EVALUATION OF MINI PARASEP® SF FAECAL PARASITE CONCENTRATOR FOR THE LABORATORY DIAGNOSIS OF INTESTINAL PARASITISM

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ABSTRACT

Objective: To evaluate the efficacy of Mini Parasep® SF commercial faecal parasite concentrator for the laboratory diagnosis of intestinal parasitism. This was compared with the modified Ridley-Allen sedimentation technique.

Methods: A total of 40 stool samples, consisting of 08 positive control samples and 32 single fresh stool samples from antenatal clinic patients attending Our Lady of Apostles (OLA) Hospital, Jos were used for the study. Wet-mount microscopic examinations were carried out on all the faecal deposits from both the formol-ether technique and the Mini Parasep® SF method using physiological saline and D'Antoni's iodine.

Results: Out of the 40 samples, 09 (22.5%) were positive using the modified formol-ether method while 14 (35.0%) were positive with the Mini Parasep® SF method. The formol-ether method detected 07 different parasites as against 08 by the Mini Parasep® SF method. The formol-ether method detected the following parasites – *Hymenolepis nana* 01 (2.5%), *Dicrocoelium dendriticum* 01 (2.5%), Hookworm 04 (10.0%), *Trichuris trichiura* 01 (2.5%), *Giardia lamblia* 01 (2.5%), *Entamoeba coli* 03 (7.5%) and *E. histolytica/dispar* 03 (7.5%). The Mini Parasep® SF method detected *H. nana* 02 (5.0%), *Schistosoma mansoni* 02 (5.0%), Hookworm 03 (7.5%), Strongyloides stercoralis 01 (2.5%), *Ascaris lumbricoides* 01 (2.5%), *E. coli* 05 (12.5%) *G. lamblia* 02 (5.0%) and *E. histolytica/dispar* 07 (17.5%). The sensitivity, specificity, positive and negative predictive values for modified formol-ether and Mini Parasep® SF techniques respectively are as follows; sensitivity (60% and 93%), specificity (80% and 96%), positive predictive values (57.4% and 91.3%) and negative predictive values (81.7% and 96.8%).

Conclusion: The study has shown that Mini Parasep® SF method can be used in place of the modified formol-ether method for the laboratory diagnosis of intestinal parasitism especially in endemic areas.

INTRODUCTION

Inadequate water, sanitation and hygiene are responsible for a major proportion of the burden of disease and death in developing countries, apart from causing hundreds of millions of people to surrender their rights to healthy and dignified lives. Intestinal parasites constitute an important health problem in many parts of the world[1]. The consequences of intestinal parasitic infestation have been shown, among others, to affect nutritional status, physical development, mental function, verbal ability and inhibition control aspects of cognitive behaviour in children[2-5].

Intestinal parasitism is endemic in Nigeria due to poor environmental situation in most communities, indiscriminate defecation, improper disposal of waste, gross environmental pollution with agrochemicals and industrial wastes plus the steady contamination of surface and underground water[6]. Poorly planned housing and human habitation patterns also contribute to environmental decay, as urbanisation in developing countries usually results in unplanned, uncontrolled and constant migration of people from the rural areas to the urban centres in search of employment opportunities.

It has also been noted that some cases of chronic diarrhoea in HIV/AIDS patients and other immunocompromised patients are due to the opportunistic coccidian intestinal parasites[7]. Therefore, the microscopic examination of faeces is essential for laboratory diagnosis of intestinal parasitism. In some infected patients, the parasite density may be small with the result that the number of the diagnostic stages (eggs, larvae and adults or part of the adult worms) may be scanty. This may lead to misdiagnosis with the attendant complications.

Presently, we make use of direct wet mount and modified formol-ether techniques for the examination of faecal samples. The formol-ether technique, apart from increasing the sensitivity of microscopy for the laboratory diagnosis of intestinal parasitism, has some disadvantages such as the use of ether which is highly flammable, needs some extra materials (funnel, gauze, centrifuge tubes etc) and it is an open system thus, increasing the chances of laboratory infections.

In the light of the devastating effects of intestinal parasites, the present study was designed to evaluate the efficacy of Mini Parasep® SF for the laboratory diagnosis of intestinal parasitism. This study compared the open method for modified Ridley-Allen concentration technique with Mini Parasep® SF, a commercial kit developed by DiaSys Limited which is an enclosed, single-use, disposable system.

MATERIALS AND METHODS

Forty faecal samples consisting of eight known positive samples and 32 fresh samples from patients were used for the study. Ethical approval and informed consent were duly obtained before the commencement of the study. The 32 samples were collected from antenatal clinic patients attending Our Lady of Apostles (OLA) hospital in Jos metropolis.

a) Modified Formol-ether method.

