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Assessment of direct wet mount and various concentration approaches for diagnosing of *Entamoeba histolytica /dispar* and *Giardia lamblia* cysts in human stool samples

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Abstract
The human gastro-intestinal tract is preferred by the parasites *Entamoeba histolytica*/*dispar* and *Giardia lamblia*. As a result, stool samples are the primary way that their stages are found. The current study's objective was to assess several approaches for locating *E. histolytica/dispar* and *G. lamblia* cysts in human stool samples. Direct wet mount and concentration procedures were used to analyse all feces samples. Both flotation and formal ether sedimentation were used as concentration techniques. *Entamoeba* and Giardia had significant prevalence rates among the Kirkuk population, with 13.7 percent and 5.1 percent, respectively. Additionally, according to the findings of this study, children from 1 month to 2 years old were the most frequently affected age group by both parasites. No differences between males and females were discovered. Both direct and concentration methods were utilized in this experiment to identify the cystic stage. The number of cases were actually found differed depending on the methodology that we applied. The cyst count was higher employing flotation and sedimentation techniques, and it was considerably higher when using saturated salt solutions and zinc sulfate for the flotation process. We came to the conclusion that the direct wet mount approach is less efficient than the concentration method; it is therefore preferable to employ the concentration method, particularly when the parasite population is low and the diagnosis is in doubt. We advise including concentration techniques based on several concepts in routine diagnostic testing in order to increase the sensitivity of the direct test.

Keywords: direct wet mount, concentration, detection, protozoa

Introduction
The protozoan parasites *Entamoeba histolytica* and *Giardia lamblia* are among the most common parasites infecting humans. They are frequently associated with gastrointestinal illnesses and diarrhea. In endemic nations, intestinal parasites cause major illness and mortality [1, 2]. Unlike helminthes, protozoan parasites can proliferate inside the human body and multiply in quantity, causing infection to worsen. [3]. These protozoa are divided into two phases. trophozoite and cyst, with trophozoite being the pathogenic stage and cyst being the infective stage, both of which are found in the patient's feces and are primarily transmitted through the oral–fecal way [3]. Microscopic examination can be used to visually demonstrate cysts and/or trophozoites in patients' feces or intestinal aspirates [4]. For the microscopic analysis of feces for the recognition and identification of intestinal parasites, direct microscopy is the most prevalent practice in the parasitology laboratory. It is particularly good for observing motile protozoan trophozoites, but it may miss ova, cysts, and larvae that are present in small numbers. Despite the fact that microscopic inspection permits visibility of the parasite and so provides a definitive diagnosis, it has a number of drawbacks [5]. Thus, microscopic examination's diagnostic sensitivity and specificity for detecting parasitic stages in feces are regarded low [6, 7]. Concentration procedures are required to remove fecal debris and retrieve parasites in low-load situations. As a result, using concentration approaches enhances the likelihood of discovering parasitic organisms, improving the sensitivity of organism identifying [8, 9]. Concentration processes, like the direct method, have a disadvantage in detecting particular stages and difficulties, such as the specific weight of solutions and some parasitic stages. So the aim of this study was to evaluate different methods for detection of *Entamoeba histolytica* and *Giardia lamblia* cyst in human stool samples.

Materials and Methods
Samples collection
In the parasitology department of Kirkuk's General Pediatric Hospital, stool samples were taken from infants and children ranging in age from a few months to 14 years. The months of January through April 2022 were eligible for sample collection. Each person experienced diarrhea and other digestive issues. The samples were prepared for microscopic examination using direct wet mount and concentration.

Direct wet mount method

Physiological saline (0.85 percent) was used to emulsify a fresh fecal sample (2 mg) before it was applied to a slide with a wooden applicator stick and covered with a cover slide for the wet mount. On the basis of their fundamental characteristics, cysts and trophozoites were identified using a high power field lens [10].

Formalin- ethyl acetate sedimentation method

A glass test tube containing 10 ml of 10% formalin and 4g of fresh stool were combined. The formalin and feces were completely combined. The amount was placed in a conical centrifuge tube after being strained through wet gauze for 10 minutes at 500g. The sediment was re-suspended in saline or formalin and centrifuged again for 10 minutes at 500 g after the supernatant fluid was decanted. I added 4 ml of ethyl acetate and gave it a good shake. 500 g centrifuged for 10 minutes. Figure 1 shows the four layers that were formed. After discarding the layers, the silt was re-suspended, and a drop was inspected with 40x objectives for protozoan stages [11].

Fig 1: Formal ether concentration method displays the formed four layers

Flotation methods

Solutions preparation

Saturated salt and sugar solutions were made by dissolving salt or sugar (300-400 g) in 1000 ml preheated distilled water, while stirring continuously, until none is dissolved, we keep adding more salt or sugar (salt or sugar remains precipitated out of solution once cooled). zinc sulfate was made by dissolving 330 g of zinc sulfate in 670 ml of distilled water [12].

