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Research Article
Mini-FLOTAC Versus Other Copromicroscopic Methods in Diagnosis of Intestinal Parasitic Infections

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Abstract

Background and Objective: Mini-FLOTAC proved to be a good promising quantitative method combining both sensitivity and low costs. This study aimed to assess the real efficiency of Mini-FLOTAC vs. other copro-parasitological methods, wet smear, sedimentation and flotation concentration for diagnosis of intestinal parasitic infections. **Materials and Methods:** Three studies were carried out. The first was used to evaluate the performance and sensitivity. The second was designed to evaluate the percent accuracy, precision and sensitivity and the third, a field study, for validating its sensitivity and predictive value. **Results:** When applying different flotation solutions (FS). Mini-FLOTAC revealed the highest sensitivity from FS1 and FS3 for detection of *H. nana* and *E. vermicularis*, FS1 for *A. lumbricoides* and FS3 for *E. histolytica*. Samples with lower level of enrichment had a higher coefficient of variation and a lower precision. The second study was designed to evaluate its accuracy (%), precision and sensitivity. The highest sensitivity was obtained for *H. nana* eggs, this value was slightly lower for *A. lumbricoides* eggs and the lowest value was obtained for *E. histolytica* cysts. A high linear relationship was revealed between outcomes. The third study, a field one for validating the sensitivity and predictive value. Out of 200 children, 38 positive cases were detected. Results revealed that FS3 showed the highest outcome. The performance of all methods on negative individuals was high (NPV>95%) for all parasites. The NPV for detection of *H. nana* eggs and *E. vermicularis* eggs with Mini-FLOTAC was 100% by the two FSs while it decreased in case of *G. intestinalis* to be 97.8 and 96.2% by FS1 and FS3, respectively. The KI for agreement among techniques showed a nearly perfect comparative relation. **Conclusion:** Mini-FLOTAC proved obvious sensitivity in diagnosis of helminths but more studies are needed to assess its capability in protozoa detection.

Key words: Helminths, protozoa, copro, parasitic infection, parasitological techniques, direct smear, Mini-FLOTAC, flotation, sedimentation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Since one person in every four harbors parasitic worms, there is always a need for accurate diagnosis for management and epidemiological investigations[1]. Microscopic examination of stools although considered the gold standard lacks sensitivity[2]. So, different concentration techniques were used[3].

Flotation procedure yields clear preparation due to separation of protozoan cysts, coccidian oocysts and certain helminths' eggs and larvae from excess debris through the use of solution with a high specific gravity. Sedimentation procedure lacks this clear field however, some helminths' eggs (operculated and/or very dense) do not concentrate well with the flotation and are revealed with sedimentation[4,5].

Formalin ether concentration technique (FECT) was routinely used for diagnosis of helminths and intestinal protozoa[6-8] where preserved stool samples can be analyzed in the laboratory several days or weeks after collection, it includes fire and explosion hazards, some parasitic elements are misdiagnosed as they might be broken or altered during the procedures so it was considered qualitative rather than quantitative[9].

Kato-Katz, although considered to be the routine method for diagnosis of soil transmitted helminths (STHs)[10-12], it lacks sensitivity if only a single stool sample is examined, particularly in light-intensity infections. Also a small number of helminth eggs, unequally excreted over days and patchily distributed in stool can be missed in the small amount of examined stool[13].

Studies suggested a copromicroscopic FLOTAC apparatus based on centrifugal flotation of fecal sample using different flotation solutions (FSs) and subsequent translation of the apical portion of the floating suspension under the microscope[14]. A central feature is that it provides counts of parasitic elements in large fecal aliquots (up to 5 g or even bigger amounts). It was initially developed for veterinary parasitology[15] and has been recently extended for the diagnosis of human intestinal helminths and protozoa[16,17].

The Mini-FLOTAC has been recently developed from FLOTAC with the advantage that it doesn't require centrifugation. This allows laboratories with limited resources to rely on a good quantitative method for both diagnostic and epidemiological purposes. It is a promising technique that can replace the FLOTAC combining sensitivity and low costs[18,19].

More studies are required for validation of these methods in diagnosis of intestinal parasites, so it was of great interest to assess the real efficiency of Mini-FLOTAC versus other copro-parasitological methods, wet smear, sedimentation and flotation concentration for diagnosis of intestinal parasitic infections.

MATERIALS AND METHODS

The study was conducted at Parasitology Laboratory, Faculty of Medicine, Al-Azhar University for Girls. All chemicals were purchased from Sigma Aldrich otherwise stated.

Stool collection for the first and second experimental studies: Stool samples from 10 apparently healthy adult volunteers were collected, each sample was preserved in 5% formalin then examined using routine examination methods (direct smear, formalin ethyl acetate sedimentation method and centrifugal flotation method using zinc sulfate ($ZnSO_4$ with specific gravity (s.g). 1.2). Negative stool samples were pooled together to get a negative stock that will be externally spiked with parasitic elements.

