Comparative Evaluation of Wet Mount and Concentration Techniques in Stool Examination for Intestinal Parasite Detection

Abstract

Background: Intestinal parasitic infections are a persistent public health concern in developing countries. Accurate diagnostic techniques are essential for timely treatment and control.

Objective: To compare the diagnostic performance of direct wet mount and Formol-ether concentration (FEC) methods for stool examination.

Methods: This 12-month observational study analyzed 350 stool samples using both saline/iodine wet mount and FEC methods at the Department of Microbiology, Sharda University.

Results: Of the 350 samples, 41 (12%) tested positive. The FEC method detected all 41 cases, while the wet mount identified only 25 (61%). *Giardia lamblia* was the most prevalent parasite (61%). Infections were more frequent among males (73%) and peaked during monsoon season (June–August), especially in children aged 11–15 years.

Conclusion: The FEC technique showed higher sensitivity and should be routinely employed with wet mount microscopy to improve detection of intestinal parasitic infections.

Commented [ho1]: Country? state?

Commented [ho2]: is more sensitive

Keywords

Intestinal parasites, ~~stool microscopy~~, wet mount, ~~Formol-ether concentration~~ Sedimentation technique, ~~Giardia lamblia,~~ diagnostic methods

1. Introduction

Parasitic diseases caused by intestinal parasites are a major public health issue in countries. Majority of these infections with parasites results from low standard of living associated with

poor sanitary and socioeconomic conditions in tropical and subtropical climates area [1]. More than 3.5 billion individuals are infected by one of the intestinal parasites, and 450 million develop illness because of parasitic infection.[2]. Organisms that live on other organism (host) and benefit from these organisms are called parasites (literally para - beside, sitos - food). It is obvious that the parasite can have no intention of doing harm to the host and that would not even be in the interest of the parasites zitself. The damage is done accidentally especially if the number of the parasites becomes too large. Parasites may do harm to their host by absorbing food intended for the host example tape worm [3]. Blood or lymph could be sucked by parasites like hookworm. Parasites like ascaris feed on the tissue of the host causing wound through which infections may enter [4]. Intestinal parasitic infections are among the major public and socio-economic concerns that adversely affect the well-being of the poor in developing countries (Christine and Christopher, 2000) and may even be more important than bacterial infections ~~2012)~~ [5]. It has been estimated that *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* infect 1,450 million, 1,300 million and 1,050 million people worldwide, respectively, while schistosomiasis affects over 200 million people. *Entamoeba histolytica* and *Giardia lamblia* are also estimated to infect about 60 million and 200 million people worldwide, respectively Considering the importance of the harmful effects of these parasites, there is need for proper diagnosis[6]. The purpose for these methods is to increase the detection rates of the infections. Concentration of parasites ova or cysts from faeces may be accomplished in a number of ways. All floatation methods depend upon mixing fecal sample with a liquid, the specific 10 gravity of which is greater than that of most of such forms yet less than the specific gravity of most of the fecal debris. Concentration of human intestinal parasites can also be achieved by sedimentation techniques which include Formol ether sedimentation and direct smear using saline and iodine preparation The difficulty in the storing, using and disposing of ether has made many laboratories to desire a concentration method that does not present the storage and disposal problems found with ether[8]. Fecal specimen examination for intestinal parasitic identification is increased by the use of concentration methods. The concentration of parasites in ova or cysts from fecal specimens can be consummate in different ways.[9].

Commented [ho3]: . the end point of the sentence

Commented [ho4]: itself

Commented [ho5]: diagnosis

Commented [ho6]: the end point of the sentence

2. Materials and Methods

Study Design: Observational study
Study Site: Department of Microbiology, Sharda Hospital, Sharda University
Duration: 12 months
Sample Size: 350 stool samples

Commented [ho7]: the country must be mentioned

Inclusion Criteria: Fresh stool samples submitted for routine parasitology examination.
Exclusion Criteria: Samples submitted more than 2 hours post-collection or from patients

receiving antiparasitic medication.

