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EVALUATION OF MIDI PARASEP® FAECAL PARASITE CONCENTRATOR FOR THE DETECTION OF INTESTINAL PARASITIC INFECTIONS: A COMPARATIVE STUDY

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Abstract

Direct stool examination is the gold standard technique for diagnosing intestinal parasitic infection. Stool examination by stained smear, concentration techniques and sometimes culture are the commonly used. The sensitivity of direct smear is low and requires repeated smears. Formalin-ethyl acetate concentration method is more sensitive, but time consuming. Midi-Parasep® concentrator is a new diagnostic tool used as a routine stool examination for intestinal parasitic infections worldwide. There's a lack of reports which evaluate the efficacy of Midi-Parasep® procedure in areas of limited diagnostic facilities and poor resource settings. Therefore, our current study represents the first report that assessed the Midi-Parasep® technique by comparing its performance to other economic standard measures like modified Ridley-Allen and formol detergent concentration techniques for detection of intestinal parasites in human stool samples. We examined 306 fecal samples using Midi-Parasep fecal parasite concentrator, modified Ridley-Allen concentration and formol detergent concentration techniques. The best over-all sensitivity (71.7%) was obtained for Midi-Parasep technique followed by formol detergent concentration (66.7%) then modified Ridley-Allen technique (51.7%). Regarding, helminths parasites, the most sensitive was formol detergent concentration technique (70%), followed by the Midi-Parasep technique (60%), and Modified Ridley-Allen technique (33.3%). Referring to the intestinal protozoa, midi-Parasep technique had the highest sensitivity (83.3%), followed by modified Ridley-Allen procedure (70%) then formol detergent concentration technique (63.3%).

Keywords: Stool examination, Parasites, Midi-Parasep®, Concentration methods.

Introduction

Intestinal parasitic infection standstill causes foremost public health obstacles in developing countries like Egypt. It affects millions of people worldwide, especially children in developing countries (PAHO, 2019). In the past, many of these infections were mainly linked to tropical and subtropical areas. Nowadays, the change in climate and vector ecology, as well as the increase in the international travel influenced the transmission of many parasitic diseases (Momčilović *et al,* 2019). Infectious intestinal parasites (IPs) including helminths and protozoans represented a subset of neglected diseases, mainly in the developing and tropical countries with efficacious and cost-effective health interventions were required to reduce the parasitic infections (Al-Rifai *et al,* 2020).

One of the strategic lines of action (2016-2022) implemented by WHO is scaling up early detection and diagnosis, as obstacles for controlling NID (WHO 2016). Therefore, the difficulty of diagnosis is the main problem in the control of intestinal parasitic infections. There are many methods for diagnosis of the intestinal parasitic infections; counting stool examination, immunological and molecular measures (Stensvold and Nielsen, 2012). The choice of a particular technique is usually influenced by affordability, simplicity, cost, sensitivity in addition to the level of technical skills involved (Garcia, 2001). Microscopic examination remains the cornerstone of parasitological diagnosis, as it helps in providing an epidemiological assessment of the parasite burden (Momčilović *et al,* 2019). However, the major drawback of microscopic diagnosis is its dependence on the morphological identification of parasites in different biological samples requiring a high level of experience for parasite detection and identification (Weerakoon and McManus, 2016).

To improve the detection rates of gastrointestinal parasites, various concentration techiques were used, such diagnostic ones must be economic and highly sensitive (Couturier *et al,* 2015). Formalin-ether sedimentation technique is commonly used in laboratories owing to its ability to isolate a large variety of parasites from fresh and preserved facial samples (Utzinger *et al,* 2010). However, it's a labor-intensive procedure and is usually associated with hazards of using the inflammable lipid solvents (Sanprasert *et al*, 2016). A locally common dish-washing detergent is used to replace diethyl ether in formalin-detergent concentration technique that was simple to perform, safe and inexpensive. However, it has less value in detecting the intestinal protozoa (Ahmadi and Damraj, 2009). The drawbacks of the standard concentration techniques have encouraged the development of new commercial kits. Such kits decrease the risks of formalin by being enclosed, in addition to the use of ethyl acetate instead of ether, as it is less flammable and more stable (Manser *et al,* 2016). Different types of commercial faecal concentrators are actively used including, Parasep® Faecal Parasite Concentrators produced by Apacor Ltd./DiaSys Europe Ltd. (Sanprasert *et al*, 2016). Parasep® is an enclosed single-use disposable system that was less hazardous procedure of comparable efficiency to the standard concentration methods (Zeeshan *et al,* 2011).

