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Diagnosis of Intestinal Parasitoses: Comparison of Two Commercial Methods for Faecal Concentration Using a Polyparasitized Artificial Liquid Stool

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Abstract

As conventional faecal concentration methods for parasite detection may carry health risks due to the toxicity of the solvents used (ether, acetyl-acetate), we compared parasite recovery obtained with the new solvent-free EasyPara® kit (Servisol, Meudon, France), which consists of a single-use tube containing a porosity gradient filter (200 to 400 µm), and that obtained with the Para-Selles® system (Fumouze Diagnostics/Sofibel, Levallois-Perret, France), an ethyl-acetate solvent sedimentation method routinely used in our laboratory.

Both kits were used as recommended by their manufacturers. Both kits concentrate parasites in a pellet, which is suspended for microscopic examination. Parasites were identified on the basis of their morphology. The numbers of parasites recovered in the total pellets were compared between the kits. To compare the detection thresholds of the kits, we tested a liquid polyparasitized stool sample prepared by pooling clinical parasitized stool samples (protozoa and helminths) from parasitized patients consulting our hospital and stored in our collection and diluting it with saline solution. Using the liquid polyparasitized stool sample, the recovery concentrations for *Entamoeba histolytica*/*dispar*, *Entamoeba coli* and *Angystrongyloides stercoralis* larvae were significantly different with the two kits but no difference was observed for *Giardi intestinalis* cysts and *Ascaris*, tapeworm egg detection.

Parasite recovery was better with the EasyPara® kit than with Para-Selles®, probably owing to the presence of an original porosity gradient filter.

Keywords: Intestinal parasitic infections; Diagnosis; Faecal concentration method; Helminth infections; Protozoan infections.

Introduction

With the increase in intercontinental travel and immigration, intestinal parasitoses are a growing public health concern in industrialized countries. Clinical manifestations can range from asymptomatic carriage to severe diarrhoea and abdominal pain. Light microscopy is the principal method used to confirm clinical diagnoses, by demonstrating the presence of cysts, trophozoites stages (protozoa), eggs or larvae (helminths) in faecal samples. However, microscopy is subject to a high rate of false-negative results, owing mainly to low parasite densities. Faecal concentration methods, based on flotation or sedimentation of parasitic elements, can help to overcome this limitation. Flotation techniques are used to bring parasitic elements to the surface of a faecal suspension, from whence they can be transferred to a microscope slide for direct examination. One limitation of this method is that nematode eggs do not float on standard flotation media and are thus better concentrated by means of sedimentation. Sedimentation techniques make parasitic elements concentrate at the bottom of the faecal sample, and are the most widely used. In many clinical parasitology laboratories, formol (formalin)-ether or formol-ethyl acetate sedimentation is the preferred method for the detection of helminth eggs and protozoan cysts in fresh and preserved faecal samples. Unfortunately, the use of formal-ether poses a chemical risk to laboratory staff: ether, used to extract debris and fat from faeces, is highly inflammable and is no longer allowed in many diagnostic laboratories, being replaced by ethyl acetate.

In our laboratory we routinely use a commercial sedimentation technique for faecal concentration (Para-Selles® kit, Fumouze, Levallois, France). Like most sedimentation techniques, this kit involves several steps, including centrifugation. We chose this kit for its operator safety, it does not use formalin, and ethyl acetate replaces ether in the detergent solution. Briefly, parasite concentration is achieved by preparing two non-miscible phases, the first aqueous (aceto-acetic buffer pH 5) and the second organic (ethyl acetate) [1]. A new sedimentation kit for parasite concentration in faeces was recently marketed (EasyPara®, Servibio, Meudon, France). This kit differs from Para-Selles® in two main respects: it uses a filter with a porosity gradient, and does not use a solvent (no ether or ethyl acetate).

Here we compared the performance of Para-Selles® and EasyPara® for the detection of parasite elements in human stools.

Materials and Methods

The study took place in the Parasitology and Mycology Laboratory of Cochin Hospital (France) in 2012. Our laboratory notably serves a travel medicine consultation and an infectious diseases ward. Over the past 10 years we have built up a permanent collection of clinical stool samples containing parasite cysts, eggs and larvae, preserved in

formalin and stored at +4°C. Samples were collected for the collection from patients' stools containing high parasite densities with typical microscopic morphologies. The collection is used as a reference material for training students and laboratory staff in microscopic identification.

Samples

- Polyparasitized artificial liquid stool.

Six preserved parasitized clinical stool specimens stored in our collection were pooled to obtain a liquid stool sample containing protozoan and helminthic parasites.