Spiking of stool samples: Stocks of eggs were prepared from *Taenia* sp. gravid segments and *A. lumbricoides* adult female worms[20]. *Hymenolepis* (*H.*) *nana* eggs, *Giardia* (*G.*) *intestinalis, Entamoeba* (*E.*) *histolytica* and *E. coli* cysts from positive stool samples[21]. Stock for *Enterobius vermicularis* eggs was also prepared[22]. Every suspension was individually added to a 20 g of weighed negative stool sample.

First study: For comparing the Mini-FLOTAC with, direct smear, centrifugal sedimentation and centrifugal flotation methods using two different FSs.

Preparation of different concentrations from stock samples: A serial of 5 dilutions (5% formol saline) was carried out for each stock sample. Each concentration was examined by the direct smear method to get 0-1 egg/cyst in the whole slide with the highest dilution. Each dilution was examined by the four methods in triplicates ending up with 15 samples for each method.

Stool examination: Using formalin (5%) preserved specimens and two FSs, modified Sheather's solution (FS1 s.g. 1.27, a modified form of FS1 s.g. 1.2) and $ZnSO_4$ (FS3 s.g. 1.2)[14], stool was comparatively examined with, direct wet mount with saline and iodine[23], FECT, $ZnSO_4$ flotation concentration[24] and Mini-FLOTAC[19].

Second study: This study was designed to test Mini-FLOTAC efficacy (percent accuracy, precision and sensitivity) using $ZnSO_4$ (s.g.1.2)[14,19].

A suspension of each parasite was prepared, 5 g from each of the stock samples used in the first study mixed with 50 mL of 85% NaCl and poured through gauze, allowed to settle then the supernatant was decanted. The whole procedure was repeated until the supernatant was clear. The final sediment was suspended in10 mL of normal saline to get the stock. Using light microscopy the number of parasitic eggs or cysts was counted per 50 µL of solution then calculated per gram of sediment. Different egg/cyst concentrations were used to spike the collected negative stool samples to get (50-100-200-400) egg/cyst per gram. Six replicates of each level of contamination were prepared and examined and the number of redetected eggs or cysts were counted. The multiplication factor used to obtain the number of redetected eggs and cysts was 10.

Third study: This study included 200 children (6-12 years old) attending two schools (Awlad Ateya and EL-Shebrawin Schools) located in rural area within a radius of 10 km from Zagazig-El-Sharkia Governorate of Egypt. Agriculture is the main job of people in this area. Temperature ranges from 25-37°C most of the year. Sample collection was done[24] with precautions[25] in a clean container with direct quick transport in formalin (5%) in a ratio of 1: 4. All samples were examined as mentioned in the first study using, direct wet smear, FECT, centrifugal flotation technique and Mini-FLOTAC using FS1 and FS3. Positive outcomes were compared and statistical analysis was done.

Statistical analysis: Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Mean and Standard Deviation (±SD) were calculated. A true positive sample was positive with all parasitological methods, while a true negative sample was negative with all methods, this criterion was defined as the gold standard for the study. Sensitivity (%)[26,27]. A one-way analysis of variance (ANOVA), probability for the level of significance (p-value) (<0.05 was significant**, <0.001 was highly significant***, >0.05 was insignificant*), the coefficient of variation percentage (CV%)[20], precision (%)[28], accuracy[29], linear regression[20], negative predictive value (NPV)[26] and Kappa index for agreement[30] were calculated.

Compliance with ethical standard: A verbal consent was taken from parents. Participation was not obligatory and withdrawal was possible at any time. Structured questionnaire was filled for every case including information about age, sex, detailed medical history and any presenting complaints. If there was any gastrointestinal troubles e.g., diarrhea, its onset, course, duration, frequency, consistency, presence of mucus or blood, in addition to some social, economic and behavioral information were included. Treatment was given to positive cases.

RESULTS

In the first study, the mean concentration values analyzed collectively for each parasite revealed a highly statistical significant outcome between the different methods in diagnosing each parasite. Mini-FLOTAC using FS3 showed best results for detection of *H. nana* eggs and *E. histolytica* cysts and *E. coli.* While Mini-FLOTAC using FS1 showed best results for detection of *A. lumbricoides* eggs and *E. vermicularis* eggs. In case of *Taenia* sp., *G. intestinalis* and *E. coli*, centrifugal sedimentation gave the best results (Table 1). The sensitivity of examined methods varied for each parasite. Mini-FLOTAC (FS1 and FS3) was the most sensitive for detection of *H. nana* eggs, the least sensitive methods were centrifugal flotation and direct smear. Sedimentation was the most sensitive for detection of *Taenia*, sp., while Mini-FLOTAC (FS3) was the least sensitive. As regards *A. lumbricoides*, the most sensitive was Mini-FLOTAC (FS1) and the least sensitive were Mini-FLOTAC (FS3) and direct smear. The sedimentation was the most sensitive for detection of *G. intestinalis* and Mini-FLOTAC (FS1 and FS3) was the least sensitive. In case of *E. histolytica*, Mini-FLOTAC (FS3) was the most sensitive and the direct smear was the least sensitive. Regarding *E. coli* cysts, the sedimentation was the most sensitive and the direct smear was the least sensitive method. As for *E. vermicularis*, floatation and Mini-FLOTAC (FS1 and FS3) were the most sensitive while direct smear was the least sensitive (Table 2).

