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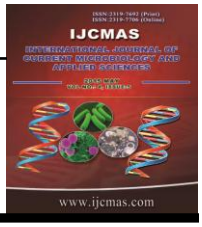

Original Research Article

Evaluation of Different Techniques of Stool Examination for Intestinal Parasitic Infections in Sulaimani City - Iraq

Hersh Ahmad Amin* and Shahnaz Abdulkader Ali

Department of Microbiology, School of Medicine, Faculty of Medical Sciences, University of Sulaimani, Kurdistan region of Iraq

**Corresponding author*

A B S T R A C T

This study was conducted: to determine the best diagnostic technique use for stool examination using three diagnostic methods, Zinc sulphate floatation, Formal-Ethyl Acetate sedimentation and direct wet mount techniques. In addition, to determine the prevalence of intestinal parasitic infections, 300 samples were collected randomly from patients with different age groups from both sexes, who attended the central laboratory in Teaching Hospital in Sulaimani City/ Iraq from June to October 2014. The results showed that Zinc sulphate floatation technique had of the highest sensitivity rate 148(49%), followed by Formal-Ethyl Acetate 130(433%) and Direct wet mount that showed lowest rate 68(22.6%). The prevalence of the intestinal parasitic infections were165 (55.6%) of which the most common parasite was *Entamoeba histolytica*(60.22%). The rate of infection for males was higher 118 (57.28%) than females 47 (50%).Children under 5 years of age had the highest prevalence of the parasitic infections 52(81.25%).

Keywords

Diagnostic techniques, Zinc sulphate floatation, Formal-Ethyl Acetate sedimentation, Direct wet mount technique, Sulaimani/Iraq

Introduction

Parasitic infections, caused by intestinal helminthes and protozoan parasites, are among the most prevalent infections in developing countries, while in developed countries, gastrointestinal infections are caused more commonly by protozoan parasites compared to helminthes. Intestinal parasites cause a significant morbidity and mortality in endemic countries. These infections are most prevalent in tropical and sub tropical regions of the developing world where adequate water and sanitation facilities are lacking (Savioli and Albonico, 2004;Cappello,2004).The usual methods that are used for detection of intestinal parasites from stool specimens are the direct wet mount using saline and iodine, but the detection can be enhanced through using concentration procedures such as salt floatation, zinc sulphate centrifugal floatation, formal ether concentration (Ritchie, 1948) and modified Baerman

sedimentation. These techniques increase the detection of protozoan cysts, helminthes eggs and larvae. However, the use of diethyl ether, an essential reagent of concentration technique, may be hazardous to laboratory workers, since it is explosive, of potential toxicity such as respiratory irritation and a cause of cardiovascular depression and narcosis (Boswell and Collins, 1996; Young *et al*., 1979).Therefore, a replacement for diethyl ether has been sought for this technique by introduced of ethyl acetate as a replacement. In view of the increasing polyparasitism in the developing countries, there is a need for sensitive diagnostic tools that are simple to apply and can detect different intestinal parasite species in the same stool sample.

The objectives of this study wereto compare three different techniques in diagnosing parasitic infections, and to determine the prevalence of intestinal parasitic infections among the patients who attended the central laboratory in Teaching Hospital Sulaimani city.

Stool Sample collection

A total of 300 fresh stool samples were collected randomly from patient with different age groups, who attended the central laboratory in teaching hospital inSulaimanicity from June to October 2014**.** Specimens were collected in sterile containers and transported to the Department of Microbiology immediately. Both the formed and unformed stools were examined immediately.

Material and methods

Each stool specimen was examined by the following techniques

Macroscopic examination: The stool specimens were examined by naked eye for presence of: The colour, consistency, blood, mucus, the nature of the faeces, and adult worms.

Direct microscopic examination using saline and iodine preparations

A small amount of the stool sample was emulsified in 1-2 drops of saline or iodine on a glass slide then a cover slip was placed on it and examined microscopically.