Each stool sample was examined macroscopically and microscopically using two techniques:

1. Direct Wet Mount (Saline/Iodine): Smears were examined under low and high-power microscopy.

2. Formol-Ether Concentration (FEC): Sedimentation technique involving formalin fixation, ether extraction, centrifugation, and examination of sediment under the microscope.

Commented [ho8]: where is the methods details?

3. Results

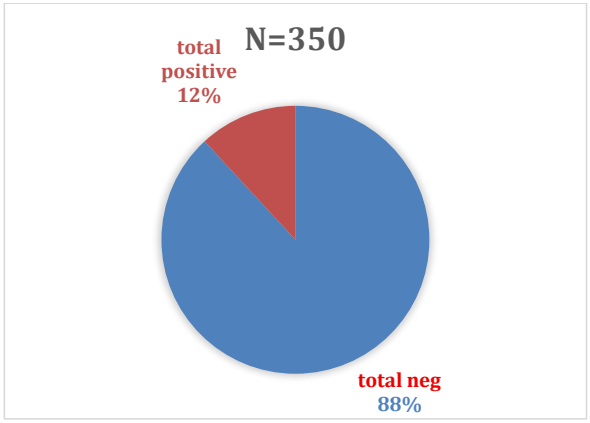
A total of 350 stool samples were processed within the study period out of which 41 were positive by either of methods, Pie chart-1

Table 1- An analysis of 350 stool samples

Commented [ho9]: there is no need for this table, its better to delete it

Total sample	Total positive	Total negative
350	41[12%]	309[88%]

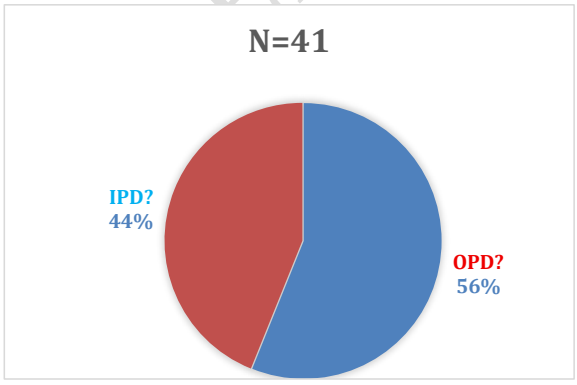
chart -1- The pie chart ~~shows the results~~ of ~~350~~ the stool samples



Out of 41 positive stool microscopy patients, majority came to hospital on OPD [56%]basis rest all[44%] were IPD samples ,Pie chart-2

~~Table 2- Out of 41 positive stool microscopy patients, the majority are in OPD, and the rest are IPD~~

Total positive	OPD	IPD
41	23[56%]	18[44%]



Commented [ho10]: ?

Commented [ho11]: Add space

Commented [ho12]: Add space

Commented [ho13]: samples, Pie

Commented [ho14]: no need for the table

chart-2- ~~Out of 41~~ positive stool microscopy patients~~, 44% are IPD and 56% are OPD~~

Out of 41 positives, the majority (30) were males, ~~i.e. 30~~, and the rest were all females. Pie chart-3

Table 3- Out of 41 positives, the majority were males (30), and the rest were females

Commented [ho15]: no need for the table

Total positives	Males	Females
41	30[73%]	11[27%]

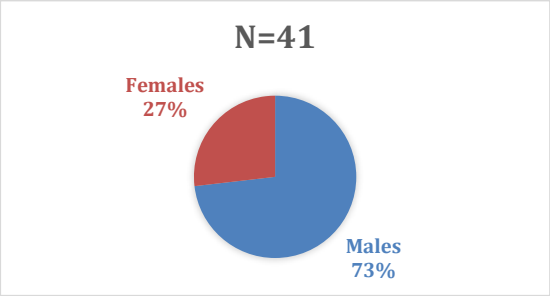


chart-3 - ~~Out of 41~~ wet mount positive, the majority were males, and ~~the rest were~~ females samples

Out of 41 positive stool samples, majority of patients were in age group of 11-15 year age group followed by 41-45Yyears ,Graph-1

