Evaluation Of Formol-ethyl acetate Concentration Method In The Diagnosis Of Intestinal Parasitic Infections

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Abstract

Introduction:Intestinal parasitic infections are widely distributed throughout the world and have been identified as one of the most significant causes of illnesses and diseases among the disadvantaged population .This study aimed to evaluate Formol-ethyl acetate concentration method in detection of intestinal parasitic infections.

Methodology / Principal Findings: **100**fecal samples were collected, preserved and examined by using formol-ethyl acetate concentration method.Formal-ethyl acetate concentration method (FECM) detectedprotozoan infections in**55%**of the samples while only **8%**helminthic infections were detected.

Conclusions/Significance: The concentration techniques recommended for the qualitative diagnosis of intestinal parasites (both helminths and intestinal protozoa) are the formol-ethyl acetate concentration method **(FECM)**. By centrifugation, it leads to the recovery of all protozoa, eggs, larvae, coccidian and microsporidia present; This method have the advantages of being rapid and suitable for fresh or preserved stool, also used for concentrating parasites on which Zinc sulfate floatation has given poor results due to excessive amounts of fats and fatty acids, and for operculated ova of some trematodes and cestodes, the morphology of most parasite is retained for easy identification and it covers most intestinal parasites.

Keywords: intestinal parasitic infections; Formol-ethyl acetate concentration.

Introduction

Intestinal parasitic diseases have a significant impact on populations and constitute an obstacle to socio-economic development in our regions. Thus, accurate diagnosis is essential for patient management and to guide the design, implementation and monitoring of programmes for community control of infectious diseases. The evaluation of the performance of the different techniques used to detect intestinal parasites helped to highlight the advantages and disadvantages of each method in the identification of species **(1)**.

The technique recommended for the qualitative diagnosis of intestinal parasites (both helminths and protozoa) is the formol-ethyl acetate concentration method (FECM) **(2).**

Flotation techniques facilitate thefloating of parasitic elements,this technique yields a cleaner preparation than does the sedimentation procedure; however, some helminth eggs do not concentrate well in the flotation method; a sedimentation technique is recommended to detect these infections **(3).**

Kato-Katz thick smears method is the diagnostic method recommended by the World Health Organization (WHO) for the quantification of STH eggs in human stool because of its simple format and ease-of-use in the field**(4)**.

Recently, fecal egg counting (FEC) techniques, such as the FLOTAC usedfor the diagnosis of helminths in parasitology **(5).** Despite their high sensitivity, the main limitation of FLOTAC techniques is the complexity of the method which involves centrifugation of the sample with a specific device **(6)**. So this study aimed to

evaluateformol-ethyl acetate concentration method in diagnosing intestinal infections.

Subjects and methods

Ethics Statement

This study was conducted from March 2017 to March 2018 after being authorized by the scientific ethics committee of our institute.

Selection criteria

100 samples were randomly collected from different laboratories.

Sample collection:

Stool samples were collected in clean, dry, wide-mouth containers with tight-fitting lids. The acceptable amount of stool required for O/P examination is at least **1g** to cover our study requirements and samples were collected avoiding contamination with urine or water.

Samples were immediately preserved after passage due to the lag time till they reach the laboratory.

Preservatives used in this study were formalin 10%

Parasitological methods

Microscopic examination was done after preparing the samples using formol-ethyl acetate concentration method**(3)**.

Statistical analysis

Data was analyzed using IBM SPSS Statistics for Windows version 25 andMedcalc version 15.8.0. Qualitative data was expressed as number and percentage. Sensitivity and negative predictive values (NPV) were calculated.

RESULTS

Protozoa were more frequent than helminths parasitizing **55% (55/100)** versus **8%** of the studied samples **(Table 1).**

	N	**%**
Helminth	**(8 /100)**	**8%**
Protozoa	**(55/ 100)**	**55%**

Table 1. Frequencies of protozoan and helminthic parasitisms (n=100).