Concentration microscopic examination

A- Zinc Sulphate centrifugal flotation: Fresh faecal specimens were formalinised by adding few drops of 10% formalin to 1g of stool sample and then emulsified in 10 parts of saline, strained through three folds of gauze. The filtrate was collected in a centrifuge tube and centrifuged at 1500rpm for 5 minutes. The supernatant was discarded and the sediment was re-suspended in water. To the sediment,3-4 mL of 33% zinc sulphate solution was added, then mixed well and filled with ZnSO4 solution to about half an inch of the rim. Several loopfuls of the supernatant fluid were removed with a bacteriological loop and put on slide, then covered with a cover slip and examined under 40X lens (Melvin and Brooke,1985).

B-Formal-Ethyl Acetate concentration: Fresh faecal specimen was emulsified by mixing about 1g of stool with7 mL of 10% formol saline and was kept for 10 minutes for fixation, then strained through three folds of gauze. The filtrate was added to 3 mL of ethyl-acetatand then centrifuged at 1500rpm for 5 minutes and allowed to settle. The supernatant was removed and two drops of the sediments on a slide, covered with a cover slip then examined under 40X for parasite detection (Neimeister *et al*., 1987).

Statistical analysis

Chi-square test was used to evaluate the statistical significance, *P*-value <0.05 was considered as statistically significant.

Results and Discussion

A total of 300 stool samples were examined, out of which 165 (55.6%) samples were positive for intestinal parasites. *Entamoeba histolytica* was the most prevalent species110 (60.22%), other parasites observed were *Blastocystis hominis* (19%), *Cryptosporidium spp* (16.43%), *Entamoeba coli* (6%), *Giardia lamblia* (2%), *Chilomastix mesnili* (2.28%), *Isospora spp* (1.36%) and *Enterobius vermicularis* (0.45%) as shown in Table 1.

From the results of three different diagnostic tests, demonstration of the parasitic infections with sensitivity of (49.3%) the Zinc sulfate flotation concentration technique was found to be most sensitive method in this study, then came Formal-Ethyl acetate at rate of (43%) and direct wet mount (22.3%) as shown in Table 2.

Interestingly males were more infected with intestinal parasites (57.28%) than females (50%).as shown in Table 3. Children under 5 years of age had the highest prevalence of the parasitic infections as shown in Table 4. The rate of poly parasitism infections seen in 56(36%) samples were infection with *Entamoeba histolytica* and *Blastocystis hominis* cysts, *Entamoeba histolytica* and *Giardia lamblia* cysts that were detected by concentration methods.

In the present study the prevalence of the intestinal parasitic infections was higher165 (55.6%) and *Entamoeba histolytica* was the most prevalent species. This was agreement with the study conducted by (Hamad and Ramzy, 2012) who reported 30% in Arbil, 61.1 % by (Farhan, 2012) in Al-Anbar and in Baghdad (23.44%) detected by (AL-Kubaisy*et al.*, 2014). Another study by (Jarallah, 2012) reported 28.87% in Basrah. *Entamoeba histolytica* was reported to be responsible for approximately 50 million cases of invasive amoebiasis and upwards of 100,000 death/year (WHO, 1997). This may be due to that *E. histolytica* are highly resistant to chlorine disinfection and their small size (range 1–17 μm) enables them to penetrate water treatment systems and cause waterborne disease even following the consumption of treated drinking-water (Graczyk *et al.*,2005).Infection is also acquired primarily through the infection of infective cyst form present in faecally contaminated water and food (Petri and Singh, 1999). Second species recorded was *Blastocystis hominis* (19.60%).This was in disagreement with studies by (Ali, 2010) (28.81%), (Rezan, 2014) (22.15%) and (Raza, 2009) 6.3% in Sulaimani and7% in Baghdad by (Mahmood *et al.*, 2014).

The prevalence rate of parasitic infections was higher in males (57%) as compared to females (50%) with no significant difference. The reason for the male preponderance in our study may be related to the daily activity rather than the sex predominance. However, many studies from Iraq showed higher prevalence of these infections among males, in Sulaimani by (Rezan, 2014), (Mariwan, 2010) in kalar Sulaimani, (AL-Kubaisy *et al.*, 2014) in Baghdad, while other studies showed increased infection among the females, (Hamad and Ramzy, 2012) in Erbil, (Raza, 2009) in Sulaimani. Nevertheless, the sex predominance for the parasite infections has still not been confirmed.

