Comparison of Para-Selles Bailenger/Kop-Color Fumouze, Para-Selles-Iodésine/Kop-Color II Fumouze diagnostic kits with conventional microscopic methods in identifying intestinal parasitic diseases in Senegal

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Abstract In the context of controlling intestinal parasites, accurate diagnosis is essential. Our objective was to evaluate the performance of new diagnostic kits compared to conventional microscopic methods in identifying intestinal parasites. Faeces collected in rural area in Senegal were subjected to several detection techniques. Thus, the sensitivity, specificity, positive and negative predictive values of new diagnostic techniques were compared to conventional merthiolate-iodine-formalin, conventional Bailenger and modified Ritchie. Furthermore, the kappa coefficient was calculated to evaluate the correlation between the new kit and those of modified Ritchie. Out of the 117 patients examined, 102 presented with a parasite, or prevalence of 87.1%. The Fumouze techniques proved to be as effective as the conventional methods in detecting flagellates and helminths with sensitivities ranging from 97 to 100%. However, conventional techniques were slightly more sensitive in identifying *Endolimax nana* and *Blastocystis hominis*. The correlation was nearly perfect ($k = 0.83$ and 1), respectively between Bailenger Fumouze, Iodesine Fumouze and modified Ritchie in identifying helminths while it was just acceptable ($k = 0.27$ and 0.28) in identifying *B. hominis*. The modified Ritchie technique routinely used in our laboratory remains a good diagnostic

tool. However, the use of kit techniques was interesting when reading the pellet after concentration and the Colour KOP staining was a considerable contribution to the diagnosis of the vegetative forms. Therefore, it would be interesting to determine the cost of a stool test using Fumouze kit techniques to provide the most cost effective way.

Keywords Bailenger Fumouze · Iodesine Fumouze · Merthiolate-iodine-formalin · Conventional Bailenger · Modified Ritchie

Introduction

Intestinal parasitic infections are a major public health problem in the world, especially in developing countries faced with overcrowding, natural disasters, lack of drinking water and inadequate sanitation infrastructure.

The World Health Organization (WHO) estimates the number of exposed subjects to be about 1.6 billion and 300 million the number of patients (OMS 2007). These parasitic diseases represent more than 40% of the disease burden attributable to tropical diseases worldwide, excluding malaria (Okyay et al. 2004; Alum et al. 2010). They have a considerable impact on the health of populations and constitute an obstacle to economic development. Apart from causing anaemia and malnutrition especially among children, intestinal infections have been associated to low educational performance of schoolchildren (Gelaw et al. 2013). In Senegal, intestinal parasitic diseases are endemic and remain a frequent reason for consultation with prevalence around 44% (Ndir et al. 2002; Faye et al. 2008). To cope with this situation, the Ministry of Health has implemented several control strategies including

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systematic deworming. This mass drug administration (MDA) with mebendazole coupled with vitamin A is carried out in advanced strategy among children less than 5 years of age in all the districts of the country. Thus, intestinal parasitic diseases are becoming increasingly rare and difficult to detect.

Diagnosing these parasitic diseases is mainly based on microscopic stool examination. The latter involves several steps including direct examination to identify, among others, vegetative forms followed by a concentration step to possibly detect parasites in the case low infestation (Truant et al. 1981). However, the process of detecting intestinal parasitic diseases requires a lot of time as well as skilled personnel, thus making it difficult to achieve under certain conditions.

In order to facilitate the diagnosis of these parasitic diseases to improve the management of cases, Fumouze laboratories have developed a kit for a better time saving and better standardization to perform a stool examination. This would obviously be a significant benefit for biology laboratories, clinicians and especially patients. It is in this context that this study was carried out, aiming to evaluate the performance of the Fumouze kit compared to conventional microscopic methods in identifying intestinal parasitic diseases.

