The Need For A Better Method: Comparison Of Direct Smear And Formol-Ether Concentration Techniques In Diagnosing Intestinal Parasites

V Oguoma, C Ekwunife

Citation

V Oguoma, C Ekwunife. *The Need For A Better Method: Comparison Of Direct Smear And Formol-Ether Concentration Techniques In Diagnosing Intestinal Parasites*. The Internet Journal of Tropical Medicine. 2006 Volume 3 Number 2.

Abstract

The study compared results of one direct smear and one formol-ether concentration examination executed on each of 103 stool samples from patients attending a hospital and a medical diagnostic laboratory in Owerri. Formol-ether concentration technique detected 65.26% of positive specimens for one or more intestinal parasites. Direct smear was 34.74% effective. A significant number of the infected population was missed by direct smear method. This accounts for the prevalence of intestinal parasites in the studied population, part of which is ensuing from misdiagnosis. Soft stool exposed most of the intestinal parasites encountered in the study more than other consistency types. Hookworm bears the highest occurrence (15.79%) in soft stools and in the entire study (24.21%) as revealed by concentration method. This study furthermore showed that the age group (9-13) has the highest prevalence for intestinal parasites. Formol-ether detected 23.16% prevalence in this age group while direct smear found 14.74%.

INTRODUCTION

Parasitic diseases caused by intestinal parasites are a major public health problem in developing countries of Africa [1]. Majority of these infections with parasites results from low standard of living associated with poor socioeconomic status, poor sanitation, and misdiagnosis of parasitic infections.

In the diagnosis of intestinal parasites a lot of techniques can be employed. Selection of a particular technique will depend on its affordability, easy to carry out, its effectiveness and level of professionalism involved. Some of these methods are DNA probes, PCR, and direct fluorescent antibody methods [2], which offer high sensitivity, but are expensive for use in the developing world. Direct stool smear, formol-ether, and salt floatation techniques in the form of stool microscopy offers many advantages over other diagnostic methods for detecting intestinal parasites. If performed correctly, it is sensitive, simple, and economical [2].

A high choice of direct stool [3] and modified formol-ether concentration [4] methods in diagnosing intestinal parasites by hospitals and researchers in developing countries, Nigeria in particular is as a result of its affordability, simplicity and sensitivity. Though each has a preference over another in certain aspects.

Due to low density of the parasites in the faeces, direct smear method is useful for the observation of motile protozoan trophozoites and the examination of cellular exudates, but is not recommended solely for the routine examination of suspected parasitic infections [5]. Therefore there is need for increased probability of finding the parasites in faecal samples to allow for an accurate diagnosis, hence there is need to concentrate. Though direct stool smear technique is quick to prepare, and is inexpensive when compared with modified formol-ether concentration method, it can miss parasites (ova, cysts, and larvae) if concentration is too low or if too much debris or fat is present. Sand, seeds, or other faecal debris can make opposition of coverslip onto slide difficult. Most diagnostic centers in Nigeria show high preference to this method, owing to the fact that it is inexpensive and non-time-consuming thereby disregarding the consequences of misdiagnosis, which has led to high prevalence of parasitic diseases and morbid conditions in Nigeria. Such complications resulting from misdiagnosis has been described [6]

The concentration procedure as described by Allen and Ridley [4] used in hospitals requires the use of ether or ethyl

acetate as a lipid removing agent and formalin as a fixative. The advantages of this method are that it will recover most ova, cysts and larvae and retain their morphology, thereby facilitating identification. There is less risk of infection from bacteria and viruses because they may not be able to survive the concentration process involved. Knight et al., [7] indicated that the concentration technique has additional advantage by allowing for transportation and storage after faeces are preserved in formalin. Conversely, it has the disadvantage of destroying trophozoites stages and distorting cellular exudates. Liquid faeces do not concentrate well, this is where direct stool smear method show great preference. Acari et al., [5] in their study stated that since the sieves sometimes are not disposable, there is a problem with cleaning for re-use. The system is also open so there is a biohazard and odour issue.

It is then clear that both methods have some advantages and disadvantages but then which method is better?

The study is therefore, aimed at comparing the direct stool smear and formol-ether concentration methods in the diagnosis of intestinal parasites for determination of a better method.

MATERIALS AND METHODS

This report is based on the examination of faecal samples obtained from individuals of different age groups attending HONIA Medical Diagnostic Laboratory and Holy Rosary Hospital, all in Owerri, Imo State.