The 40 faecal samples were prepared for microscopy using the Modified Formol-ether method and in this method, the filtration process was skipped. About 1gm of the sample is emulsified in 7ml of 10% formol-saline and 3ml of ether was added, covered and vortexed/mixed. The preparations were then centrifuged at 1500 rpm for 2 minutes. The fatty plug was dislodged and the supernatant decanted into a disinfectant jar. The deposit was subsequently examined microscopically using ×10 and ×40 objectives.

b) Mini Parasep® SF method

The 40 faecal samples were also prepared using the Mini Parasep® SF method.

The tubes and the sedimentation cones were labelled with the specimen identification numbers. A level faecal sample was introduced into each tube containing 3.3ml of 10% formol-saline using the spoon on the end of the Mini Parasep® SF filter. The Mini Parasep® SF was sealed by screwing in the filter/sedimentation cone unit. This was then vortexed to emulsify with the sedimentation cone pointing upwards. The Mini Parasep® SF was then inverted and centrifuged at 1500 rpm for 2 minutes. The mixing chamber and the filter was then unscrewed and discarded for incineration while the supernatant in the sedimentation cone was decanted. The deposit was then examined microscopically using physiological saline and D'Antoni's iodine for the eggs, trophozoites and larvae of intestinal parasites. Each of the preparations was examined systematically for a minimum of 5 minutes microscopically.

Statistical comparisons were performed using Chi-square analysis and 'P' values of <0.05 were accepted as statistically significant.

RESULTS

Out of the 40 samples examined, 09 samples (22.5%) were positive using the modified formol-ether method while 14 (35.0%) were positive with the Mini Parasep® SF method; but the difference is not statistically significant ($\chi^2 = 1.1304$, $P > 0.05$). The formol-ether technique identified 07 different parasites while the Mini Parasep® SF method identified 08 different parasites (Table 1).

Table 1 Number of Positive Samples in relation to the methods used (n = 40)

Technique	No. (%) Positive	No. (%) Negative	Types of Detected Parasites
Modified Formol-Ether	09 (22.5%)	31 (77.5%)	07
Mini Parasep® SF	14 (35.0%)	26 (65.0%)	08

$\chi^2 = 1.1304$, $P > 0.05$

Table 2 shows the distribution of the intestinal parasites in relation to the methods used in the analysis of the faecal samples.

Table 2 Distribution of the Intestinal Parasites in relation to the methods used (n = 40)

Intestinal Parasite	Number (%) Positive	
	Modified Formol-Ether Technique	Mini Parasep® SF Technique
Hymenolepis nana	01 (2.5)	02 (5.0)
Dicrocoelium dendriticum	01 (2.5)	Nil
Schistosoma mansoni	Nil	02 (5.0)
Hookworm	04 (10.0)	03 (7.5)
Strongyloides stercoralis	Nil	01 (2.5)
Ascaris lumbricoides	Nil	01 (2.5)
Trichuris trichiura	01 (2.5)	Nil
Entamoeba coli	03 (7.5)	05 (12.5)
E. histolytica/dispar	03 (7.5)	07 (17.5)
Giardia lamblia	01 (2.5)	02 (5.0)

With the modified formal-ether technique, the following parasites were identified - *Hymenolepis nana* 01 (2.5%), *Dicrocoelium dendriticum* 01 (2.5%), Hookworm 04 (10.0%), *Trichuris trichiura* 01 (2.5%), *Giardia lamblia* 01 (2.5%), *Entamoeba coli* 03 (7.5%) and *E. histolytica/dispar* 03 (7.5%).

With the Mini Parasep® SF technique, the following parasites were detected - *H. nana* 02 (5.0%), *Schistosoma mansoni* 02 (5.0%), Hookworm 03 (7.5%), Strongyloides stercoralis 01 (2.5%), *Ascaris lumbricoides* 01 (2.5%), *E. coli* 05 (12.5%) *G. lamblia* 02 (5.0%) and *E. histolytica/dispar* 07 (17.5%).

Tables 3 and 4 show the Sensitivity, Specificity, Positive and Negative predictive values of the two methods. Mini Parasep® SF method recorded a sensitivity of 93%, specificity of 96%, positive predictive value of 91.3%, and negative predictive value of 96.8%. The modified formol-ether technique recorded a sensitivity of 60%, specificity of 80%, positive predictive value of 57.4%, and negative predictive value of 81.7%.

Table 3 Comparison of Modified Formol-Ether and Mini Parasep® SF techniques for the laboratory diagnosis of Intestinal Parasitism.

Parameter (%)	**Formol-Ether Technique**	**Mini Parasep® SF Technique**
Sensitivity	60	93
Specificity	80	96
Positive Predictive Value	57.4	91.3
Negative Predictive Value	81.7	96.8

Table 4 Detection of Intestinal Parasites using Modified Formol-Ether and Mini Parasep® SF Techniques.