Materials and methods

Study area and population

Our study was conducted in a Koranic school attended by about 130 children supervised by a dozen teachers in the village of Bentégné, located in the rural community of Diass, about 60 km from the capital city, Dakar. This was a descriptive cross-sectional prospective study from the period 23 to 30 November, 2012. The study population was represented by all children aged 0–15 years attending Koranic school as well as adults found on site.

Collection of socio-demographic data

A questionnaire was developed and allowed us to collect information for each individual sampled. It included, an identification number, the surname and name of the individual, sex, age, symptomatology, and type of water consumed. All information collected was also recorded in registers made for the occasion. Different participants or their guardians (in the case of children) should first answer the questionnaire before providing their stool.

Collection of stool samples

Faeces were collected at the Koranic school in appropriate sealed, labelled and clean pots. After direct examination carried out on site, the samples were stored at 4 °C in coolers for transport to the Laboratory of Parasitology of the *Centre Hospitalier National Universitaire (CHNU) de Fann*. The stools that were not examined directly due to lack of time were to be kept in a merthiolate-iodine-formalin (MIF) staining solution.

Parasitological stool examination

All stool samples received at the laboratory were examined macroscopically and microscopically. The stools that were examined directly at the school were directly concentrated while those stored in the MIF solution were first examined between slide and slip cover prior to the concentration stage. The following steps were followed.

Direct examination

Direct examination in a fresh state is performed by depositing on an object-carrying slide, a drop of physiological water (salted to 9‰) mixed with a fragment of stool collected at several locations of the sample.

Direct examination after staining All stools examined in a fresh state were then stained with traditional Lugol and Kop-Colour.

Kop-Colour is a method for differential staining of parasitic elements using a mixture of colouring agents including Lugol. Its use facilitates the detection of parasitic elements that appear to be yellow, orange-yellow or yellow-brown on more or less dark blue background. It involves mixing on a object-carrying slide 10 µL of Kop-Colour II with a drop or 25 µL of a stool suspension in physiological water. The observation is made using a microscope with a 10× and 40× objective.

Microscopic examination after concentration

All stool samples were concentrated using the five techniques, i.e. the conventional methods (conventional MIF, conventional Baileys and modified Ritchie) and those of the kit which included a Para-Selles Baileys/Kop-Colour and a Para-Selles-Iodine/Kop-Color II.

Para-Selles Baileys/Kop-Color Fumouze This is a two-phase concentration method using ether as the organic solvent and pH 5 aceto-acetic buffer solution as dilution liquid. It involves mixing 5 mL of the supernatant of the stool in the preparation of the aceto-acetic buffer with 3 mL of ether. The examination of the pellet is performed

after staining with 10 μ L of kop-Color II deposited on the blade.

Para-Selles-Iodesine/Kop-Color II Fumouze This is a two-phase concentration method using ether as an organic solvent and an Iodesine solution as dilution liquid. The Iodesine concentration technique replaces that of the MIF concentration. The basis for Iodesine is a mixture of ethanol (<25%) and acetone (<20%). The basis for Iodesine and Kop-Color II contains neither formaldehyde, nor mercury derivative, in order to improve safety at the workplace. It consists of a very small portion of stool (3–4 g or 3–4 mL if the stool is liquid) in the Iodesine solution already prepared by mixing 200 μ L of Lugol and 20 mL of Iodesine Base. Subsequently, 3 mL of ether were added to the 5 mL of the supernatant of the preparation. After centrifugation, the pellet was directly read microscopically.

Conventional Bailerger It is a two-phase concentration technique. The dilution liquid is represented by the Bailerger solution containing acetic acid buffer at pH 5.

Conventional MIF It is a two-phase concentration technique. The liquid dilution is the MIF solution temporarily prepared MIF.

Modified Ritchie (formalin-ether concentration technique) This is a two-phase concentration technique. The dilution liquid is a 10% formalin solution.