STUDY AREA

The study was carried out in two neighboring L.G.As. in Owerri. HONIA Medical Diagnostic Laboratory and Holy Rosary Hospital in Owerri Municipal and Owerri North L.G.As., respectively. Samples were collected from both laboratories but analysis was carried out solely in HONIA Medical Diagnostic Laboratory.

SAMPLING TECHNIQUE

Stool samples were collected from 103 individuals attending HONIA Medical Diagnostic Laboratory and Holy Rosary Hospital in Owerri Municipal and Owerri North L.G.As., respectively. The samples were examined parasitologically for the presence of parasite ova, cysts, larvae and trophozoites. Egg count for each method is carried out as that described by Monica [8].

PARASITOLOGICAL TECHNIQUE

The method adopted was that supplied by WHO [9] and described by Ukaga et al., [10]. It includes:

- Direct Smear Method.
- Formol-ether Concentration Technique.

IDENTIFICATION OF INTESTINAL PARASITES

The parasites were identified using the bench aid for diagnosis of intestinal helminthes [9] and confirmed by Dr. C.A. Ekwunife of Parasitology and Entomology Department UNIZIK Awka. A medical laboratory scientist also helped in the identification of protozoa and helminth parasites.

STATISTICAL ANALYSIS

The data obtained from samples were statistically analysed using chi-square (X2) test to establish whether diagnosis is dependent on the technique employed and if distribution of intestinal parasites in stool is related to the consistency of the stool.

RESULTS

A total of 103 samples collected from the same number of individuals were examined for intestinal parasites using the direct smear method and the formol-ether concentration technique. Sixty two (65.26%) was recorded for formol-ether concentration technique and 33 (34.74%) for direct smear method (Table 1). The different types of intestinal parasites are also shown in Table 1.

Figure 1

Table 1: Number and percentage of different parasites found

Intestinal Parasites	Formol-ether conc./(%)	Direct smear/(%)	Overall/(%)
Hookworm	23 (24.21)	11 (11.58)	34 (35.79)
A. lumbricoides	9 (9.47)	5 (5.26)	14 (14.74)
T. trichiura	5 (5.26)	2 (2.11)	7 (7.37)
S. mansoni	2 (2.11)	0 (0)	2 (2.11)
Entamoeba coli	14 (14.74)	9 (9.47)	23 (24.21)
E. histolytica	6 (6.32)	3 (3.16)	9 (9.47)
Giardia lamblia	3 (3.16)	3 (3.16)	6 (6.32)
Total	**62 (65.26)**	**33 (34.74)**	**95 (100.00)**

Table 1 shows that the higher number of helminth found were Hookworm 24.21% for formol-ether concentration and 11.58% for direct smear ($p<0.05$). The higher number of protozoa found was E. coli (14.74%) for formol-ether concentration and 9.47% for direct smear ($p<0.05$). The total

number of parasites identified with formol-ether concentration technique was 62 (65.26%) while direct smear was 33 (34.74%) ($p<0.05$).

Figure 2

Table 2: Distribution of parasites by age.

Parasite	Formol-ether conc. / Age group (yrs)						Direct smear/ Age group (yrs)					
	4-8	9-13	14-18	19-23	24-28	29-33	4-8	9-13	14-18	19-23	24-28	29-33
Hookworm	-	11	9	2	1	-	-	7	3	1	-	-
A. lumbricoides	2	3	2	1	1	-	1	2	2	-	-	-
T. trichiura	-	3	1	1	-	-	-	1	-	1	-	-
S. mansoni	-	-	2	-	-	-	-	-	-	-	-	-
Entamoeba coli	3	1	1	1	3	5	2	1	2	-	1	3
E. histolytica	2	2	-	1	-	1	1	1	-	-	-	1
G. lamblia	-	2	1	-	-	-	-	2	1	-	-	-
Total Infected	7	22	16	6	5	6	4	14	8	2	1	4
% Prevalence	*7.37*	*23.16*	*16.84*	*6.32*	*5.26*	*6.32*	*4.21*	*14.74*	*8.42*	*2.11*	*1.05*	*4.21*

The results in Table 2 demonstrate the distribution of parasites by age. Amongst the six age groups studied, Hookworm and E. coli showed the highest prevalence in both methods.

