may account for the female predominance.

In the current study, recovery of parasite was mainly (69.23%) from the age group between 20-50yrs.A previous study conducted also showed a positivity of 25.2% in the age group of 21-30 years (Bineshlal *et al.*, 2015). This implies that the maximum burden of the intestinal parasites was carried by the economically productive age group. In the clinical conditions, most of the patients from whom the parasites were recovered belong to the immunocompromised group (64%) thereby showing that intestinal parasitic infections are very common in this group of patients irrespective of whether they are symptomatic or asymptomatic.

Remaining burden was borne by patients who have anaemia and diarrhea. As hookworm is a known cause of anaemia, routine screening to treat and eradicate it is necessary both in adults as well as children as anaemia can be a major cause of morbidity and mortality to the population.

Limitations

Since only formol ether concentration technique was performed due to lack of resources, all the different types of concentration methods could not be performed on the stool samples and hence comparison of the techniques wasn't possible. As this is an adult based study, we were not able to look at the recovery rate in children which would have made a better comparison of the technique. This is a hospital based study and the recovery of different parasites might not reflect the distribution of intestinal parasites which we might see in a community based population and hence its comparison.

Intestinal helminths are very much prevalent, of which STH still contributes a major burden. The recovery of intestinal parasite was more by using concentration technique, which is formol ether concentration in our study. It showed that concentration technique increases the detection of intestinal parasites which otherwise would have been missed by the direct wet mount microscopy and is a useful adjunct to the routine direct microscopy of stool samples.

So, using concentration technique over and above direct stool microscopy on a routine basis is advisable for patients living in the endemic area and will help to catch the intestinal parasite infections in patients having low burden. Adding this method routinely will go a long way in reducing the morbidity due to parasites showing silent infection and hyper infection. It will also help a lot in the preventive measures and control of intestinal parasites.

Funding

The authors would like to thank Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry for supporting this

study through an intramural grant (JIP/Res/Intra-M.Sc/phs1/01/2016-17 sl no 1)

Acknowledgement

The authors would like to thank Mrs Lakshmi D for her immense help during the study.

References

Adams V J, Markus M B, Adams J F A, Jordaan E, Curtis B, Dhansay M A, *et al.*, 2005. Paradoxical helminthiasis and giardiasis in Cape Town, South Africa: epidemiology and control. Afr Health Sci, 5: 131-136.

Akinseye J F, Nmorsi O P G, Akinbolaji T J, 2015. Human Soil-Transmitted Helminthiasis among Adolescents in Ilara-Mokin Community, Ondo State, Nigeria; A Case Study of *Ascaris lumbricoides*, *Trichuris trichuira* and Hookworm. Int J Life Sci Res, 3: 108-12

Al-Mekhlafi H, Atiya A S, Lim Y, Mohammed Mahdy AK, Ariffin W W, Abdullah H C *et al.*, 2007. An unceasing problem: soil-transmitted helminthiases in rural Malaysian communities. SAJTMPH, 38: 998-1007

Ananthakrishnan S, Das P K, 2001. Integrated Programme for Control of geohelminths: A Perspective. Natl Med J India, 14:148-53.

Angraini R, Dimyati Y, Lubis B, Pasaribu S, Lubis C P, 2005. Association between soils transmitted helminthiasis and hemoglobin concentration in primary school children. Paediatrica Indonesiana, 45: 24-30

Bineshlal Y, Thiyagarajan A, and Jayakumar S. Comparison of Different Concentration Techniques of the Stool Examination for Detecting Intestinal Parasites. RJPBCS. 2015; 6: 873-8

Chatterjee KD. Parasitology, 2009. 13th ed. New Delhi: CBS publishers and distributers Pvt. ltd.

Garcia LS, 2007. Diagnostic Medical Parasitology. 5th ed .USA: ASM Press American Society for Microbiology.

Greenland K, Dixon R, Khan SA, Gunawardena K, Kihara JH, Smith JL *et al.*, 2015. The epidemiology of soil-transmitted helminths in Bihar state, India. PLoS Negl Trop Dis, 20; 9: e0003790

Hotez PJ, Bundy DAP, Beegle K, Brooker S, Drake L, de Silva N, *et al.*, 2006. Helminth Infections: Soil-transmitted Helminth Infections and Schistosomiasis In: Jamison DT, Breman JG, Measham AR, *et al.*, editors. Disease Control Priorities in Developing Countries. 2nd ed Washington (DC) World Bank.

Kaliappan S P, George S, Francis M R, Kattula D Sarkar R, Minz S *et al.*, 2013. Prevalence and clustering of soil transmitted helminth infections in a tribal area of south India. Trop Med Int Health, 18: 1452-62

Nkengazong L, Njioko U F, Wanji S, Teukeng F, Enyong P, Asonganyi T *et al.*, 2010. Prevalence of soil transmitted helminths and impact of albendazole on parasitic indices in Kotto Barombi and Marumba II villages (South-West Cameroon). Afri J of Envir Sci and Tech., 4: 115-21

Ojurongbe O, Oyesiji KF, Ojo JA, Odewale G, Adefioye OA, Olowe AO *et al.*, 2014. Soil transmitted helminth infection among primary school children in lle- lfe south west, Nigeria: A cross sectional study. Int Res J Med Med Sci., 2: 6-10

Parameshwarappa KD, Chandrakanth C, Sunil B, 2012. The Prevalence of Intestinal Parasitic Infestations and the Evaluation of Different Concentration Techniques of the Stool Examination. J Clin Diag Res, 6: 1188-91

Rajkumari N, Jayaseelam V, 2016. Burden of intestinal helminthic infection among school going children in a south –Asian nation. NJLM, 5: 6-11

Sam-Wobo S O, Akintola O, Atungwu J, Ekpo U F, Adeleke M A, Mafiana C F *et al.*, 2013. Prevalence and effect of Schistosome and soil-transmitted helminth infection on labour input in rice-growing communities of Ogun State, Nigeria. Epi Biost Pub Heal, 10: e8841-7

Sheyin Z, Bigwan E I, Galadima M, 2013. Comparison of Formol-Ether, Direct smear and Nigrosine Methylene Blue for the diagnosis of human Intestinal Parasites. J Micr Res and Rev, 1: 30-4.

Shivekar S, Chand P, Rangasamy G, 2011. Soil transmitted helminths in a rural population of Puducherry- A Hospital based study. Internl J Pharma and Biosciences 2; 293-7

Someshwaran R, Nachammai S M, Aravazhi A N, 2015. A rare case report of intestinal hymenolepiasis and ascariasis double infection in a symptomatic immunocompetent host from south India. Int J Med Sci Clin Inven, 2: 1443-7

Tadesse G. The prevalence of intestinal helminthic infections and associated risk factors among school children in Babile town, eastern Ethiopia. Ethiop J Health Dev, 19: 140-7

Terefe A, Shimelis T, Mengistu M, Hailu A, Erko B, 2011. *Schistosoma mansoni* and soil-transmitted helminthiasis in Bushulo village, southern Ethiopia. Ethiop. J. Health Dev, 25: 46-50

How to cite this article:

Liji, A.S., Nonika Rajkumari, Subhash Chandra Parija and Harichandrakumar, K.T. 2019. Comparison of Direct Stool Microscopy with Formol Ether Concentration in the Isolation of Soil Transmitted Helminths in Adult Population. *Int.J.Curr.Microbiol.App.Sci.* 8(08): 2133-2143. doi: https://doi.org/10.20546/ijcmas.2019.808.249