In the second study six replicate readings from each sample were calculated excluding *Taenia* sp., *G. intestinalis* and *E. coli* samples which showed no positive results in (50-100-200-400) concentrations per gram with Mini-FLOTAC. The mean numbers of *H. nana* eggs per gram was higher than that for *A. lumbricoides* and *E. histolytica* (Table 3). With different outcomes, the percentages of recovered eggs/cysts for the whole apparatus were increased when the dose of contamination was increased. Samples with little enrichment had a higher coefficient of variation and a lower precision than samples with higher enrichment (Table 4). For every level of enrichment, six replicates were examined. There was a highly significant outcome in comparing the one to whole chamber for each parasite with different enrichments. The best sensitivity was obtained for *H. nana* eggs, this value was

Table 1: Collective diagnostic performance of the different methods for all spiked parasitic samples

	Mean±SD					ANOVA	
Parasites	Direct wet	Sedimentation	Flotation	MFT(FS1)	MFT(FS3)	ANOVA	p-value
H. nana	5.13±0.61	19.20±1.97	13.87±0.42	22.53±0.81	29.20±1.73	153.186	<0.001***
Taenia sp.	11.73±1.67	47.13±1.55*	6.27±0.12	12.87±0.64	6.20±0.72	532.129	
A. lumbricoides	2.40±0.40	13.07±1.55	6.60±0.69	20.20±0.00*	3.87±0.58	240.715	
E. vermicularis.	4.60±1.15	13.73±3.43	17.00±4.25	28.73±7.18	25.47±6.37	80.683	
G. intestinalis	15.93±1.15	74.80±3.14*	53.53±0.76	5.47±0.61	4.13±0.23	1236.366	
E. coli	9.00±1.51	55.87±1.36**	31.20±2.40	14.80±1.22	42.13±0.90	457.872	
E. histolytica	5.47±0.23	22.80±1.97	14.40±0.72	16.60±2.16	33.67±1.03	161.552	

*Highest values revealed

Table 2: Collective sensitivity (%) for each spiked sample for each method

Parasites	Direct wet (%)	Sedimentation (%)	Flotation (%)	MFT(FS1) (%)	MFT(FS3) (%)
H. nana	66.7	93.3	80.0	100	100.0
Taenia sp.	80.0	100	66.7	80	60.0
E. vermicularis.	66.7	80	100.0	100	100.0
A. lumbricoides	60.0	80	66.7	100	60.0
G. intestinalis	73.3	100	80.0	60	46.7
E. coli	60.0	100	80.0	80	86.7
E. Histolytica	60.0	100	80.0	80	100.0

Table 3: Diagnostic performance of Mini-FLOTAC for detecting *H. nana* eggs, *A. lumbricoides* eggs and *E. histolytica* cysts

	Mean±SD			
Parasite	50	100	200	400
H. nana	22.6±1.8	52.3±3.20	125±3.60	248±4.70*
A. lumbricoides	10.0± 1.4	26.0±1.86	85±3.17	150±3.29*
E. histolytica	-	-	33±3.40	88±3.20*

*Highest values revealed

Table 4: Percent accuracy, coefficient of variation and precision for parasitic infection of *H. nana, A. lumbricoides* and *E. histolytica*

	50			100			200			400		
	Recovery	CV	Precision	Recovery	CV	Precision	Recovery	CV	Precision	Recovery	CV	Precision
Parasites	(%)											
H. nana	45.5	8	92	52.3	6	94	62.5	2	98	62.0	1	99
A. lumbricoides	20.0	14	86	26.0	7	93	42.5	3	97	37.5	2	98
E. histolytica	-	-		-	-		16.5	10	90	22.0	3	97

CV: Coefficient of the variation

Table 5: Limit of quantification (the lowest level of detection of contents in which occurred normal distribution) of *H. nana, Ascaris* and *E. histolytica* for Mini- FLOTAC basic technique