Figure 3

Table 3: Distribution of parasites with respect to the consistency of the stool samples

Parasite	Formol-ether conc. / Stool consistency				Direct smear/ Stool consistency			
	Formed	Soft	Loose	Watery	Formed	Soft	Loose	Watery
Hookworm	7	15	1	-	2	4	3	2
Ascaris lumbricoides	3	5	-	1	-	2	1	2
Trichuris trichiura	2	3	-	-	-	2	-	-
Schistosoma mansoni	-	2	-	-	-	-	-	-
Entamoeba coli	1	3	7	3	1	2	3	3
Entamoeba histolytica	-	2	3	1	-	-	1	2
Giardia lamblia	-	-	2	1	-	-	-	3
Total Infected	**13**	**30**	**13**	**6**	**3**	**10**	**8**	**12**
% Prevalence	*13.68*	*31.58*	*13.68*	*6.32*	*3.16*	*10.53*	*8.42*	*12.63*

Table 3 shows the distribution of parasites with respect to the consistency of the stool samples. Throughout the study, soft stool samples were more frequently encountered than other consistency types and it sampled all the parasites encountered in the study as indicated by the formol-ether concentration method.

DISCUSSION

In Hospital/Medical laboratory based studies, repeated stool examinations would be expensive, time consuming in cases of emergency, and operationally difficult due to lack of motivation among technicians in performing stool microscopy. Therefore, the present study was conducted to compare two methods of stool microscopy mostly available in Hospitals and Medical laboratories in developing countries. The evaluation was conducted on direct smear and formol-ether concentration techniques using fresh stool samples collected from a population known to be endemic with intestinal parasites and the result of the study confirmed that human parasitic infections existed in the population studied.

Prevalence estimates of the two diagnostic methods revealed the distinctive superiority of formol-ether concentration technique over direct smear ($p<0.05$). This is in agreement with the work of Amal et al., [11], whose work shows 10.7% and 5.35% for formol-ether concentration and direct smear methods respectively. Their work also found that the sensitivity of formol-ether concentration was higher than direct smear. This in part, accounts for high parasitic burden among the individuals between age group of 9-18, and morbidity rate as a result of misdiagnosis. The age group 9-13 had the highest prevalence of Hookworm infection. This is in contrary to the study of Mbanugo and Okakpu, [12], where the peak of infection was in the age group 5-8 years.

In the detection of Giardia lamblia, both methods were effective, but formol-ether concentration sampled the cysts while the direct method was effective in detecting the trophozoites as well as the cysts. Therefore this study has shown that direct smear has a higher specificity in the detection of G. lamblia and other protozoan parasites (especially in cases where exposure of trophozoites is the primary interest). Though Akujobi's et al., [13] study on the comparative evaluation of both methods in the identification of Cryptosporidium species showed that there was no statistical difference between the two methods. Amal et al., [11] is also contradictory, where they found a statistical difference in both methods in diagnosing for G. lamblia with formol-ether concentration showing greater effectiveness than direct smear.

This study has also shown that formol-ether concentration is a very useful method in diagnosing intestinal helminths. By

comparison, formol-ether concentration sampled 24.21% hookworm, 9.47% A. lumbricoides, 5.26% T. trichiura and 2.11% S. mansoni while direct smear indicated 11.58% hookworm, 5.26% A. lumbricoides, 2.11% T. trichiura and 0% S. mansoni ($p<0.05$). This complied with the findings of Knight et al., [7], where they posited that the modified formol-ether concentration technique is more successful in detecting light infections. Direct smear missed 12.63%, 4.21%, 3.15% and 2.11% of infected individuals with hookworm, A. lumbricoides, T. trichiura and S. mansoni respectively.

In analyzing the distribution of parasites with respect to the consistency of the stool samples, Soft stool samples were encountered most against other consistency types with the highest number 42 of 103 examined samples. It revealed both protozoal and helminthic parasites with formol-ether concentration again showing a greater preference to direct smear technique. The study did not go into unraveling the relationship between the prevalence of soft stool among the population and the distribution of intestinal parasites. There is also a discrepancy between both methods in diagnosing for parasites in watery stool. Direct smear was effective in detecting for intestinal parasites in watery stools than formol ether concentration.