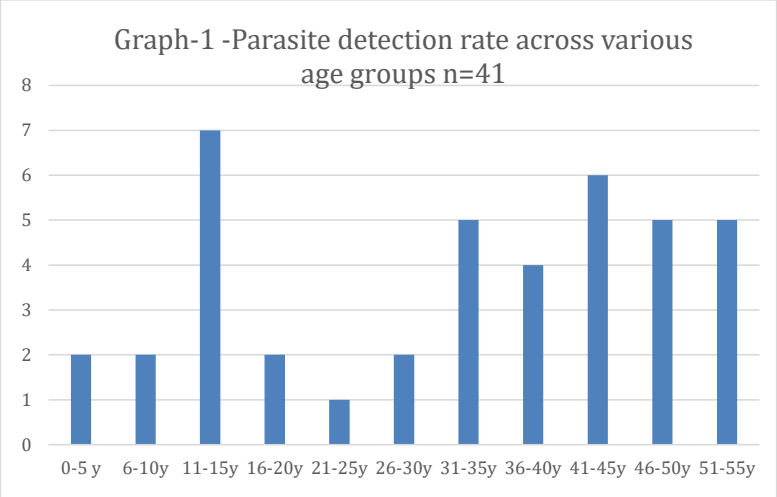
~~AGE WISE DISTRIBUTION OF POSITIVE FINDINGS~~

Commented [ho16]: no need for the table

0-5Y	6-10Y	11-15Y	16-20Y	21-25Y	26-30Y	31-35Y	36-40Y	41-45Y	46-50Y	51-55Y

2	2	7	2	1	2	5	4	6	5	5

Table 4- Analysis of Age-Wise Distribution of Positive Findings

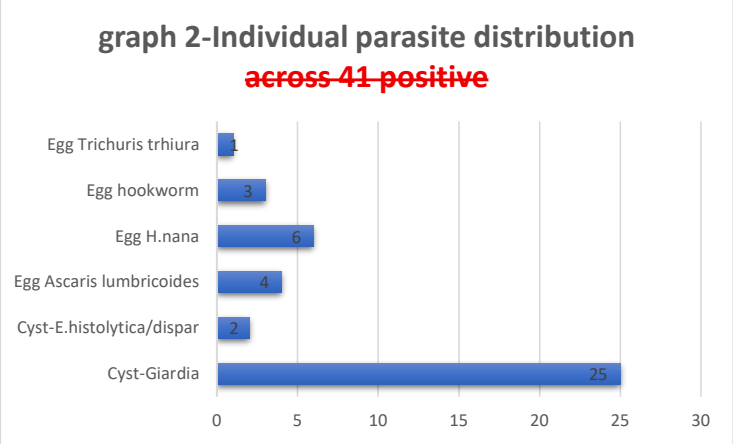


Graph 1: Analysis of Age-Wise Distribution of Positive Findings

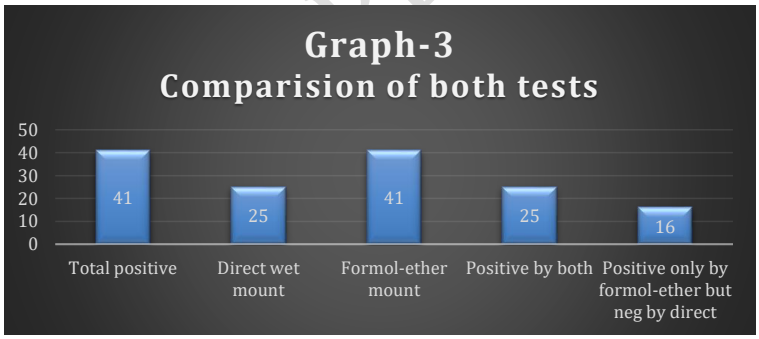
- Parasite detection rate across various age groups,n=41

The 41 positive stool were positive for single parasitic egg or cyst, polymicrobial parasitic infections were not seen. Graph-2 describes spectrum of parasitic eggs/cysts seen

Commented [ho17]: stools



The Formal-ether sedimentation technique revealed 41 positive stool samples whereas out of these 41 samples direct wet-mount was positive in 25 only and negative in 16 samples. All 25 direct wet mount positive were positive by Formol-ether concentration technique(Graph-3)