As representative protozoan intestinal parasites we chose cysts of *Giardia intestinalis* (synonyms: *G. lamblia*, *G. intestinalis*) and cysts of *Entamoeba histolytica*/*dispar*, as they are the most common causes of parasitic diarrhoea. We also included *Entamoeba coli*, a non pathogenic amoeba, because its cysts can be confused with those of *Entamoeba histolytica*/*dispar*. As representative helminthic intestinal parasites, we chose eggs of *A. lumbricoides* and *Ancylostoma duodenale*, because they are frequent soil-transmitted nematodes. We also included eggs of *Taenia solium* and larvae of *Strongyloides stercoralis* in order to test the performance of the EasyPara® filter for large parasites (>100 µm for *S. stercoralis* and around 40 µm for *Taenia solium*).

The liquid stool sample was diluted ½, ¼, 1/10, 1/20, 1/100 and 1/400 in 0.9% sodium chloride solution. One milliliter (EasyPara®) or three milliliters (Para-Selles®) of each sample was tested in parallel with each kit after vortexing for at least 2 minutes. We noted results obtained with one specimen of each dilution tested.

The polyparasitized artificial sample was stored at +4°C.

Faecal concentration kits

Both concentrator kits were used as recommended by their manufacturers.

EasyPara®: EasyPara® consists of disposable, closed, single-use tubes with a built-in filter, as well as reagent solutions (aceto-acetate buffer pH 5 and 0.1% Triton-X). Each kit is composed of 3 parts: a mixing chamber, a porosity gradient filter (200 to 400 µm) and a sedimentation chamber (Figure 1). The device is assembled and sealed by screwing the filter thimble onto the sedimentation chamber and mixing chamber. Briefly, 1 mL of artificial stool or 1 g of fresh stool was mixed with 8 ml of aceto-acetate buffer solution in the mixing chamber. One drop of Triton-X was added to the mixture to solubilize fat particles. The filter was immediately sealed by screwing it onto the sedimentation chamber and then to the mixing chamber. The faecal suspension was vortexed for 15 seconds, and then the device was inverted and centrifuged for 3 min at 500 g. The supernatant was discarded and the pellet was used for microscopic examination (Figure 2).

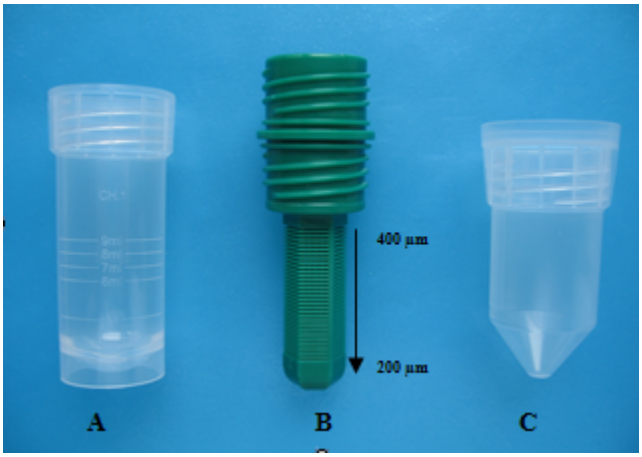

Figure 1: Easy-Para® faecal concentrator kit. A: Mixing chamber, B: Filter with porosity gradient and C: Sedimentation chamber.

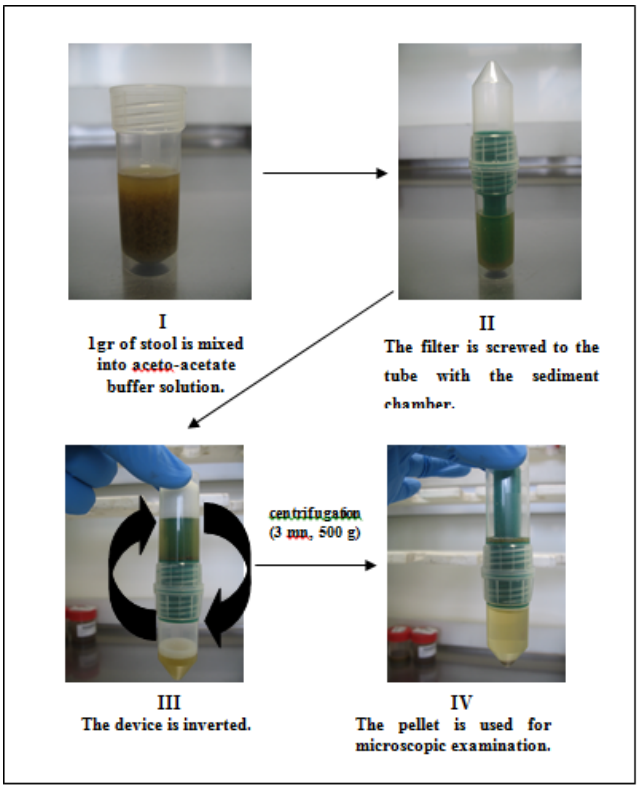

Figure 2: Easy-Para® faecal concentrator kit.