Statistically no significant difference in the percentage of intestinal parasites infections were according to the age of the patient (p>0.05).

Table.1 Total Prevalence of parasitic infections

parasites	Number of (+ve) case (n=300)	**%**
Entamoeba histolytica	110	50.16
Blastocystis hominis	43	19.6
Cryptosporidium spp.	36	16.41
Entamoeba coli	15	6.84
Giardia lamblia	6	2.73
Chilomastix mesnili	5	2.28
Isospora spp.	3	1.36
Enterobius vermicularis	1	0.45
Total	219	

Table.2 Sensitivity of different parasitic examination techniques

Techniques	No. positive for parasites (Total No. 300)	%
Zinc sulphate floatation	148	49.3
Formal-Ethyl Acetate	130	43.3
Direct wet mount	68	22.6

Table.3 Sex prevalence of parasitic infections

Sex	Examined No.	No. positive for parasites	%
Male	206	118	57.28
Female	94	47	50
Total	300	165	55.6

$\chi 2 = 0.409$, *P*-value >0.05

Table.4 Age prevalence range of parasite infections

Age	Examined No.	Positive	%
< 5	64	52	81.25
6-10	14	8	57.14
11-20	58	28	48.27
21-30	102	49	48.03
31-40	55	23	41.8
41-50	7	5	71.8
Total	300	165	55.6

$\chi 2 = 6.79$, *P*-value >0.05

The prevalence rate in all age groups is high, but children who were between 1-5 years of age had the highest prevalence rate of parasitic infections. The high prevalent rate could be attributed to poor personal hygiene and environmental exposure. Similar rates were recorded by (AL-Kubaisy *et al.*, 2014), and (Farhan, 2012), which showed that the

largest number of intestinal parasitic infections were found in children aged ≤5 years and that infection declined progressively with increasing age groups. Conversely our results disagree with the result of (Raza, 2009) in Sulaimani, (Mohamed and Yahya, 1999) in kerkuk who recorded a higher prevalence rate of parasitic infection in children from 6-9 years old.

The present study found that the Zinc sulphate floatation concentration technique was more sensitive with49.3% of the cases were detected, followed by Formal-Ethyl Acetate sedimentation (43.3%) and then direct wet mount technique showed the lowest detection rate (22.6%). Results of our study is in agreement with studies that found 60% by (Mahmood *et al.*, 2014) in Baghdad by the flotation, 58.7% by sedimentation and 39.3% by direct wet mount. Other studies (Raza, 2009) in Sulaimai, (Allan *et al*., 1981) in United states-Texas, found that the concentration by flotation methods were more sensitive in intestinal parasitic diagnosis. Flotation technique permits the separation of protozoan cysts and certain helminthes eggs from excess debris through the use of a liquid with a high specific gravity, where the parasitic elements are recovered from the surface film and the debris remains in the bottom of the tube. This technique yieldsa cleaner preparation and morphology of the parasites than the sedimentation procedure (Garcia, 2001), (Melvin and Brooks, 1985). A study from India by (Parameshwarappa *et al.*, 2012) showed that the formol-ether concentration technique was more sensitive as compared to the other methods and found that 64.5% of the cases were detected by the formol-ether method and 55% by Zinc sulphate floatation. This may be due to that Sedimentation procedure leads to the recovery of all protozoa, eggs and larvae present. However, the preparation contains more debris than the flotation procedure, though Ethyl acetate is used as an extractor of debris and fat from the faeces and leaves the parasites at the bottom of the suspension. Concentration by formalin-ethyl acetate sedimentation is recommended because it is the easiest to perform, allows recovery of the broadest range of organisms and is the least subject to technical error (Neimeister *et al.,* 1987). In our study the direct wet mount showed the lowest sensitivity from the concentration methods, but the advantage of this method is to provide a quick diagnosis of a heavily infected specimen, to check organism motility and to diagnose parasites that may be lost in concentration techniques (Robyn, 1997).

In conclusion, our results indicate that the Zinc sulphate floatation method is more sensitive technique from Formal-Ethyl Acetate sedimentation and direct wet mount but over all, these methods are important for diagnosis. The prevalence of parasitic infections is high and *Entamoeba histolytica* was the most prevalent species.

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