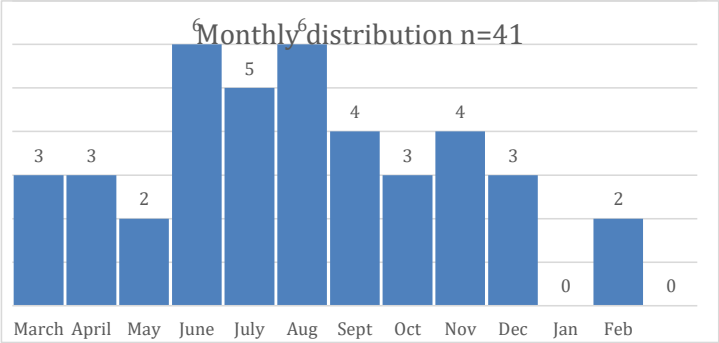


Seasonal trends

During the study period of one year out of 41 positive stool microscopy maximum number of positive samples were seen in month of June ,July and August whereas least number of positive samples were seen in January and February(Graph-4)

~~Table 5- During the study period of one year out of 41 positive stool microscopy~~

~~March~~	~~April~~	~~May~~	~~June~~	~~July~~	~~Aug~~	~~Sep~~	~~Oct~~	~~Nov~~	~~Dec~~	~~Jan~~	~~Feb~~
3	3	2	6	5	6	4	3	4	3	0	2



Graph-4- Monthly distribution of positive samples

4. Discussion

DISCUSSION

Commented [ho18]: the discussion is weak and needs stronger explanations

In our study two methods for stool parasite examination were compared i.e Direct mount and Formol ether sedimentation technique. A total of 350 samples were received from which 41 were positive in total for parasitic eggs/cysts, various parasitic eggs /cysts were seen like cyst of Giardia cysts of Entamoeba coli/dispar, Eggs of *H.nana* and *Ascaris lumbricoides* etc.

Total positivity rate found in our study was 12%(i.e 41/350), Previous studies from South India have shown that the prevalence rate of individual GI? parasitic infections in India ranges from 12.5% to 66% [11].

Among the 41 positive stool samples in our study,25(61%) were positive for cyst of *Giardia lamblia* which was found to be highest, similar findings were observed in a study from south India[12]. In a study in Thailand, *G. lamblia* was the most frequent parasite (18.4%).[12] Furthermore, it was more common in the asymptomatic population than the symptomatic ones.

Among the STH?, hookworm is by far the most common in India, as also in our study (7.31%), leading to intractable anaemia in rural people who are accustomed to walking barefoot in contaminated soil. However, the prevalence of hookworm infestation is now grossly decreased in our country compared to that in the 1980s. Puducherry study in 2016 (8.7%)[13] compared to study by Parija and Rao in 1987 where it was 10.5%[14]. This may be due to more use of footwear among farmers, laborers, and other common people in the present days and also gross improvement of sanitation and a commendably higher use of sanitary latrines in villages and among people of poor socioeconomic status due to vigorous campaigning, financial and technical helps by the government in recent years.

Age-related trends: Across age groups, the highest prevalence of intestinal parasites was found in the age group of 11-15yr(17.07%) whereas the lowest prevalence (2.4%) was in the age group 21-25yr? . When common parasites, with prevalence more than 0.1 per cent, were analyzed, the proportions differed by age group

Pre-school and school-going children have been found to be at the greatest risk for intestinal parasitic infections in some studies[15], but others have reported higher prevalence in older age groups[16].

In our study, a significantly higher proportion of samples from male patients was found to be positive compared to females (73% vs. 27%). Although males have generally been found to be more predisposed to be positive for intestinal parasites[17], the differences observed in the prevalence rates of these infections have not always been significant[18]. This difference in the prevalence and intensity of parasite infections has been attributed to increased exposure to certain parasites in males because of occupation and activity and also hormonal and immunological mechanisms such as the immunomodulatory effects of testosterone in males which increases their susceptibility to certain parasitic infections[19]. It has been established that females generally exhibit higher levels of immune responses as compared to males[20].