CONCLUSION

Though the concentration technique was weighed down with some shortcomings like cost of running the test, which involves the need for a centrifuge, constant electric supply, a well ventilated work space, adequate water supply, a standard light microscope and reagents (which are expensive as such), but still is reserved as the best method for diagnosing intestinal parasites in resource-poor countries like Nigeria, where a variety of non-microscopic methods for diagnosing intestinal parasites exist or is unaffordable. Such non-microscopic methods include antigen detection in faeces, direct fluorescent antibody methods, and molecular biological techniques such as DNA probes and polymerase chain reaction (PCR) [2]. There is a possibility of finding ova, cysts and larvae among low excretes with concentration technique than direct smear method since it involves the use of 1g faeces.

Therefore, formol-ether concentration technique should be adopted for routine faecal examination since it exposes a higher percentage of infection missed by direct smear method, which is the implemented method by Medical diagnostic laboratories and hospitals in developing countries (e.g., Nigeria). This will pave a way in reducing the prevalence of intestinal parasites resulting from misdiagnosis.

Generally, there should be an outstanding concern for stool microscopy because lack of motivation among stool microscopists, non-recognition of work of stool microscopists by peers, ineffective supervision by laboratory consultants, improper collection of specimens, empirical treatment by doctors and a host of other factor as described by [2], are contributing factors towards misdiagnosis of intestinal parasites.

Health personnel (laboratory technicians/scientists and clinicians) in developing countries who rely on direct smear method should be aware that a person is not to be classified as uninfected on the basis of a simple negative examination by the use of direct smear.

Finally, if formol- ether concentration technique is adopted as a routine method in diagnosing intestinal parasites or is incorporated with direct smear method, stool microscopy will continue as the most important diagnostic method and at the same time reducing the traces of prevalence of intestinal protozoan and helminth parasites ensuing from misdiagnosis.

References

1. Oduntan, S.O. The health of Nigerian school children of school age (6 - 15 years). II Parasitic and infective conditions, the special senses, physical abnormalities. Annals of Tropical Medicine and Parasitology, 1974; 68:145-156.
2. Parija, S.C., and Srinivasa, H. Viewpoint: The neglect of stool microscopy for intestinal parasites and possible solutions. Tropical Medicine and International Health. 1999; 4(7): 522-4.
3. Beaver, P.C. The standardization of faecal smears for estimating egg production and worm burden. J. Parasitol; 1950; 36: 451-6.
4. Allen, A.V.H., and Ridley, O.S. Further observations on the formol-ether concentration technique for faecal parasites. J. Clin. Pathol; 1970; 23: 343-352.
5. Arcari, M., Boxendine, A., and Bennett, C.E. Diagnosing medical parasites through coprological techniques. 2000. Available online at http://www.soton.ac.uk/~ceb/Diagnosis/vol1.htm
6. Barnabas, M.M., and Aboi, J.K.M. Missed dignosis of schistosomiasis leading to unnecessary surgical procedures in Jos University Teaching Hospital. Tropical Doctor. 2005; 35: 96-97
7. Knight, W.B., Hiatt, R.A., Cline B.L., and Ritchie, L.S. A modification of the formol-ether concentration technique for increased sensitivity in detecting Schistosoma mansoni eggs. Am. J. Trop. Med. Hyg; 1976; 25(6): 818-23.
8. Monica Cheesbrough. Medical laboratory manual for tropical countries. Vol.1. 1981
9. World Health Organization. Basic laboratory methods in

Medical Parasitology. W.H.O. 1991. Geneva.
10. Ukaga, C.N., Onyeka, P.I., and Nwoke, B.E.B. Practical medical Parasitology. 1st edition. Avan Global publications; 2002; p. 18-26.
11. Amal, M.N., Azza, H.A., Hager, A.S., and Ayman, A.E. Evaluation of parasep (faecal parasite concentrator) for detection of Giardia lamblia in faeces. Sci. Med. J. ESCME; 2003; 15(3): 1-4.
12. Mbanugo, J.I., and Okakpu, V.O. Epidemiological survey of helminthic infections in primary school children in southeastern Nigeria. Journal of Environmental Health; 2004; 1(2): 64-8.
13. Akujobi, C.N., Iregbu, K.C., and Odugbemi, T.O. Comparative evaluation of direct stool smear and formol-ether concentration methods in the identification of Cryptosporidium species. Nigerian Journal of Health and Biomedical Sciences; 2005; 4(1): 5-7.

Author Information

V. M. Oguoma, B.Sc.
Department of Parasitology and Entomology, Nnamdi Azikiwe University

C. A. Ekwunife, Ph.D.
Department of Parasitology and Entomology, Nnamdi Azikiwe University