	Number of eggs/cysts per gram of sample						
Parasites	50	100	200	400	ANOVA	p-value	Sensitivity (%)
H. nana							
One chamber	20.00±21.91	46.67±27.33	123.33±23.38	230.00±37.42	67.03	<0.001***	87.5
Whole	23.33±15.06	53.33±10.33	126.67±15.06	248.33±27.14	185.72	<0.00***1	100.0
A. lumbricoides							
One chamber	3.33±8.16	23.33±23.38	76.67±23.38	136.67±23.38	50.21	<0.001***	70.8
Whole	1.67±4.08	26.67±18.62	85.00±21.68	150.00±17.89	90.89	<0.001***	75.0
E. histolytica							
One chamber	0.00±0.00	0.00±0.00	3.33±8.16	100.00±40.00	35.24	<0.001***	29.2
Whole	0.00±0.00	0.00±0.00	3.33±5.16	88.33±20.41	103.10	<0.001***	33.3

slightly lower for *Ascaris* sp. eggs and the lowest value was obtained for *E. histolytica* (Table 5). In all cases, the whole chamber had a greater sensitivity and predictive value as calculated from linear regression than examination of one chamber. A high linear relationship was revealed between outcomes (Fig. 1).

In the third study, out of 200 children, 38 positive cases were detected. The highest prevalence was that of

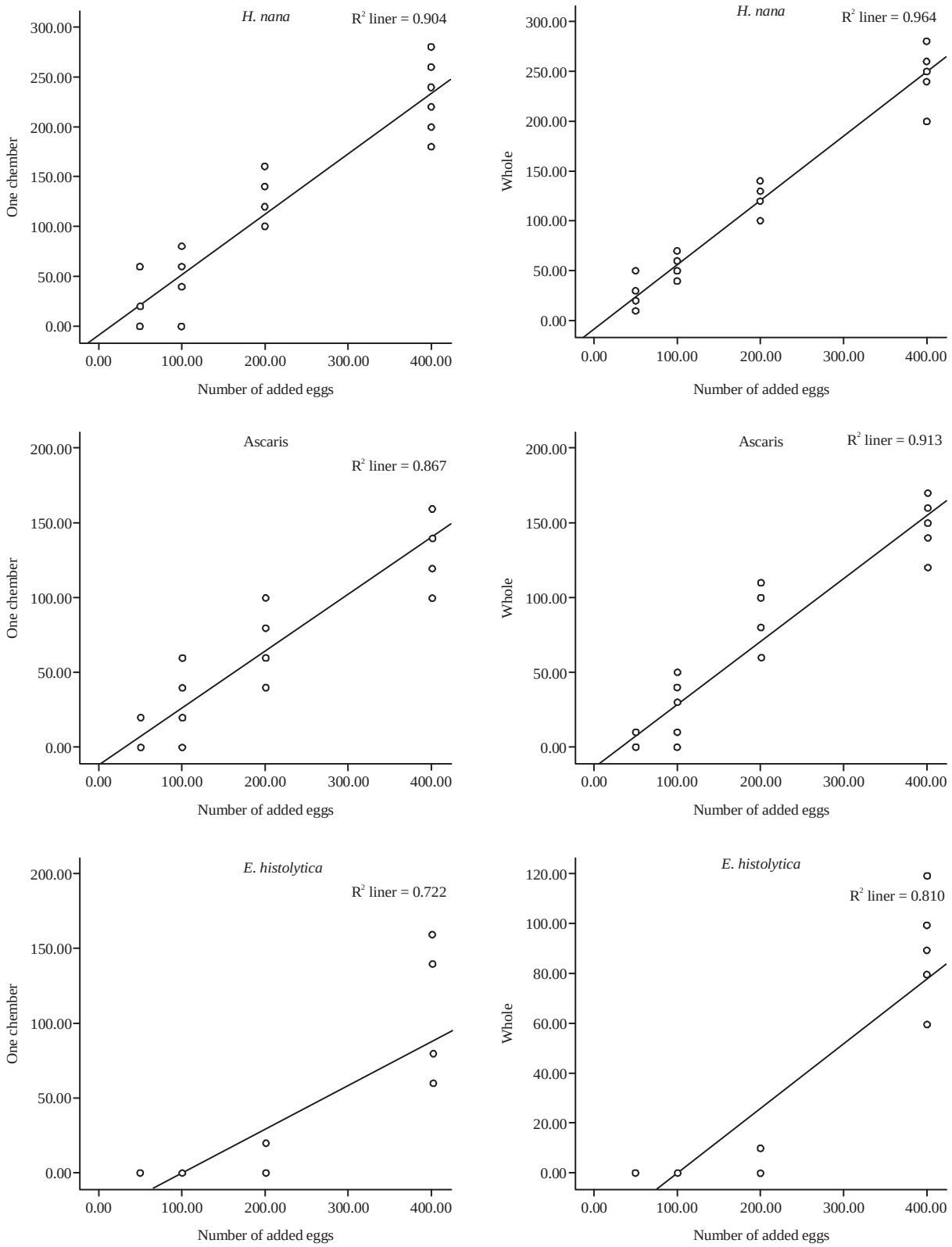

Fig. 1: Estimation of the linear range of Mini- FLOTAC basic technique for detection of *H. nana, Ascaris* and *E. histolytica* in one vs. whole chamber