Our study also looked at whether there was any seasonality in the prevalence of intestinal parasites. For STH infections such as hookworm and for *Giardia* species, the peak was in June to August(41.4%). These peaks corresponded to the months of high humidity levels and rainfall in our setting. Although our study was from a hospital setting, this seasonality was likely also a reflection of the picture in the community. For many intestinal parasites, variation in temperature, humidity and rainfall seems to affect the intensity of infections. This has especially been seen for parasites where there are external larval stages which develop in the soil[21].

Commented [ho19]: was

Commented [ho20]: what was the study?

Commented [ho21]: This phrase is not clear, should be rewritten

Among total 41 samples positive by Formol ether concentration method ,only 25(60%) by direct stool microscopy. An additional 16(39.02%) samples were positive by Formol ether technique which were negative by direct wet mount. All samples positive by wet mount were positive by Formol ether technique aswell

Commented [ho22]: as well

The present study findings were in agreement with the findings of Khyati Jain et al.,[22] and Parameshwarappa? ~~KD~~ et al.,[101]. In their studies 23% parasites were found by direct wet mount while it increased to 36% after concentration of stool sample and 38% parasites were found by direct wet mount which increased to 64.8% after concentration of stool sample respectively

This result is also similar with other studies conducted in Ethiopia among pregnant women previously[24].This confirmed that using FEC technique for routine stool examination is essential for better detection of intestinal parasite and good management intestinal parasite infections. Intestinal parasite infected pregnant women may develop anaemia; this may lead to infant underweight.

Our study finding showed that direct wet mount exhibited very low positivity for the detection of intestinal parasites as compared to the FEC. This was similar with another study.[25]. This suggested that the use of direct wet mount alone as an intestinal parasitic infections identification is insufficient and may lead to false negative results

5. Conclusion

The prevalence of intestinal parasites was under-reported by direct wet mount microscopy. The diagnostic performance of Formol Ether Concentration technique for the diagnosis of intestinal parasites was significantly high as compared to Wet Mount technique in the present study. Therefore, the FEC method should be used in combination with WM as a routine diagnostic technique in health facility laboratories for the diagnosis of intestinal parasites, ~~furthermore~~ atleast ~~three stool samples should be tested per patient to find out true prevalence of intestinal parasites~~.

Commented [ho23]: at least

6. References

This source can be added instead of any old source.

Hiro Mohammed Obaid. Assessment of direct wet mount and various concentration approaches for diagnosing of Entamoeba histolytica /dispar and Giardia lamblia cysts in human stool samples. International Journal of Biology Research, www.biologyjournal.in, ISSN: 2455-6548. Volume 7, Issue 3, 2022, Page No. 17-21.

1. V. M. Oguoma, C. A. Ekwunife: The Need For A Better Method: Comparison Of Direct Smear And FormolEther Concentration Techniques In Diagnosing Intestinal Parasites. The Internet Journal of Tropical Medicine. 2007. Volume 3 Number
2. http://www.ispub.com/ostia/index.php?xmlFilePat Daumerie D, Savioli L. Working to Overcome the Global Impact of Neglected Tropical Diseases: First WHO Report on Neglected Tropical Diseases. Vol. 1. World Health Organization; 2010.
3. Comparison of Formol-Ether, Direct Smear and Nigrosine Methylene Blue for the Diagnosis of Human Intestinal Parasites Sheyin z1*., Bigwan EI1 ., Galadima M2 . 1Department of Medical Laboratory science, University of Jos, Nigeria. 2 Federal University of Technology, Minna, Nigeria. E- mail for Correspondence:Benesh DP (2013)
4. Parental effects on the larval performance of a tapeworm in its copepod first host. Journal of Evolutionary Biology. 26 (8): 1625-1633.3.
5. Christine AN and Chistopher S (2000). Definition of a parasite. British Medical Bulletin. 56 (1): 193-2018
6. Liza AN., Mengistu L., Mulugeta B., Konjit T., Kebreten M and Chanda M (2011). Intestinal parasitic infections Among Under-Five Children And Maternal Awareness about the Infection in Shesha Kekele Wondo Genet Southern Ethiopia. Ethiop. J. Health Dev. 24 (3): 186-190
7. Parameshwarappa KD., Chandrakanth C and 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(7): 1188-1191 Raina A., Yattoo GN., Wani FA., Para RA., Changal KA and Parry AH (2013). Pancreatitis secondary to ascaris lumbricoides: A case series analysis. Int J Med Res Health Sci. 2(3):673-677
8. Moges F., Belyhun Y., Tiruneh M., Kebede Y., Mulu A and Kassu AV (2010). Comparison of the formol-acetone concentration method, the direct iodine preparation and formol-ether concentration methods for the examination of stool parasites. Ethiop J Health Dev. 24(2):148-51.
9. Demeke G, Mengistu G, Abebaw A, Toru M, Yigzaw M, Shiferaw A, Mengist HM, Dilnessa T. 2018Wet Mount and Concentration Techniques of Stool Examination for Intestinal Parasites Identification specialized Hospital, Ethiopia
10. Jayalakshmi S & Dharanidevi S.The Prevalence of Intestinal Parasitic Infections in a Tertiary Care Hospital in Southern India - A Retrospective Study. International Journal of Current Microbiology and Applied Science,2016 5(10): 718-723. 89.