Flotation technique

A total of 10 ml of saline (0.85% NaCl) and 4 g of fresh stool were combined well. The mixture was centrifuged for 10 minutes at 500 g while being filtered through moist gauze. We decanted the supernatant fluid, re-dissolved the debris in 0.85 % NaCl, and continued washings until clear supernatant was obtained. The sediment at the bottom of the tube was mixed with 2 to 3 ml of flotation solution (zinc sulfate, saturated sugar, or salt solutions), centrifuged, and then filled to the tube's rim. The tube was then left to stand for 10 to 15 minutes. A drop from the tube's top was placed on a slide and screened with 40x magnification [12].

Cysts count

15 samples were chosen from the positive samples for *Entamoeba* and *Giardia* in order to compute the number of the cystic stages and the average difference between the numbers obtained using the direct method and the concentration method. Utilizing a hemocytometer chamber, the number of cysts was determined using 0.1 ml of the sample solutions made for the direct technique and concentration procedures. The sensitivity and specificity of each test was determined [13].

Results

Entamoeba histolytica/dispar and *Giardia lamblia* each had a prevalence rate of 13.7 and 5.1 percent, respectively, according to table 1's findings from this study. without a discernible difference between the months under study.

Table 1: *Entamoeba* and *Giardia* prevalence in studied months

Months	NO. of examined samples	*Entamoeba* +ve samples (%)	*Giardia* +ve samples (%)
January	178	34 (19.1)	15 (8.4)
February	271	26 (9.6)	13 (4.8)
March	131	23 (17.6)	7 (5.3)
April	272	34 (12.5)	8 (2.9)
Total	852	117 (13.7)	43 (5.1)

The most common age range for *Entamoeba* and *Giardia* infections, as reported in table 2, was 1 month to 2 years old. In the 11–14 years, fewer positive instances were reported.

Table 2: *Entamoeba* and *Giardia* prevalence according to the age

Age in year	NO. of examined samples	*Entamoeba* +ve samples (%)	*Giardia* +ve samples (%)
1month-2year	178	74 (41.6)	22 (12.3)
3-6 year	271	22 (8.1)	10 (3.7)
7-10 year	131	14 (10.7)	7 (5.3)
11-14 year	272	7 (2.6)	4 (1.5)
Total	852	117 (13.7)	43 (5.10

There was no discernible difference between the sexes, with both males and females reporting prevalence rates that were roughly the same, table 3.

Table 3: Prevalence *Entamoeba* and *Giardia* according to the sex

Gender	No. of examined samples	*Entamoeba* +ve samples (%)	*Giardia* +ve samples (%)
Female	419	56 (13.4)	20 (4.8)
Male	433	61 (14.1)	23 (5.3)
Total	852	117 (13.7)	43 (5.1)

The sensitivity of the laboratory techniques used varied; the concentration by sedimentation method had the maximum sensitivity at 100%, followed by the flotation concentration technique utilizing zinc and salt. The results of the sugar-based concentration method were nearly identical to those of the direct method, with no changes in the methods' specificity levels, table 4.

Table 4: Sensitivity and specificity of the used method

No. of examined samples	***Entamoeba histolytica/ dispar***					
	No. of +ve by direct wet mount	**No. of +ve by flotation method**			**No. of +ve by sedemintation method**	**Total +ve samples**
		Zinc sulfate	**Saturated sugar**	**Saturated salt**		
852	94	115	98	101	117	117
%	11.1	13.7	11.5	11.9	13.7	13.7
Sensitivity	80.3	98	83.7	86.3	100	----
Specificity	100	100	100	100	100	----
	Giardia lamblia					
No. of examined samples	No. of +ve by direct wet mount	No. of +ve by flotation method			No. of +ve by sedemintation method	Total +ve samples
		Zinc sulfate	Saturated sugar	Saturated salt		
852	40	40	34	39	43	43
%	4.8	4.7	3.9	4.6	5.1	5.1
Sensitivity	93	93	79	90.1	100	----
Specificity	100	100	100	100	100	----

The cystic stage numbers calculated using the various methods under study revealed that the sedimentation method is the most effective at producing high cysts numbers, followed by the flotation method using zinc and salt. The direct method and the sugar solution produced the lowest numbers, fig. 2, table 5.

Table 5: Mean of cysts count in the used method

Mean of cysts count in *Entamoeba histolytica/ dispar*					
No. of examined samples	**Direct wet mount**	**Flotation method**			**Sedimentation method**
		Zinc	**Sugar**	**Salt**	
15	1100	5150	1300	4350	5700
Mean of cysts count in *Giardia lamblia*					
No. of examined samples	Direct wet mount	Flotation method			Sedimentation method
		Zinc	Sugar	Salt	
15	1650	4000	1500	3450	4450