By using FECM Cryptosporidium was found in **24%** of all samples and E. histolytica in **14%**, E. coli in **9%**, G. intestinalis in **9%**, Cyclosporacayetanensis in **7%,**E. nana in **6%**, H. nana in **5%**, C. mesnili in **3%**, A. lumbercoides in **2%**, Blastocystis sp. in **2%**, E. hartmanni in **2%**, E. vermicularis in **2%**, Isospora belli in **1%**, Microsporidia sp. in **1%** and Pentatrichomonashominis**0%(Table 2).**

Parasite	Negative No. (%)	Positive No. (%)
Protozoa		
Cryptosporidium	**76 (76%)**	**24 (24%)**
E. histolytica	**86 (86%)**	**14 (14%)**
E. coli	**91 (91%)**	**9 (9%)**
Giardia intestinalis	**91 (91%)**	**9 (9%)**
Endolimax nana	**94 (94%)**	**6 (6%)**
Cyclosporacayetanensis	**93 (93%)**	**7 (7%)**
Chilomastixmesnili	**97 (97%)**	**3 (3%)**
Entamoebahartmanni	**98 (98%)**	**2 (2%)**
Blastocystis sp.	**98 (98%)**	**2 (2%)**
Isospora belli	**99 (99%)**	**1 (1%)**
Microsporidia sp.	**99 (99%)**	**1 (1%)**
Pentatrichomonashominis	**100 (100%)**	**0 (0%)**
Helminths		
Hymenolepis nana	**95 (95%)**	**5 (5%)**
Enterobiusvermicularis	**98 (98%)**	**2 (2%)**
Ascarislumbercoides	**98 (98%)**	**2 (2%)**

Table 2. Distribution of the studied samples according to detected parasites by formal-ethyl acetate concentration method (FECM)(N. = 100).

Concerning to helminthic infections,sensitivity of FECM was 66.7% for total helminthic infections, 71.4% for *H.nana*, 100% for *E.vermicularis* and 66.7%for *A.lumbricoides***(Table 3).**

Parasite type	Sensitivity	NPV	Accuracy
Helminths	**66.7%**	**95.7%**	**96%**
Hymenolepis nana	**71.4%**	**97.9%**	**98%**
Enterobiusvermicularis	**100%**	**100%**	**100%**
Ascarislumbercoides	**66.7%**	**99%**	**99%**

Table 3.Sensitivity and negative predicative value (NPV) of formal-ethyl acetate concentration method (FECM) in helminths detection.

Concerning to protozoal infections sensitivity of FECM was **98.2%** for all protozoa infections, **100%** for *Cryptosporidium*, **87.5%** for *E.histolytica*, **90%** for *E.coli*, **90%** for *G.intestinalis*, and **87.5%** for *E.nana* **(Table 4).**

Parasite type	Sensitivity	NPV	Accuracy
Protozoa	**98.2%**	**97.8%**	**99%**
Cryptosporidium	**100%**	**100%**	**100%**
E. histolytica	**87.5%**	**97.7%**	**98%**
E. coli	**90%**	**98.9%**	**99%**
Giardia intestinalis	**90%**	**98.9%**	**99%**
Endolimax nana	**87.5%**	**98.9%**	**99%**

Table 4.Sensitivity and negative predicative value (NPV) of formal-ethyl acetate concentration method (FECM) method in protozoa detection.

Discussion

Reliable, sensitive and practical diagnostic tests are an essential tool in disease control programs for mapping, impact evaluation and surveillance. So there is a great need to provide a global assessment of the relative performance of these diagnostic tools for the detection of different parasitic infections **(6).**

Examination of **100** stool samples collected randomly from different laboratories in Sohag governorate showed that **FECM** revealed different protozoa species in **55%** while helminthic infection in **8%**.

The results show **FECM** was sensitive for protozoa diagnosis **(sensitivity=98.2%),**these results agree with the study done by **Barda *et al.*, (2013)**who found that FECM sensitivity for protozoa was **(88%) (7),** while other studies done by **Hussein *et al.*, (2017)**showed that FECM sensitivity for protozoa was **(100%) (8).**

Considering the cost-benefit and the feasibility FECM took average 2 min to prepare the sample, 5 min of centrifugation and 10 min to read the slide (a total of 17 min).

As for the cost of the technique, FECM requires a centrifuge, formol and ethyl-acetate that are not always easy to purchase, especially in laboratories with limited resources.

RECOMMENDATIONS

Low prevelance of helminh infections included in the study, causes the study to be based on limited species of helminths. So further studies are needed to provide more information about the use of FECM for a wider range of helminth species.

Other studies should be done to compare FECM with other types of concentration techniques.

To conclude, this study demonstrates that FECM is definitely a sensitive, relatively simple andstill a gold standard technique for the qualitative diagnosis of helminth and protozoalinfections..

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