Para-Selles®: Para-Selles® consists of 2 conical plastic tubes (30 ml and 10 ml) and 2 solutions (aceto-acetic buffer and an ethyl acetate-based organic phase). Briefly, 3 mL of artificial stool or 3 g of fresh stool was mixed with 20 ml of aceto-acetate buffer in the 30-ml tube then vortexed for 15 seconds. After 3 minutes of sedimentation to eliminate coarse faecal debris, 5 ml of the supernatant was removed and placed in the 10-ml tube containing 3 ml of ethyl acetate. The tube was vortexed and centrifuged for 3 min at 500 g, then the supernatant

was discarded and the pellet was used for microscopic examination (Figure 3).

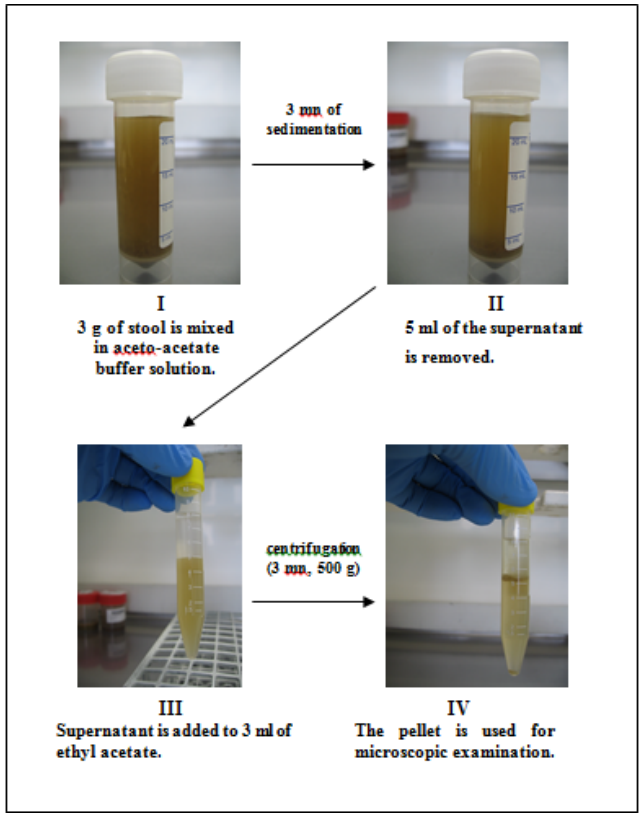

Figure 3: Para-Selles® faecal concentrator kit.

Study design

Between 1 and 3 drops of 0.9% sodium chloride solution were added to the pellets obtained with the kits, and the entire resulting suspension was deposited on slides (1 to 3 slides) depending on the volume of suspension) and coverslipped. The slides were observed under a microscope at 10X and 40X magnifications by a biologist with extensive experience in the morphological identification of intestinal parasites. The number of cysts, larvae and eggs observed in the entire suspension was recorded.

Statistical analysis

We applied paired Student's test to log-transformed values in order to compare the results obtained with the two kits on the polyparasitized artificial stool sample.

Results

As expected, the number of parasites detected in the concentrated samples fell in parallel with the dilution factor, from ½ to 1/400 (Tables 1A and 1B). The highest positive dilution (last positive dilution) for protozoa was around 1/100, and contained similar numbers of parasites with the two kits (7 cysts of *E. histolytica/dispar* with Para-Selles® and 9 with EasyPara®; 2 *E. coli* cysts detected with Para-Selles® and 3 with EasyPara®). The number of *G. intestinalis* cysts detected with EasyPara® was 3-fold higher than that detected with Para Selles® at dilutions ½, ¼ and 1/20, although the difference was not statistically significant but the number of *E. histolytica/dispar*, *E. coli* cysts detected with EasyPara® was significantly higher than that detected with Para Selles®. For helminths, the last positive dilutions for *A. lumbricoides*, *T. solium* and *S. stercoralis* were 1/10, 1/10 and 1/100, respectively with both kits. *S. stercoralis* larvae were about twice as numerous with EasyPara® than with Para-Selles® at dilutions ½, ¼ and 1/10 and the difference was significant. No significant difference between the kits was observed for *A. lumbricoides* or *T. solium*.

