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### A COMPARISON BETWEEN THE EFFICIENCY OF FORMAL GASOLINE CONCENTRATION TECHNIQUE AND OTHER TECHNIQUES USED FOR THE DETECTION OF INTESTINAL PARASITES

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#### ABSTRACT

Formal-ether technique is considered as a gold standard method for detection of most intestinal parasites; however, because of its low safety and hazardous impact, a need for a better technique has a paramount importance. Objective to evaluate the efficiency of formal-gasoline procedure in detecting parasite ova, cyst, or larvae compared with the direct wet preparation and formal-ether concentration methods. A total of 300 stool samples were collected from Khartoum state in 2009, and preserved in 10% formal saline. The samples were processed and examined using wet preparation, formal-ether, and formal-gasoline concentration techniques. Out of 300 stool specimens examined, 97 cases were found to be positive for different parasites. Formal-ether, formal-gasoline, and direct wet preparation detected 79, 90, and 51 positive cases of the total specimens respectively with  $p < 0.01$ . In this study gasoline and diethyl ether were both good in maintaining characteristic morphology however, gasoline was considerably superior to diethyl ether in its sensitivity (i.e. the mean number of detected parasites/gm of stool), *Hymenolepis nana* egg was rarely seen by formal-ether. For safety, low hazard, availability, low cost, and high sensitivity of gasoline, this new method should be used as an alternative choice for formal-ether concentration method which is considered the gold standard.

**Key Words:** Formal-ether technique, Intestinal parasites.

#### INTRODUCTION

Fecal concentration has become a routine procedure as a part of complete parasite examination. It allows the detection small of organisms that may be missed by using only direct wet smear [1, 2]. The formalin-ether concentration technique is commonly used in laboratories to concentrate parasite eggs, cyst, and larvae in stool specimens [3]. This technique, also, provides certain advantages including less alteration to organisms and increased recovery of helminthes eggs and protozoan cysts [4]. Unfortunately, diethyl ether, an essential component in the formal ether technique is extremely flammable, is highly volatile, produce anesthetic vapor, and forms explosive peroxides when exposed to light [5].

Moreover, it can be a possible cause of mutagen, if inhaled, or absorbed through skin with harmful long term health effect like neurotoxicity or cancer [6]. To overcome these disadvantages, several other chemicals have been evaluated as substitutes for diethyl ether like ethyl acetate, acetone and gasoline [7, 8, 9]. Among these solvent, gasoline has been rated as safe in comparison to the ether effect on health and has also an equivalent detection rate of parasites [10]. Therefore, in accordance with this assumption, in areas where there is high prevalence of parasites and need of replacement of ether by a hazard free solvent as well as effective diagnostic technique has a paramount importance. This study aimed at evaluating the efficiency of formalin-gasoline concentration method as newly introduced technique in comparison with the routinely used formalin-ether concentration technique for the detection of parasites.

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## MATERIALS & METHODS

### Study type and approach

This is a descriptive facility based case study.

### Study area

This study was carried out in Brashear hospital, which is located in Mayo area, Ombada hospital which is located in Ombadaarea, Omdurman ,and Elzain medical center which located in salha area ,Omdurman.

### Study population

The study was conducted on outpatients who attended the above mentioned hospitals. The population was categorized according to gender and age groups.

### Sample size

The study was carried out on 300 outpatients attending these hospitals

### Data collection

A questionnaire was designed to collect data.

### Techniques used

#### Direct wet smear

Small amount of specimen was placed on slide, the sample was covered with cover glass and pressed gently. The preparation was examined under microscope using 10x objective with condenser iris closed sufficiently and the 40x objective was used to identify the parasites.

#### Formal ether concentration techniques

##### Principle

Ether is used as an extractor of debris and fat from the feces and leaves the parasite at the bottom of the suspension.

##### Method

1. 1ml of preserved feces in 10%formal saline was emulsified in about 4ml 10%formal water in a centrifuge tube.
2. Further 3-4ml of 10%v\ v formal water was added. The tube was capped and mixed well.
3. Emulsified feces were sieved; and sieved suspension was collected in a beaker

4. The suspension was transferred into a centrifuge tube. 3ml of ethyl acetate was added.
5. The tubes were stoppered; and shaken vigorously for 1minute.
6. The stopper was loosened by the piece of gauze warped around the top of the tube
7. The centrifuged suspension was immediately at 1000g for 1minute.Four layers were formed; a small amount of sediment (containing the parasites) in the bottom of tube; a layer of formalin; a plug of fecal debris on top of the formalin layer; and a layer of ethyl acetate at the top.
8. The plug of fecal debris was removed by ringing the plug with an applicator stick; all of the supernatant fluid was decanted.
9. All sediment was transferred to a slide, and covered with a cover glass.
10. The entire preparation was examined microscopically by using 10xobjective with the condenser iris closed sufficiently to give good contrast. The 40x objective was used to identify the small cysts and eggs. If cysts are present, small drop of iodine was added under the cover glass to confirm their identification.
11. The number of different stages of (eggs, cysts and larvae) the parasites were counted to give an approximate number of the parasites stage per 1gm of feces.