This study aimed to evaluate the Midi-Parasep® for the detection of intestinal parasites in human stool samples compared with the widely used standard diagnostic techniques: modified Ridley-Allen technique and formol detergent concentration technique.

Materials and Methods

Sample collection: was carried from August 2018 to May 2019. A total of 306 fresh stool samples were collected from patients of different ages, and sex, attended the Pediatric and Tropical Medicine Outpatient Clinics of Zagazig University Hospitals. They suffered from different gastrointestinal troubles.

The stool samples were macroscopically examined for determination of consistency (formed, semi-formed, soft or watery), color (yellowish, greenish, brownish), odor (normal or offensive and presence of blood and/or mucous.

Each sample was examined by: 1- Direct smear method: Unstained and stained smears with the Lugol's iodine and eosin methods (Fleck and Moody, 1988) were used to identify the helminthic eggs or larvae and the protozoan cysts, oocysts or trophozoites. 2- Modified Ridley-Allen technique (MRAT): About 1gm of the fecal sample was emulsified in 7ml of 10% formol-saline and 3ml of ethyl acetate was added, covered and vortexed for 15-30 seconds. The preparations were then centrifuged at 1500rpm for 3 minutes. The fatty plug was removed and then supernatant fluid was decanted. Deposit was examined microscopically using X10 & X40 objectives (Manser *et al,* 2016). 3- Formol detergent concentration technique (FDCT): About 0.5ml of the fecal specimen was mixed in 10ml of stock formol detergent solution (10ml of 37% formaldehyde, 10ml of detergent liquid solution and 480ml tap water). Subsequently, it was strained through 2 layers of gauze into a 15ml centrifugation tube which was then filled to the 13ml mark with the formalin-detergent solution and shaked vigorously for 30 seconds. The suspension was allowed to stand undisturbed overnight, the supernatant fluid was removed and then discarded. The sediment was examined microscopically with a magnification of X10 & X40 (Kightlinger and Kightlinger, 1990).
4- Midi-Parasep® Faecal Parasite Concentration technique: Midi-Parasep® Faecal Parasite Concentrator (MP): (Apacor Ltd. Unit 5 Sapphire Centre, Fishponds Road, Wokingh am, Berkshire RG41 2QL, England) was tested for the concentrated fecal parasites. This kit was composed of a sedimentation cone, two-stage filtration matrix, a mixing chamber and Parasep lid (Fig. 1). There was

also a debris trap, so that rejected particles were trapped to prevent extrusion into the sedimentation cone during centrifugation. The device was assembled and sealed by-screwing the vertical filter onto a sedimentation cone and the mixing chamber.

Briefly, the fecal sample was obtained by using the spoon on the end of the Midi-Parasep® filter then mixed with 6ml of the fixative (10% formol-saline). Ethyl-acetate (2ml) was added to the mixing chamber. The Parasep was immediately sealed by screwing the filter thimble onto the sedimentation cone afterwards to the mixing chamber. The seal was an air/liquid seal which prevented the release of biohazardous material. The mixture was vortexed for 15 seconds, and the Parasep was inverted for allowing the mixture to be filtered through the filter thimble. The device was centrifuged at 1200g for 3 minutes. The mixing chamber and the filter thimble were unscrewed and discarded. The supernatant fluid was poured off and the sediment recovered was examined microscopically for ova, cysts or larvae (Fig.2).

Statistical analysis: Data were tabulated and analyzed using SPSS version 22 software (Armonk, 2013). Sensitivity (Se), negative predictive value (NPV) and Kappa index (KI) for each method were calculated. Sensitivity was defined as a probability to detect a true positive case, while negative predictive value (NPV) was defined as a true negative case. The agreement between the results of every method was calculated with the KI. Interpretation of KI was as follows: ≤0 = poor, 0.01-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 substantial, & 0.81-1.00 almost perfect agreement (Sim and Wright, 2005). The combined results from the individual methods (any positive from the three tests was considerded as positive) as the diagnostic "gold standard" method. The accepted level of significance was when 0.05 ($P< 0.05$ was considered significant).

Ethical considerations: The study was approved by the Ethical Committee at the Faculty of Medicine, Zagazig University. Informed consents were obtained from the patients before the starting of the study.