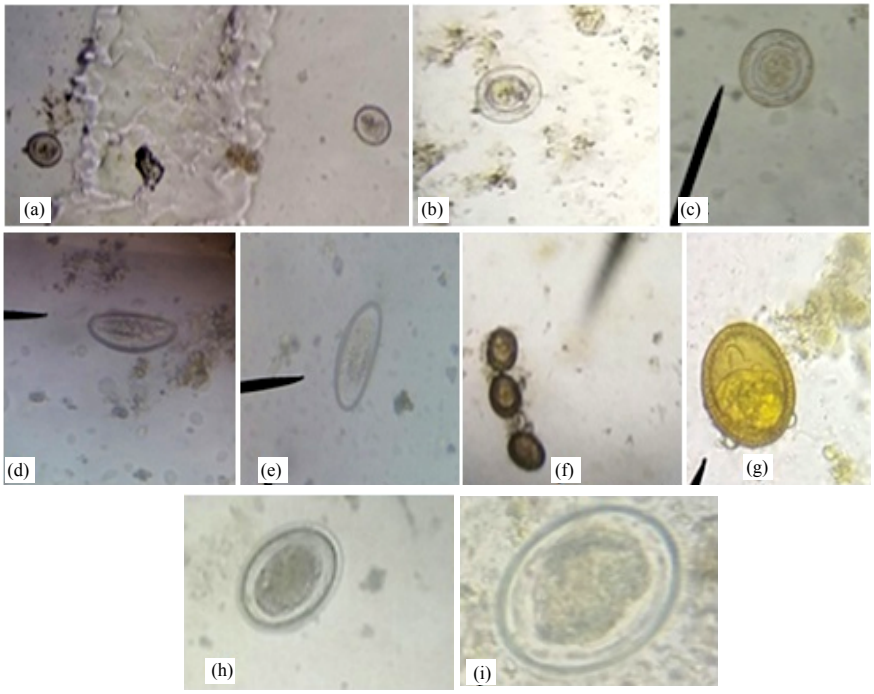

Fig. 2(a-i): Slides with positive helminthic findings (objective x), (a) *H. nana*-Mini FLOTAC (10x), (b) *H. nana*-direct smear (40x), (c) *H. nana*-flotation (40x), (d) *E. Vermicularis*-Mini-FLOTAC (40x), (e) *E. Vermicularis*-flotation (40x), (f) *Taenia* sp. Mini-FLOTAC (10x), (g) *Taenia* sp. direct smear (40x), (h) *Ascaris lumbricoides* direct smear (40x) and (i) *Ascaris lumbricoides* Mini-FLOTAC (10x)

Table 6: Prevalence of parasitic infection among examined children

Parasite	Number of cases	Prevalence (%)
H. nana	7	3.5
E. vermicularis	3	1.5
Taenia sp.	1	0.5
Giardia intestinalis	21	10.5
E. histolytica	4	2.0
Mixed *Giardia intestinalis* and *E. histolytica*	2	1.0
Total	38	19.0

G. intestinalis and the least was for *Taenia* sp. (Table 6). The five diagnostic methods used in detecting *H. nana*, *E. vermicularis, E. histolytica,* mixed infection of *G. intestinalis* and *E. histolytica* from patients revealed that Mini-FLOTAC (FS3) showed the highest outcome, while the least one was obtained from direct wet smear. Regarding *G. intestinalis* the highest outcome was obtained from the sedimentation method and the least one was obtained from the Mini-FLOTAC (FS3) (Table 7). All methods were 100% sensitive for mixed infection with *G. intestinalis* and *E. histolytica.* The performance of all methods on negative individuals was high (NPV >95%) for all parasites. The NPV for *H. nana* and *E. vermicularis* with Mini-FLOTAC was 100% by the two FSs while it decreased in case of *G. intestinalis* to be 97.8 and 96.2% by Mini-FLOTAC FS1 and FS3, respectively (Table 8). The KI for agreement among techniques showed a nearly perfect comparative relation between the gold standard and all used methods (Table 9).

Examples of slides with positive parasitic findings with the different methods are illustrated (Fig. 2, 3).

DISCUSSION

The diagnostic accuracy of Mini-FLOTAC changes according to the FS used[14,29]. In light of previous studies $ZnSO_4$ (s.g. 1.2) (FS3)[21,31] and Sheather's (s.g. 1.27) (FS1) were chosen[32,33].