Sample No.	Formol-Ether Method	Mini Parasep^{R} SF
01	*H. nana*	*H. nana*
02	*E. coli, E. histolytica/dispar, D. dendriticum*	*E. coli, E. histolytica/dispar, Hookworm, S. stercoralis*
03	*E. coli, E. histolytica/dispar*	*E. coli, E. histolytica/dispar*
04	*Hookworm*	*Hookworm*
05	*Nil*	*H. nana, S. mansoni*
06	*Hookworm*	*Hookworm, S. mansoni, E. histolytica/dispar*
07	*G. lamblia*	*G. lamblia*
08	*Hookworm*	*Hookworm*
09	Nil	Nil
10	Nil	Nil
11	Nil	Nil
12	Nil	Nil
13	Nil	Nil
14	Nil	Nil
15	Nil	Nil
16	Nil	Nil
17	Nil	Nil
18	Nil	Nil
19	Nil	Nil
20	Nil	*G. lamblia*
21	Nil	*E. coli, E. Histolytica/dispar*
22	Nil	Nil
23	Nil	Nil
24	Nil	Nil
25	Nil	*E. histolytica/dispar*
26	*E. coli, E. Histolytica/dispar*	*E. coli, E. Histolytica/dispar*
27	Nil	Nil
28	Nil	*E. coli, E. Histolytica/dispar*
29	Nil	Nil
30	Nil	Nil
31	Nil	Nil
32	*Hookworm, Trichuris trichiura*	Nil
33	Nil	Nil
34	Nil	Nil
35	Nil	Nil
36	Nil	Nil
37	Nil	*E. histolytica/dispar*
38	Nil	Nil
39	Nil	Nil
40	Nil	Nil

DISCUSSION

This study evaluated the efficacy of the Mini Parasep® SF method for the laboratory diagnosis of intestinal parasitism by comparing it with the modified formol-ether technique that is routinely used in some diagnostic laboratories. Routinely, the filtration step in the formol-ether technique is being skipped by most laboratories because it is cumbersome.

Overall, the recovery of parasites by both methods as shown in Table 1 is comparable, provided the microscopy is done expertly. There is no significant difference ($P > 0.05$) between the two methods.

As shown in Table 3, the sensitivity, specificity, positive and negative predictive values of the Mini Parasep® SF method are all higher than the corresponding values with respect to the modified formol-ether method. This might be due to the skipping of the filtration process in the modified formol-ether method, as the filtration process makes the procedure very cumbersome especially where large number of specimens are involved (either routinely or for research surveys). Thus, in endemic areas where the prevalence of intestinal parasitism is high, the use of Mini Parasep® SF method is desirable.

However, the rapidity and safety of the laboratory personnel are added advantages of the Mini Parasep® SF method. The standard open Ridley-Allen formol-ether sedimentation method uses several apparatus (centrifuge tubes, funnel, surgical gauze and applicator sticks) which are labour intensive in terms of disinfecting and cleaning. This is in addition to the use of diethyl ether which is highly flammable with the attendant hazards to the laboratory personnel which, in some cases, especially in the rural communities, are not adequately trained. Secondly, some of the diagnostic laboratories may not afford to to procure diethyl ether as a Winchester bottle of ether costs about $100 (#15,000). The practice in these laboratories is to use only the direct wet mount resulting in the misdiagnosis of some positive cases.

The Mini Parasep® SF technique has the advantage of being a disposable enclosed system thus minimizing the apparatus used especially with the filtration process in the modified formol-ether technique. This ultimately speeds up the procedure making it easier to handle and more user friendly. In terms of laboratory safety, it has an air/liquid seal and safety lock; the seal preventing the release of aerosols and other hazardous materials while the lock ensures that the mixing chamber and filter are removed together for safe disposal after centrifugation. It is also a single use device and so minimizes the risk of sample contamination.

In terms of cost, presently laboratories charge from #150 - #700 ($1 - $4.60) for stool microscopy. The laboratories that charge lower amounts, in most cases, cannot afford to use the formol-ether sedimentation technique and so will miss out some positive samples especially when the parasite density is low. If the intended transfer price of #200 ($1.30) for the Mini Parasep® SF method is assumed then it is definitely more cost-effective and can be used in all categories of diagnostic laboratories.

It is also suggested that for diarrhoeic samples from immunocompromised patients, smears should be prepared from the deposits before staining by modified Ziehl-Neelson method for the coccidian parasites.

In conclusion, the recovery of intestinal parasites (diagnostic stages) by the modified Ridley-Allen sedimentation technique and Mini Parasep® SF, a commercial faecal parasite concentrator is comparable, although the latter is preferred due to the inherent advantages enumerated earlier.

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