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# Intestinal helminths in immigrants in Naples (Italy): a comparison between two different diagnostic techniques

Silvano Esposito<sup>1\*</sup>, Silvana Noviello<sup>1</sup>, Sebastiano Leone<sup>1</sup>, Renato Pascale<sup>1</sup>, Enrico Russo<sup>1</sup>, Luciano Gualdieri<sup>2</sup>

<sup>1</sup>Department of Infectious Diseases, University of Salerno, Italy

<sup>2</sup>Ascalesi Hospital, Naples, Italy

## PEER REVIEW

### Peer reviewer

Francesco Menichetti, MD, Director of the Infectious Diseases Unit of Pisa Hospital.

Tel: +39050995431

E-mail: menichettifrancesco@gmail.com

### Comments

This is a valuable and original research that might contribute to improve the diagnosis of a largely widespread infection.

Details on Page 443

## ABSTRACT

**Objective:** To compare two different methods for detection of intestinal parasitic in immigrants from high risk geographic areas for intestinal parasitic diseases.

**Methods:** A total of 307 stool specimens were analysed by Ridley method and FLOTAC, a new technique performing a direct count of all parasitic elements.

**Results:** Compared to Ridley method, FLOTAC technique led to fewer negative results ( $P < 0.05$ ), index of a higher sensibility.

**Conclusions:** Performing a more accurate detection of parasites appears a goal to reach in terms of public health.

## KEYWORDS

FLOTAC, Intestinal parasites, Traditional technique

## 1. Introduction

Faecal egg count (FEC) techniques are widely used for parasitological diagnosis in humans and animals to assess the number of parasitic elements (eggs, larvae, oocysts) per gram of faeces (EPG/LPG/OPG). They are also used to measure the prevalence and intensity of infections for epidemiological surveys, to quantify the efficacy of chemotherapies, and to detect anthelmintic resistance<sup>[1]</sup>. The FLOTAC is a novel apparatus patented for a multivalent copromicroscopic technique, the FLOTAC technique<sup>[2]</sup>. Based on flotation in centrifuge and translation of the apical portion of the floating suspension, it is capable of giving egg/larva/oocyst counts in quantities up to 1 g of faeces (volume=10 mL). The aim of the present study is to carry out a comparative study on stool samples, obtained by asymptomatic immigrant individuals in Naples, and examined by both FLOTAC and traditional technique.

## 2. Materials and methods

### 2.1. FLOTAC apparatus

The cover slip used in traditional flotation methods is removed from the faecal suspension and placed on the microscope slide. Besides the flotation solution used, there is no way to insure that: i) the total quantity of parasitic elements float to the top of the suspension; ii) all the floated parasitic elements adhere to the underside of the coverslip. The FLOTAC takes advantage of the facts that when flotation takes place in a centrifuge, all parasitic elements float to the top; in addition, if the top portion of the flotation suspension is transversally cut (translated), all parasitic elements can be collected and examined under a microscope.

The FLOTAC apparatus has been designed to easily carry out the flotation in centrifuge and the translation of the apical portion of the floated suspension. It is a cylindrical-shaped device composed of three principal

\*Corresponding author: Prof. Silvano Esposito, Department of Infectious Diseases, University of Salerno, Largo Citta' di Ippocrate, 84100 Salerno, Italy.

Tel: +39 089 642420, +39 333 6940183

E-mail: silvanoesposito@libero.it

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physical components: the base, the translation disc, and the reading disc. The FLOTAC apparatus holds two sample flotation chambers, 5 ml each, for a total volume of 10 ml, with two ruled grids. Each grid contains 12 equidistant ruled transparent lines, permitting the counting of parasitic elements under them. Where the dilution of faeces is 1 in 10, the reading of 1 grid (total volume is 5 mL=0.5 g of faeces) gives an analytic sensitivity of 2 EPG/LPG/OPG, the reading of 2 grids (total volume is 10 mL=1.0 g of faeces) gives an analytic sensitivity of 1 EPG/LPG/OPG.

## 2.2. Diagnostic procedure

Ten grams of faeces are suspended in 90 mL of tap water (dilution ratio=1:10). The suspension is accurately homogenized, filtered through a wire mesh (aperture=350 mm), and the debris are discarded. Ten ml of the filtered suspension are placed into a tube and centrifuged for 2 min at 1500 r/min. The supernatant is poured off, leaving only a pellet in the tube. The tube is then filled with the flotation solution to its previous 10 mL level, and slowly agitated. The two flotation chambers are filled; then, the FLOTAC apparatus is centrifuged for 5 min at 1000 r/min. Flotation in centrifuge causes the debris to sink to the bottom of the chambers, and the parasitic elements to float up to the top under the two ruled grids. After centrifugation, the translation disc and the reading disc are simultaneously 45° right rotated using the FLOTAC key, thus allowing the parasitic elements to be easily viewed under a microscope while they are still in the FLOTAC apparatus.

The stool samples, fixed in sodium acetate–acetic acid–formalin solution, were simultaneously examined by two independent expert microbiologists in double blind using both the ether–concentration method and the FLOTAC technique.

## 3. Results

From October 2006, at the Ospedale Ascalesi of Naples (southern Italy), a parasitological service has been activated for immigrants having a temporary residence permit. So far, 307 stool samples have been collected from asymptomatic immigrants from the following countries: Senegal ( $n=191$ ), Pakistan ( $n=20$ ), Bangladesh ( $n=19$ ), Nigeria ( $n=14$ ), Guinea ( $n=10$ ), other countries ( $n=53$ ). Table 1 shows the results of the comparison between the different diagnostic approaches. Of the 307 stool samples obtained by asymptomatic immigrant individuals, 12 (3.90%) was positive when examined with the FLOTAC technique, 3 (0.90%) after examination of ether concentration method. The helminth eggs detected in the stool were of *Hymenolepis nana*, *Trichuris trichura* and *Ancylostoma duodenale*.