#### Formal gasoline sedimentation concentration technique

As mentioned above except in step number 4, 3ml of gasoline was added instead of the ether Procedure notes for formal ether or Formal gasoline concentration technique:

- ✓ Only one gauze is used
- ✓ B-After the fecal debris is rimmed and excess fluid is decanted, the sides of the tube can be swabbed with cotton –tipped applicator stick while the tube is still upside down to remove excess ethyl acetate or gasoline
- ✓ C-If there is excess ethyl acetate or gasoline in the smear of the sediment prepared for examination, bubbles will be present, which will obscure the material of interest. This step is more important in gasoline because gasoline dose not evaporate like ether; so bubbles must be cleared carefully.

## RESULTS

**Table 1. Overall prevalence rate of parasites using different technique**

No. examined	No. positive	prevalence
300	97	32.33%

**Table 2. Detection rates of different technique used**

Technique	No. positive	No. detected	Detection rate
Wet preparation	97	51	52.57%
Formal ether	97	79	81.4%
Formal gasoline	97	90	92.7%

P=0.00019

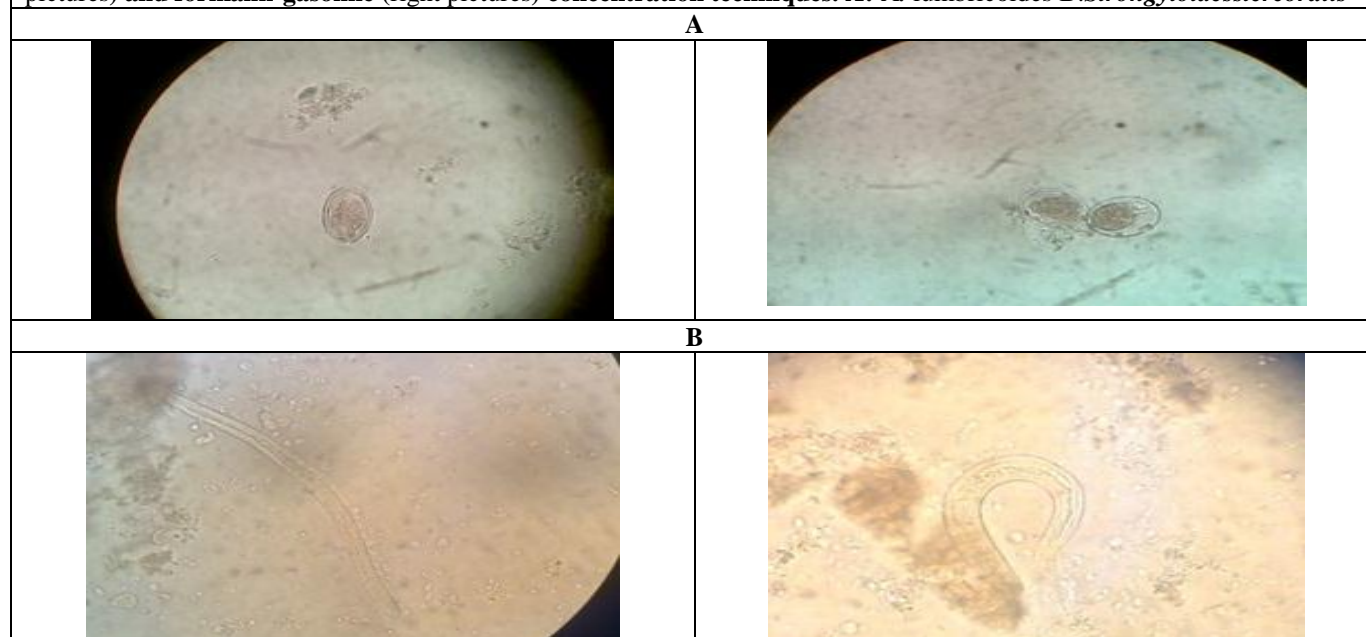
**Table 3. The mean number of parasitic stages per gm of stool by the formal ether and gasoline techniques.**

Technique	Mean
Formal ether	29.9
Formal gasoline	48.5

P=0.0001 .

**Table 4. Comparison of physical and chemical characteristics of diethyl ether and gasoline**

Properties	Ether (diethyl)	Gasoline
Flammable limits, % in air	1.9-36.0	1.4-7.6
Explosive limits, % in air	1.7-48	1.3-6.0
Boiling point (°C)	34.5	38-204
Flash point (°C)	-45	-42.8
Melting point (°C)	-116.2	Less than -60
Auto ignition temperature (°C)	160-180	280-456
Solubility in water	Yes	No
Specific gravity	0.714	0.72-0.78
Relative cost (reagent grade)	More than 10	1
Access	Limited access or less easy	Easy access
Anesthetic vapor formation	Yes	No
Safety precautions	Keep away from sources of ignition, oxidizers, iodine, chlorine; use in very well-ventilated areas	Keep away from sources of ignition, peroxides, nitric acid and perchlorates; use in well-ventilated areas

**Figure 1. Microscopic photographs of the eggs and larva of recovered parasite species by the formalin-ether (left pictures) and formalin-gasoline (right pictures) concentration techniques. A: A. lumbricoides B: Strongyloides stercoralis**

## DISCUSSION AND CONCLUSION

The diagnosis of parasitic infections in humans is challenging and requires the recognition of parasite stages based on size, morphology, color, and movement. Parallel examination of total stool samples by the routine formalin-ether (original) and by the formalin-gasoline techniques resulted in identical distribution of positive slides and morphology of recovered parasite species.

However, the easy availability of gasoline (wherever gas stations are present) and its low cost in comparison to ether makes gasoline superior to ether for use in concentration of stools by the sedimentation method in laboratories, including laboratories with limited material resources and also laboratories present in small cities and rural health centers.

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