11. Nazer H, Greer W, Donnelly K, Mohamed AE, Yaish H, Kagalwalla A, Pavillard R. The need for three stool specimens in routine laboratory examinations for intestinal parasites. Br J Clin Pract. 1993 Mar-Apr;47(2):76-8. PMID: 8334067
12. Manochitra K, Padukone S, Selvaratthinam AP, Philips A, Parija SC. Prevalence of intestinal parasites among patients attending a tertiary care centre in South India. Int J Curr Microbiol Appl Sci 2016;5:190-7.
13. Wongstitwilairoong B, Srijan A, Serichantalergs O, Fukuda CD, McDaniel P, Bodhidatta L, et al. Intestinal parasitic infections among preschool children in Sangkhlaburi, Thailand. Am J Trop Med Hyg 2007;76:345-50.
14. Manochitra K, Padukone S, Selvaratthinam AP, Philips A, Parija SC. Prevalence of intestinal parasites among patients attending a tertiary care centre in South India. Int J Curr Microbiol Appl
15. Bogitsh BJ, Carter CE, Oeltmann TN. Human Parasitology. 5th ed. London, UK: Elsevier; 2013.
16. Intestinal Tapeworms; Parija SC, Rao RS. Prevalence of parasitic infections in Pondicherry. Indian J Parasitol 1987.
17. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, DiemertD,etal.Soil transmitted helminthinfections:Ascariasis, trichuriasis, and hookworm. Lancet 2006
18. Abu-Madi MA, Behnke JM, Doiphode SH. Changing trends in intestinal parasitic infections among long-term-residents and settled immigrants in Qatar. Parasit Vectors 2010
19. Lifson AR, Thai D, O'Fallon A, Mills WA, Hang K. Prevalence of tuberculosis, hepatitis B virus, and intestinal parasitic infections among refugees to Minnesota. Public Health Rep 2000
20. Smith H, Dekaminsky R, Niwas S, Soto R, Jolly P. Prevalence and intensity of infections of Ascaris lumbricoides and Trichuris trichiura and associated socio 57 demographic variables in four rural Honduran communities. Mem Inst Oswaldo Cruz 2001
21. Klein SL. Hormonal and immunological mechanisms mediating sex differences in parasite infection. Parasite Immunol 2004
22. Schuurs AH, Verheul HA. Effects of gender and sex steroids on the immune response. J Steroid Biochem 1990
23. Weaver HJ, Hawdon JM, Hoberg EP. Soil-transmitted helminthiases: Implications of climate change and human behavior. Trends Parasitol 2010
24. Jain K, Arjun S. Gurjar and Madhurendra S. Rajput. A cross sectional study for prevalence of intestinal parasitic infestation by using saline, iodine, glycerol-iodine, KOH and LPCB wet Mount preparations of stool samples from patients attending AIIMS, Dewas MP(2018)
25. Parameshwarappa K D, Chandrakanth C, Sunil B. —The Prevalence of intestinal parasitic infestations and the evaluation of different concentration techniques of the stool examination. Journal of Clinical and Diagnostic Research, 2012 September (Suppl)