In the first study, Mini-FLOTAC using FS3 gave the best performance (100% sensitivity) for detection of *H. nana* eggs. Steinmann *et al.*[34] showed similar results out of nine FSs. While Barda *et al.*[27] revealed that Mini-FLOTAC using FS2 (NaCl s.g. 1.2) was more sensitive. The Mini-FLOTAC using FS1 gave second best performance and was still 100% sensitive. This was followed by approaching results from sedimentation for *H. nana* eggs detection. These results were similar to Steinmann *et al.*[34] in comparison to FECT.

Regarding *Ascaris* sp., Mini-FLOTAC FS1 revealed the best performance (100% sensitivity), while FS3 showed 60%

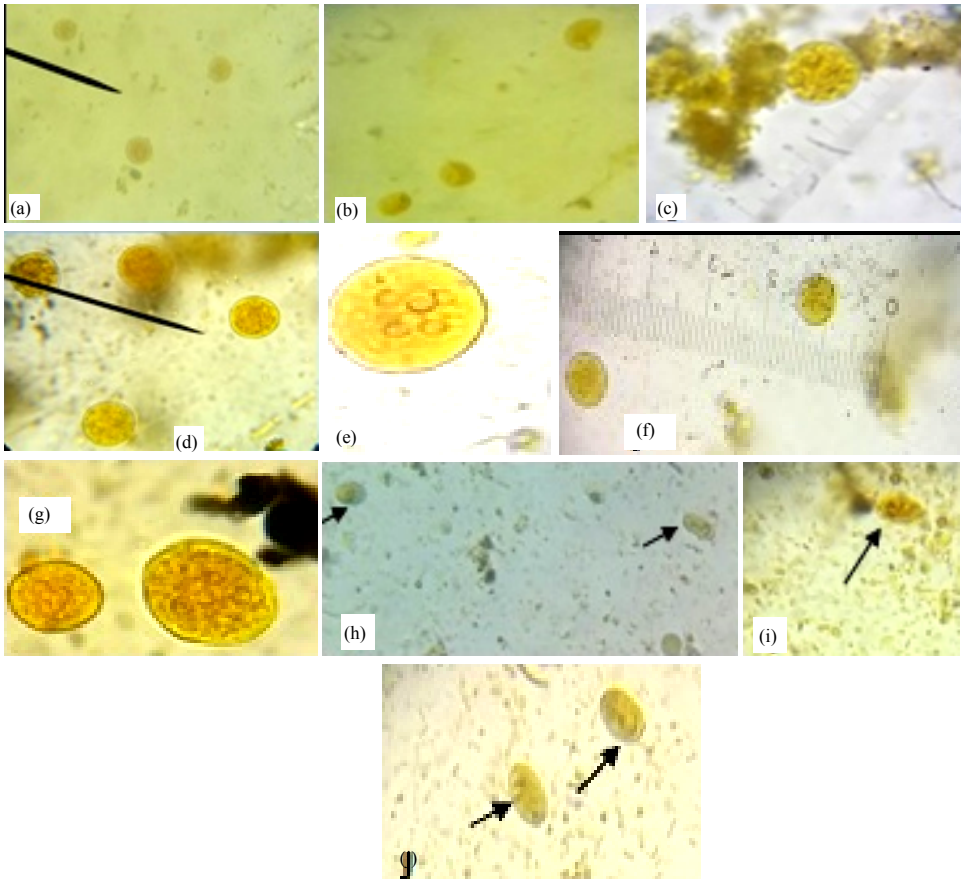

Fig. 3(a-j): Slides with positive protozoal findings (objective x), (a) *E. coli* cysts -Mini-FLOTAC (40x), (b) Rupture of *E. coli* cysts after 15 min-Mini-FLOTAC (40x), (s) *E. coli*-direct smear (oil), (d) *E. coli*-centrifugal flotation (oil), (e) *E. coli*-sedimentation (oil), (f) *E. histolytica/dispar* cysts-Mini-FLOTAC (40x), (g) *E. histolytica/ dispar* and *E. coli* cysts-sedimentation (oil), (h) *G. intestinalis* cysts-Mini-FLOTC (40x), (i) *G. intestinalis* cysts-direct smear (40x) and (j) *G. intestinalis* cysts-centrifugal flotation (40x)

Table 7: Comparative diagnostic performance of the different methods from field study

	Mean±SD					
Parasites	Direct wet	Sedimentation	Flotation	MFT(FS1)	MFT(FS3)	p-value
H. nana	2.43±3.100	12.43±6.190	8.43±5.910	28.57±21.81	34.43±23.28	<0.001***
E. vermicularis	3.00±3.000	10.33±5.860	14.33±9.290	19.33±9.290	24.67±12.50	
Giardia intestinalis	19.57±15.67	114.14±77.90	82.62±57.04	8.67±7.750	5.67±5.620	
E. histolytica	7.00±5.290	53.00±34.82	43.75±32.50	30.75±24.54	65.75±45.60	
Mixed *Giardia intestinalis* and *E. histolytica*	60.00±28.28	222.50±67.18	175.00±35.36	170.00±42.43	235.00±77.78	