Results

The overall sensitivity of modified Ridley-Allen, Formol detergent concentration and Midi-Parasep® techniques for the diagnosis of all intestinal parasites were 51.7%, 66.7% and 71.7% respectively. Also, the negative predictive values (NPV) in those techniques were 59.2%, 67.8% & 71.2% respectively. The specificity for each technique was 100% and the positive predictive value (PPV) for detecting the different intestinal parasites were 100% in all the three tests (Tab. 1).

Table 1: Comparison between three stool concentration techniques to detect intestinal parasites in stools.

Technique	No. of samples tested			Total	Sensitivity %	Specificity %	NPV %	PPV %
	Positive	Positive	Negative					
MRAT	Positive	31[a]	0[b]	31	51.7%	100%	59.2%	100%
	Negative	29[c]	42[d]	71				
FDCT	Positive	40	0	40	66.7%	100%	67.8%	100%
	Negative	20	42	62				
MP	Positive	43	0	43	71.7%	100%	71.2%	100%
	Negative	17	42	59				

Se= [a/(a+c)]×100; Sp=[d/(b+d)]×100; NPV= [d/(c+d)]×100; PPV=[a/(a+b)]×100. MRAT: Modified Ridley-Allen technique; FDCT: Formol Detergent Concentration Technique; MP: Midi-Parasep®.

The most sensitive technique for diagnosing helminth species was the formol detergent concentration technique (70%), followed by the Midi-Parasep technique (60%), but modified Ridley-Allen technique was the least sensitive one (33.3%). For *A. lumbricoides* infection, the most sensitive technique was the Midi-Parasep® (90%), followed by formol detergent concentration technique (70%), and Modified Ridley-Allen technique (40%). The recovery of *H. nana* eggs by both Midi-Parasep® and formol detergent concentration methods (80%) was higher than that of modified Ridley-Allen procedure (30%). Concerning the *A. duodenale* and *T. trichiura* ova, the formol detergent concentration technique

gave highest sensitivity (60%), followed by modified Ridley-Allen procedure (30%) and then Midi-Parasep® technique (10%).

Midi-Parasep® technique gave the highest sensitivity (83.3%), followed by the modified Ridley-Allen and then formol detergent concentration procedures (70% & 63.3% respectively). Midi-Parasep® and modified Ridley-Allen procedures showed higher sensitivity (80%) in detecting *E. coli* cysts as compared to the formol detergent concentration one (70%). *E. histolytica/ dispar* cysts was better with Midi-Parasep® procedure (90%). The *G. lamblia cysts* were more detected by the Midi-Parasep® method (80%), than by the modified Ridley-Allen (60%) or the formol detergent concentration procedures (70%).

Table 2: *S*pecific sensitivity and negative predictive values of techniques to detect intestinal parasites in stools.

Intestinal parasite and Technique	No. of samples tested		Positive samples by different methods		
	Positive	Negative*	MRAT	FDCT	MP
Helminths (ova)	30	21	10	21	18
Sensitivity%	100%	100%	33.3%	70%	60%
NPV%	100%	100%	52.2%	70%	63.6%
A.lumbricoides	10	7	4	7	9
Sensitivity%	100%	100%	40%	70%	90%
NPV%	100%	100%	53.8%	70%	87.5%
H. nana	10	7	3	8	8
Sensitivity%	100%	100%	30%	80%	80%
NPV%	100%	100%	50%	77.8%	77.8%
(*A.duodenale* &*T.trichiura)*	10	7	3	6	1
Sensitivity%	100%	100%	30%	60%	10%
NPV%	100%	100%	50%	63.6%	43.8%
Protozoa (cysts)	30	21	21	19	25
Sensitivity%	100%	100%	70%	63.3%	83.3%
NPV%	100%	100%	70%	65.6%	80.8%
E.coli	10	7	8	7	8
Sensitivity%	100%	100%	80%	70%	80%
NPV%	100%	100%	77.8%	70%	77.8%
E.histolytica/dispar	10	7	7	5	9
Sensitivity%	100%	100%	70%	50%	90%
NPV%	100%	100%	70%	58.3%	87.5%
G.lamblia	10	7	6	7	8
Sensitivity%	100%	100%	60%	70%	80%
NPV%	100%	100%	63.6%	70%	77.8%

Kappa index (Tab 3), showed substantial for *A. lumbricoides* infection between formol detergent concentration and modified Ridley-Allen and Midi-Parasep® techniques. There was agreement among them to detect of *E. coli* infection (> 80%, P<0.001).