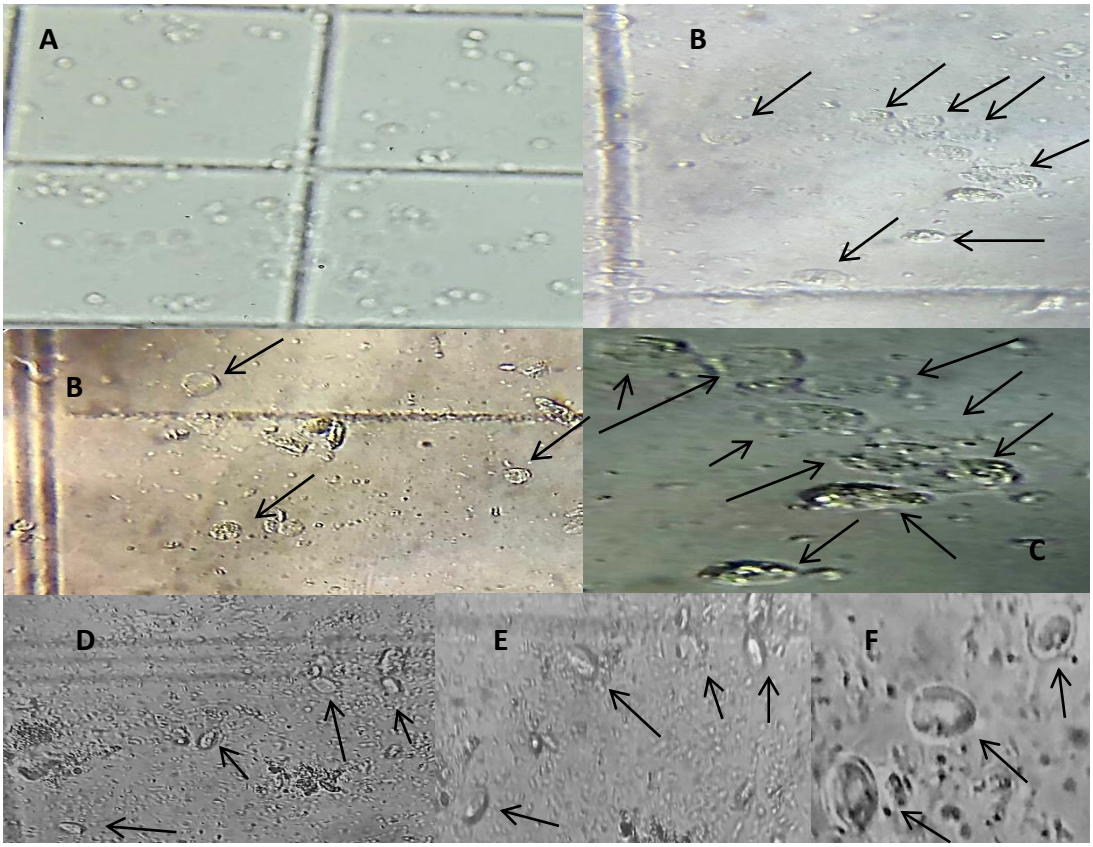

Fig 2: Cysts on counting chamber, A (*Entamoeba* cysts 100X), B (*Entamoeba* cysts 400X), C (*Entamoeba* cysts 1000X), D (*Giardia* cysts 100X), E (*Giardia* cysts 400X), F (*Giardia* cysts 1000X), the arrows refer to the cysts.

Discussion

The parasites *Entamoeba histolytica/dispar* and *Giardia lamblia* favor the human gastro-intestinal tract. Therefore, the main method for determining their stages is by stool samples. In our survey group, age was a substantial and crucial factor influencing the occurrence of both G. lamblia and E. histolytica/dispar. The majority of G. lamblia and E. histolytica/dispar positive samples were discovered in the 1 month to 2 year age range; there was no discernible difference in the incidence of parasites between males and females, which was consistent with earlier findings [15, 16]. Unlike our outcome, according to earlier studies, gender is another variable that greatly influenced the prevalence of these protozoan parasites [17]. The rate and prevalence of the disease may differ in one group from another depending on factors including the experience group, the location or environment, and the technique of diagnosis. The formol-ether approach was determined to be the most sensitive in the current investigation since a large percentage of the cases were discovered to be present. The formol-ether concentration technique with the standard iodine preparation and the formol acetone concentration techniques performed better results. They demonstrated that the formol-ether concentration method was more accurate than the alternative techniques [18]. The results of the current investigation indicated that the number of parasites found using concentration approaches had increased compared with the other studied methods. Therefore, normal concentration procedures may be used for routine parasite examination. Concentration enables the detection of organisms that are present in little quantities, direct wet mounts may miss these [6, 11]. For the precise detection of such parasites, wet mount technique must be used, which has a very low sensitivity for protozoan cysts. The Wet mount method has become a common diagnosis since it is easy to use, affordable, and quicker than the alternatives. But wet mount approach has a relatively low detection rate for parasites in a single stool examination because of its limited sensitivity [19, 20]. By separating parasites from fecal waste, concentration techniques make it possible to detect parasitic organisms, even in small amounts [16, 18]. Since the conclusion that a significant portion of the cases were found to be present by formol-ether method the most sensitive in the current investigation. Its recommended that, the sensitivity may be boosted by including one or two distinct concentration approaches into the standard diagnostic procedures, may increase the sensitivity of the parasite identification by routinely used method. Especially all three techniques are economical and can be used in remote areas with only the most basic infrastructure.

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