Table 8: Sensitivity (%), negative predictive values and p-value of different methods in the field study

	Direct wet		Sedimentation		Flotation		MFT (FS1)		MFT (FS3)		
Parasite	Percentage	NPV%	Percentage	NPV%	Percentage	NPV%	Percentage	NPV%	Percentage	NPV%	p-value
H. nana	70.0	98.5	100	100	87.5	99.5	100	100.0	100	100.0	0.007**
E. vermicularis	75.0	99.5	100	100	100.0	100.0	100	100.0	100	100.0	0.004**
Giardia intestinalis	87.5	98.4	100	100	95.5	99.4	84	97.8	75	96.2	0.021**
E. histolytica	80.0	99.5	100	100	80.0	99.5	80	99.5	100	100.0	0.047**
Mixed *Giardia intestinalis* and *E. histolytica*	100.0	1.00*									

NPV: Negative predictive value

Table 9: Kappa index for agreement between the diagnostic methods

Parasites	Direct wet	Sedimentation	Flotation	MFT (FS1)	MFT (FS3)
H. nana	k = 0.816 (p<0.001)	k = 1.000 (p<0.001)	k = 0.931 (p<0.001)	k = 1.000 (p<0.001)	k = 1.000 (p<0.001)
E. vermicularis	k = 0.855 (p<0.001)	k = 1.000 (p<0.001)	k = 1.000 (p<0.001)	k = 1.000 (p<0.001)	k = 1.000 (p<0.001)
Giardia intestinalis	k = 0.925 (p<0.001)	k = 1.000 (p<0.001)	k = 0.974 (p<0.001)	k = 0.902 (p<0.001)	k = 0.838 (p<0.001)
E. histolytica	k = 0.886 (p<0.001)	k = 1.000 (p<0.001)	k = 0.886 (p<0.001)	k = 0.886 (p<0.001)	k = 1.000 (p<0.001)

Strength of Agreement: Scott's Kappa, 0.20: Slight agreement, 0.21-0.40: fair agreement, 0.41-0.60: Moderate agreement, 0.61-0.80: Substantial agreement, 0.81-1: Nearly perfect agreement

sensitivity this was attributed to the high density of eggs. Sugar solutions were the most effective flotation media for eggs of different parasites in different host species[32,35]. Sedimentation revealed second best results after Mini-FLOTAC, as documented before[22,36,37].

Concerning *Taenia* sp., the performance of Mini-FLOTAC using FS1 and FS3 wasn't the best with sensitivity 80 and 60% respectively. Although the s.g. of FS1 (1.27) is slightly higher than that of the *Taenia* sp. eggs (1.225), it didn't give the expected results. This may be related to the narrow difference in specific gravities or due to preservation of these eggs in formalin rendering it denser. Also, the increased viscosity of the sugar solution might impede egg recovery in a simple flotation[32] and the downward force created by the centrifugation enhances the flotation of the eggs in the viscous solution and drives them to the surface meniscus where they are concentrated and result in greater parasite recovery.

The *E. vermicularis* eggs were detected using Mini-FLOTAC (FS1 and FS3) with a higher mean than sedimentation, centrifugal flotation and direct smear. Similar to Bartlett *et al.*[38] using samples preserved in formalin for less than one month, centrifugal flotation gave second best results in diagnosing *E. vermicularis* after Mini-FLOTAC. This study revealed that FS1 was superior to FS3 and this was revealed before[14,37]. In previous studies, FS3 floated light eggs like *E. vermicularis* with better results than sedimentation[39].

Mini-FLOTAC using FS3 gave best performance with sensitivity (100%) for the recovery of *E. histolyica/dispar* cysts. Unlike, Becker *et al.*[40] and Zajac *et al.*[31] who proved better results with FS1 in diagnosis of intestinal protozoa while FS3 caused distortion to some cysts. Sedimentation revealed second best performance. In contrary, Barda *et al.*[37] recorded that FECT was the most sensitive method for diagnosis of *E. histolytica/dispar, E. coli* and *G. intestinalis* (88%), followed by direct fecal smear (70%) and lastly Mini-FLOTAC. They stated that Mini-FLOTAC made it more difficult to have a flawless visibility of the internal structures.