Table 3: Kappa index among three techniques to diagnose parasites in stools:

A. lumbricoides	MRAT	FDCT	MP
MRAT	------	0.61 (S)	0.43(S)
FDCT	0.61(S)	-------	0.77(S)
MP	0.43(S)	0.77(S)	-------
H.nana	MRAT	FDCT	MP
MRAT	-------	0.39(S)	0.39(S)
FDCT	0.39(S)	-------	1(HS)
MP	0.39(S)	1(HS)	------
*Other helminths	MRAT	FDCT	MP
MRAT	--------	0.56 (S)	0.45(S)
FDCT	0.56(S)	------	0.21 (NS)
MP	0.45(S)	0.21 (NS)	-------
E.coli	MRAT	FDCT	MP
MRAT	------	0.88(HS)	1(HS)
FDT	0.88(HS)	------	0.88(HS)
MP	1(HS)	0.88(HS)	-------
E.histolytica/dispar	MRAT	FDCT	MP
MRAT	-----	0.75(S)	0.77(S)
FDT	0.75(S)	------	0.54(S)
MP	0.77(S)	0.54(S)	------
G. lamblia	MRAT	FDCT	MP
MRAT	------	0.88 (S)	0.76 (S)
FDT	0.88 (S)	---	0.88 (S)
MP	0.76 (S)	0.88 (S)	----

NS: Non-Significant ((P>0.05) , S: Significant (P<0.05),HS: Highly significant (P<0.001, *Other helminths: (*A. duodenale*, *T. trichiura*).

Regarding parasite morphology, the better morphological structures of them were given by the Midi-Parasep® method, as it retained the morphology of the detected parasites. The clarity of the sediment in the modified Ridley-Allen procedure was better than that of formol detergent concentration method. However, both techniques had more fecal debris backgrounds in comparison to the Midi-Parasep® method (Figs. 3 & 4).

Discussion

Intestinal parasitic infections constitute a major public health and socio-economic concerns (WHO, 2017). Several environmental, social and geographic factors are responsible for the persistence of intestinal parasites in tropical and subtropical countries like; poor sanitation conditions and low levels of education (Adu-Gyasi *et al,* 2018). Generally, fecal parasites were detected by direct microscopic examination after sample concentration. The most concentration techniques used were the sedimentation principle and chemical reagents to dissolve fecal solids (Nicolas *et al,* 2006). However, the inability of the standard concentration techniques to detect multiple-species parasitic infection with a high level of accuracy made it necessary to develop new diagnostic tools (Sudré *et al*., 2006). The use of commercial kits has been marketed (Perry *et al,* 1990), for limiting the use of toxic reagents especially, ethyl-acetate and ether (Saez *et al,* 2011). So, alternative methods are required for early and accurate diagnosis of intestinal parasites.

In the present study, Midi-Parasep® technique showed higher sensitivity (71.7%) and negative predictive value (71.2%) for detection of intestinal parasites compared to modified Ridley-Allen concentration technique with sensitivity of (51.7%) and a negative predictive value of (59.2%). This may be attributed to the clear fecal sediment obtained by the Midi-Parasep® technique which allowed a better parasitic identification. The present results agreed with Ikeh and Elujola (2015) who found higher sensitivity (93%), specificity (96%), positive (91.3%) and negative predictive values (96.8%) achieved by the Mini Parasep® SF method in comparison to modified formol-ether sedimentation technique in diagnosis intestinal parasites. Also, Sanprasert *et al.* (2016) found that in school-age children, Mini Parasep® SF was the most sensitive (56.38%) diagnostic tool for detection of intestinal parasites followed by direct smear (40.4%) and formalin-ethyl acetate concentration technique (37.3%). These data disagreed with Kitvatanachai and Rhongbutsri (2017) who found that Mini Parasep®SF kit gave the least efficacy (55.2%), in detecting intestinal parasites when compared to direct smear (74.2%) and modified formalin-ether concentration technique (65.7%). Funk *et al.* (2013) reported that the formalin-ethyl acetate concentration technique (FECT) was more efficient than Mini Parasep®SF procedure despite the statistically insignificant difference.

In the current study, Midi Parasep® was the most sensitive technique in diagnosing *A. lumbricoides* ova (90%) than Formol detergent concentration technique (80%) in detection of *H. nana* eggs. Adugna *et al*. (2017) reported better performance of Mini Parasep® SF fecal concentrator than Kato-Katz and McMaster techniques for detection of *Schistosoma mansoni, A. lumbricoides* and *H. nana* in stools.