**Table 1**

Frequency and prevalence values regarding the helminths (eggs) detected by the two techniques.

Coproscopic technique	Helminths frequency (n=307)			Total*
	<i>Hymenolepis nana</i>	<i>Trichuris trichura</i>	<i>Ancylostoma duodenale</i>	
Ether–concentration method	1 (0.30%)	1 (0.30%)	1 (0.30%)	3 (0.97%)
FLOTAC	3 (0.97%)	6 (1.90%)	3 (0.97%)	12 (3.90%)

\*Significant difference ( $P<0.05$ ;  $\chi^2$  test).

## 4. Discussion

Worldwide, there are approximately 3.5 billion infections with the parasitic geohelminths and hookworm<sup>[3]</sup>. Most infections occur in developing countries. The high prevalence of soil–transmitted helminth infections in the tropics and subtropics is explained by climatic factors coupled with poor hygiene standards and the lack of adequate sanitation<sup>[4]</sup>. However, the increasing number of immigrants from high risk geographic areas for intestinal parasitic diseases (IPD) represents a major concern for public health<sup>[5,6]</sup>. In fact several epidemiological studies show the need to pay more attention to immigrants and travelers to endemic areas as potential carriers of major parasitic infections in the community<sup>[4,7,8]</sup>. Even if the low prevalence of the parasites among travelers demonstrated the significance of health education before travelling in order to avoid this kind of infection<sup>[9]</sup>. Considering that often no particular or specific correlations could be observed between clinical manifestations and pathogen agents, a specific etiologic diagnosis is crucial to establish a correct therapeutic strategy<sup>[10,11]</sup>.

The definitive diagnosis of a IPD rests on microscopic visualisation of eggs in stool. The ether–based concentration method is widely used for diagnosis of helminths and intestinal protozoa and has the advantage of fixing the eggs or cysts<sup>[12]</sup>. However, the ether–based concentration and others traditional technique have a low sensitivity, particularly in cases of low infection intensities. Recently, Raso et al. showed that the sensitivities of the ether–based concentration method and Kato–Katz technique were 58.6% and 77.8%, respectively<sup>[13]</sup>. Same results were observed in Malawi by Dacombe. The author showed that the comparison of 988 stool samples subjected to the Kato–Katz and ether concentration method revealed prevalences of 27.3% and 19.3%, respectively<sup>[14]</sup>. The FLOTAC technique is a new sensitive technique for the diagnosis of IPDs<sup>[2]</sup>. More recently, Utzinger et al. compared the diagnostic performance of three methods (Kato–Katz, ether concentration and FLOTAC techniques) in stool samples obtained from 102 schoolchildren in Côte d’Ivoire<sup>[15]</sup>. The authors showed that the hookworm prevalences as assessed by the FLOTAC, Kato–Katz and ether concentration techniques were 65.7%, 51.0% and 28.4%, respectively. Moreover, the FLOTAC technique showed a sensitivity of 88.2% compared with 68.4% for the Kato–Katz and 38.2% for the ether concentration techniques. This new technique does not require the extrapolation that is necessary when using other techniques, e.g. the Kato–Katz technique and the standard or modified McMaster technique<sup>[16]</sup>. In addition, since the FLOTAC technique examines a large amount of faeces (suspended in 10 mL), its analytic sensitivity is high and thus, it is less likely to give false negative results. This is very important because many types of research require highly reliable FECs; for example, studies of the efficacy of anthelmintics, clinical vaccine trials, studies of helminth resistance, and studies of host resistance. The technique has also been used for diagnosing helminths and protozoa in other domestic ruminants, as well as in wild ruminants, horses, rabbits, and pets<sup>[2,17–19]</sup>. There is a clear need for the standardization of FEC techniques. The accuracy of the FEC in terms of how well the observed values agree with the true values depends on three main factors:

1) the analytic sensitivity in terms of the maximum volume of the instrument (slide, apparatus, etc.); 2) the choice of the flotation solution, since solutions vary with respect to density, capability to float parasitic elements and debris, negative impact on parasitic elements, production of bubbles, and clarity; and 3) the care with which the procedures (weighing, dilution, filtration, loading, etc.) are carried out on the faecal sample. Due to the high analytic sensitivity and reliability of the FLOTAC technique, it is a candidate for the standard FEC technique.

### Conflict of interest statement

We declare that we have no conflict of interest.

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### Comments

#### Background

Helminths are estimated to infect roughly half of the human population worldwide and represents a major concern in terms of public health. Immigrants from endemic areas are increasing in developed countries and to screen them for helminth infection, also in the absence of symptoms, is advisable. More sensitive methods might contribute to reduce the burden of disease.

#### Research frontiers

The paper reports on the comparison of a new technique with the traditional method for the detection of parasites in stool samples, showing a higher sensitivity of the new method, which might represent a turning point in the diagnosis of parasitic infections.

#### Related reports

Ether-concentration methods are used worldwide for the diagnosis of parasitic infection but have a low sensitivity.

#### Innovations & breakthroughs

The paper reports on a new method for the diagnosis of parasitic infections which resulted more sensitive than traditional method.

#### Applications

Helminths are a major public health concern especially in developing countries and a cheap, more sensitive method for diagnosis has a huge potential of application.

#### Peer review

This is a valuable and original research that might contribute to improve the diagnosis of a largely widespread infection.

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