Regarding *E. coli* cysts, sedimentation gave best performance with sensitivity (100%). Mini-FLOTAC using FS3 gave second best performance with sensitivity (86.7%), while FS1 showed sensitivity 80%, this decrease in sensitivity may be due to the destruction of *E. coli* cysts which was noticed microscopically. Similar results were shown from, Becker *et al.*[40] (2011) where FECT was more sensitive. Centrifugal flotation gave third best results with sensitivity 80%. This was similar to Parameshwarappa *et al.*[41]. Another study revealed that centrifugal flotation with FS3 gave same results like sedimentation in case of *E. histolytica/dispar* while sedimentation was more sensitive in case of *E. coli*[38]. Again this had been attributed to the samples preserved in formalin where the s.g. might be affected.

Regarding *G. intestinalis* cysts, our study revealed that Mini-FLOTAC gave the least performance with sensitivity 60 and 46.7% using FS1 and FS3 respectively, those results may be attributed to specific parasite structure being too delicate and light (s.g. 1.05) to float in FS3 perfectly without centrifugation, also a dense medium like FS1 may cause distortion and collapse because of the high osmotic pressure. Sedimentation showed high performance in detecting *G. intestinalis* cysts with 100% sensitivity. Also, similar results were shown when comparing the diagnostic accuracy of the FLOTAC and FECT where FECT proved to be more sensitive (8.3% vs. 6.5%)[40]. The low sensitivity of the Mini-FLOTAC was attributed to the less transparent base part of the device which made the view a bit vague so the smaller particles, like *G. intestinalis* were less detectable[42].

In the second study, Mini-FLOTAC wasn't able to detect *Taenia* sp. eggs, *G. intestinalis* and *E. coli* cysts at the used levels of contamination. In case of *H. nana* and *A. lumbricoides* the accuracy (%) for Mini-FLOTAC decreased from the 200 and 400 levels. This lack of a dose response was unexpected. This was similar to a study done by Noel *et al.*[28], who found that the accuracy of Mini-FLOTAC was decreased between the 500 and 1,000 enrichment levels during determining equine Strongyle egg count and stated that this phenomenon needs further explanation. In our study, *A. lumbricoides* showed a 20% reduced detection rate at 400 than 200 enrichment levels. This was similar to Ruzicova *et al.*[21] who showed that the accuracy became relatively high at low infection intensities. At low level of parasitic elements some may be lost during the preparation[20],

however, published results using the FLOTAC method, for detection of *Ancylostoma caninum*[43] and *Hymenolepis* sp.[34], provided CV% values with very low levels (about 5% for the most efficient FS) which again may be attributed to centrifugation during the preparation of samples.

Current study showed that *E. histolytica/dispar* cysts started to disintegrate in FS3 within 20 min and that was stated before[44] so, it is desirable to minimize the time for sample processing and reading in Mini-FLOTAC as much as possible. Similar results were shown when 9 FSs were evaluated from which FS3 was the most suitable for trophozoite detection, while FS1 was selected as most suitable for cysts[21]. This may explain the low accuracy of *E. histolytica/dispar* detection in the second study.

Comparing the one to whole chamber/s showed respective results from those theoretically assumed by the authors of the FLOTAC[14]. Different outcomes are due to theoretical assumption that all parasitic elements present in the examined feces should be detected[20].

The field study results ran parallel to the first study. Mini-FLOTAC was the best method for detection of *H. nana* and *E. vermicularis* eggs with decreased efficiency in detection of *G. intestinalis* cysts and *E. histolytica*. Mini-FLOTAC was more effective in detecting all true negative cases.

The agreement between all tested methods and the gold standard was nearly perfect, while in a previous study, the agreement between the Mini-FLOTAC (FS2 and FS7), FECT and direct smear was generally moderate in detection of *E. coli*, *E. histolytica/dispar*, *G. intestinalis* and hookworm[22]. Low agreement using the same set of parasites has been observed in a previous study. Prevalence of intestinal parasitic infections can alter agreement between methods. The diagnostic agreement between the European reference centers was only moderate using FECT for *E. histolytica/ dispar* and *G. intestinalis*. These observations enlighten that microscopic identification is still challenging even in reference laboratories[45].

CONCLUSION

Mini-FLOTAC (FS3) showed the best results in diagnosis of *H. nana* and *E. vermicularis*. It came first in diagnosing *A. lumbricoides* when using FS1. It was the best in diagnosis of *E. histolytica* using FS3 but it came after sedimentation in diagnosis of *E. coli* and it wasn't effective in diagnosis of *G. intestinalis*.

SIGNIFICANCE STATEMENT

The Mini-FLOTAC had the highest sensitivity for helminth detection although it was not very promising in diagnosis of intestinal protozoa lacking the benefit of centrifugation. This study proved that Mini-FLOTAC could be considered a step forward in solving the problems of diagnosis and assessment of prevalence. In different degrees, it proved obvious sensitivity in diagnosis of intestinal helminths, but more studies are needed to assess its capability in protozoa detection.

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