Concentration	Protozoa								
	Entamoeba. histolytica/dispar			*Entamoeba coli*			*Giardia intestinalis*		
	D	P.S	EP	D	P.S	EP	D	P.S	EP
1/2	23	70	111	10	20	76	11	11	45
1/4	20	55	85	11	19	35	4	9	31
1/10	13	41	62	6	18	38	4	10	26
1/20	2	9	19	1	3	9	0	0	7
1/100	0	7	9	0	2	3	0	1	0
1/400	0	1	2	0	0	0	0	0	0
Student's test	p	0.0009		p	0.01		p	0.11	
D: Direct Microscopy (before concentration); P.S: Para-Selles®; EP: EasyPara®.									

Table 1A: Liquid stool sample. Results of Para-Selles® and EasyPara® according to the dilution in normal saline. Number of parasites recorded for the whole concentration pellet.

Concentration	Helminths								
	Strongyloides stercoralis			*Ascaris lumbricoides*			*Taenia solium*		
	D	P.S	EP	D	P.S	EP	D	P.S	EP
1/2	13	31	61	0	0	3	1	2	3
1/4	8	13	21	0	1	1	0	2	3
1/10	5	9	22	0	2	1	0	3	2
1/20	0	3	10	0	0	0	0	0	1
1/100	0	1	2	0	0	0	0	0	0
1/400	0	0	0	0	0	0	0	0	0
Student's test	p	0.01		p	0.54		p	0.23	
D: Direct Microscopy (before concentration); P.S: Para-Selles®; EP: EasyPara®.									

Table 1B: Liquid stool sample. Results of EasyPara® according to the dilution in normal saline. Number of Helminths recorded for the whole concentration pellet.

Discussion

Faecal parasites are generally detected by direct microscopic examination after sample concentration. Most concentration methods

use the sedimentation principle and chemical reagents to dissolve faecal solids [6]. Ready-to-use commercial kits have been marketed in recent years [7], and have the advantage of limiting the use of toxic reagents (especially ethyl-acetate and ether) [8]. Here we compared the new EasyPara® kit with the older Para-Selles® kit that we have been using routinely in our laboratory for 10 years.

Kit	Method	Reagents	References
MidiParasep® (DiaSys Ltd, Workingham, England)	- Centrifugation 100 g/1 min or 500 g/5 min -425-µm pore diameter filter	Ethyl acetate, Triton X.	[3,5,9-11]
MidiParasep®SF (Solvent Free) (DiaSys Ltd, Workingham, England)	- Centrifugation 200 g/3 min - Two-stage filtration with a perforated fat-dispersion chamber and 425-µm pore diameter filter	Triton X.	[8]
TF-test® (ImmunoAssay, Irupeva, Brazil)	- Centrifugation 1500 rpm/ 1 min - Double filtration system with 2 filters of 600 and 220 µ pores diameter	Ethyl acetate	[2,4]
Paratest® (Diagnostek, Itu, Brazil)	- No centrifugation - One-step filtration with a 266-µm pore diameter filter	No	[1]

Table 2: Faecal parasite concentrator kits. Main characteristics and references.

Filter-based faecal parasite concentrators have been available for at least two decades, and differ notably by the filter pore size (Table 2). Several studies have shown that the performance of these kits depends on several parameters, including the type of filter, the volume of stool, and the centrifugation time [4,8,9,11,12]. Some authors have found that solvent-based kits are more efficient than those that do not use solvents [8,9] and those based on filtration [7,11].

With the artificial liquid stool sample, EasyPara® and Para-Selles® showed different performances. The performance of EasyPara® might be improved by sample dilution, which removes debris and facilitates parasite detection. In contrast, sample dilution could reduce the performance of Para-Selles® by reducing the parasite concentration, because the initial sedimentation step, designed to remove debris, might also remove some parasites (Table 1B).

One original feature of EasyPara® is its use of a porosity gradient filter (from 200 to 400 µm) and the absence of solvents, which avoids toxic exposure of laboratory staff. In conclusion, the results of this preliminary study suggest that EasyPara® is suitable for the detection of intestinal parasites in diarrhoeal stools. Further tests, using a broader spectrum of parasites (*Schistosoma*, flukes, etc), are needed to confirm the performance of this new kit.

Acknowledgements

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Conflict of interest

A. Paugam is member of ADERMEPT (Association pour le Développement de la Recherche en Médecine Parasitaire et Tropicale). ADERMEPT received funds from Servibio (Meudon, France) to test the kit and pay the publication costs.

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