In the present study, Mini Parasep® SF fecal concentrator showed the least sensitivity (10%) in diagnosing eggs of *A. duodenale* and *T. trichiura.* Failure of Mini Parasep® SF faecal concentrator to detect eggs of hookworms and *Opisthorchis viverrini* were reported by Sanprasert *et al.* (2016). Funk *et al.* (2013) found that Kato-Katz technique had a significantly higher fecal egg count and sensitivity for both hookworm and *T. trichiura* as compared to Midi Parasep® technique.

The present study showed that the Midi-Parasep® technique gave the highest sensitivity (83.3%) and NPV (80.8%), in identifying

more positive protozoan cysts. Also, Useh *et al.* (2011) reported that the modified formol ether concentration technique was more efficient in detecting helminth infections, and Mini-Parasep® SF method was better in detecting protozoan infection. Sanprasert *et al.* (2016) found that the Mini Parasep® SF detected more protozoa than either direct smear or FECT. The Midi Parasep® technique gave the highest sensitivity to diagnose *E. histolytica/dispar* and *G. lamblia* (90% & 80% respectively). For diagnosing *E. coli,* this technique was as sensitive as Modified Ridley-Allen concentration method (80%). The Mini Parasep® method in diagnosing intestinal protozoa agreed with Mewara *et al.* (2019) who found a better yield of *E. coli* and *G. lamblia* by Mini Parasep® compared to the direct smear or form- ol-ether concentration methods.

The highest kappa value was found between the Midi Parasep® and FDCT procedures for *H. nana* diagnosis (KI= 1.0), and for *A. lumbricoides* (KI= 0.77), whereas the least value (KI= 0.21) was found among the same techniques for the detection of *A. duodenale* & *T. trichiura* ova. This agreed with Adugna *et al*. (2017) who reported that the highest Kappa value was found between The Mini Parasep® SF procedure with Kato-Katz thick smear and McMaster techniques for the detection of *H. nana* (KI= 0.94) and *A. lumbricoides* (KI= 0.93)*,* followed by *T. trichiura* (KI= 0.68)*,* and hookworms (KI=20).

Sedimentation clarity, less debris & background uniformity was important considerations for detection of parasites in concentrated faecal samples (Perry *et al,* 1990). However, the wet mount prepared from the Midi-Parasep® procedure had less background fecal debris compared to the standard concentration methods. These results agreed with Khanna *et al*. (2018) and Mewara *et al.* (2019). But, Sanprasert *et al.* (2016) found the larger size and denser fecal debris concentrated in Mini Parasep® Solvent Free (SF) than that concentrated in Formalin-ethyl acetate concentration technique.

Conclusion

The midi-Parasep fecal parasite concentrator has the advantages of being highly sensitive, closed system, safe and rapid. Hence, it should be used as one of the appropriate faecal examination methods for surveillance and monitoring of intestinal parasitic infections.

The Midi-Parasep® procedure proved to be efficient, simple and rapid compared to the other conventional stool examination methods. It holds the potential for application as a routine concentration technique, especially for examining high numbers of stool samples in limited time.

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Explanation of Figures

Fig. 1: Midi-Parasep®Faecal Parasite Concentration kit. (A) Sedimentation cone; (B) Two stage filtration matrix; (C) Mixing chamber and (D) Parasep lid.

Fig. 2: Flow diagram depicting steps of Midi-Parasep® technique. (A) Fecal sample obtained using spoon on filter end; (B) Faecal sample mixed with 6ml of the fixative and 2 ml of ethyl-acetate; (C) Filter thimble screwed onto sedimentation cone afterward mixing chamber and vortexed with sedimentation cone facing upward; (D) Parasep device inverted; (E) Fecal debris blocked by filter thimble after centrifugation; (F) Sediment used for microscopic examination.

Fig. 3: Qualitative Comparison of some eggs detected by Midi-Parasep® (MP) and Formol Detergent Concentration (FDCT) Techniques. (A1) and (A2): *A. lumbricoides*; (B1) and (B2): *T. trichuria* (X100).

Fig. 4: Qualitative Comparison of some cysts detected by Midi-Parasep® (MP) and Modified Ridley-Allen (MRAT) techniques. (C1) and (C2) *Entamoeba coli* (black arrows) (X400); (D1) and (D2) *Giardia lamblia* (black arrows, X 1000).