Entry and data analysis

The data were entered into ExcelTM and analyzed with the TM R2.15.0 software (R Foundation for Statistical Computing, Vienna, Austria). Qualitative variables were described in terms of numbers, percentage of data provided and confidence intervals calculated at 95%. For calculating prevalence, the positive identification of the species by at least one of the techniques was considered as a positive result. Statistical comparisons were made using the Chi square test or Fisher test depending on the conditions of applicability. The test was considered as significant if p was less than 0.05.

For evaluating performance techniques in the detection of intestinal parasitic diseases, the sensitivity, specificity, positive and negative predictive values of each method were calculated. They were expressed as percentages with confidence intervals at 95%. In the absence of a standardized reference method, the results obtained after the combination of several techniques both on direct examination and examination after concentration was considered as a reference. The kappa coefficient to assess the level of correlation between the Fumouze kit and modified Ritchie techniques routinely used in our laboratory was also calculated. The kappa measure was interpreted as follows: $k = 0.21$ – 0.40 ,

acceptable correlation; $k = 0.41$ – 0.60 , moderate correlation; $k = 0.61$ – 0.80 , significant correlation; and $k = 0.81$ – 1 , almost perfect correlation (Glinz et al. 2010; Becker et al. 2011).

Ethical considerations

The legal representatives of the Daara children involved in this study were informed about the use of their pathological material collected as part of their infection assessment and they all gave an oral approval.

Results

Socio-demographic and clinical characteristics of the patients

Overall, 117 samples were collected and examined in the laboratory. The age of our study population ranged from 2 to 67 years with a mean age of 11.49 ± 10.9 years. Children aged 5–10 years were more representative (43.6%). The sex ratio was 1.16 with 53.8% of male participants and 46.2% of female. In terms of water consumption, almost all the participants (92.3%) drank well water.

Clinically, the most frequently observed symptoms were represented by diarrhoea (34.2%) and abdominal pain (26.5%). It should however be noted that 11.1% of individuals had not shown any symptoms.

Prevalence of intestinal parasites

Out of the 117 patients tested, 102 presented with a parasite through at least one of the various techniques performed, thus giving intestinal parasite prevalence at 87.1%. Of the 102 patients with parasites, 75 (73.5%) were identified as infected with at least two species of parasites. *Blastocystis hominis* was the most frequently isolated species with an overall prevalence of 58.1% followed by *Entamoeba coli* (54.7%), *Endolimax nana* (35.9%) and *Giardia intestinalis* (34.2%). Among pathogenic protozoa, *G. intestinalis* was the most frequent species followed by *Entamoeba histolytica/dispar* (8.5%) and *Trichomonas intestinalis* (2.6%). Other non-pathogenic protozoa found were present in relatively low proportions. These are especially *Dientamoeba fragilis* (7.7%), *Entamoeba hartmanni* (6.8%), *Chilomastix mesnili* (6%), *Pseudolimax butschlii* (2.6%) and *Enteromonas intestinalis* (0.9%). Conversely, helminths were scarcely found in stool with *Trichuris trichiura* which was the most isolated species with a prevalence of 2.6%, followed by *Ascaris lumbricoides* and *Hymenolepis nana* which both 1.7% (Fig. 1).

Fig. 1 Distribution of parasitic species

Comparison of diagnostic methods

Direct examination with physiological water and after Lugol staining presented high sensitivity rate in identifying intestinal protozoa as shown in Table 1. Indeed, direct sensitivities to direct examination with physiological water and Lugol were 100% (95% CI 100–100) in identifying *E. nana* and *G. intestinalis*, 93.3% (95% CI 80.7–100) and 100% (95% CI 100–100) in identifying *B. hominis*. While the percentages for the KOP colour examination were 93.3% (95% CI 84.4–100), 92.9% (95% CI 85.1–100) and 86.7% (95% CI 69.5–100), respectively in identifying *E. nana*, *G. intestinalis* and *B. hominis*. Specificities as well as positive and negative predictive values ranged from one technique to another (Table 1).

In the diagnosis of helminths, all techniques proved to be very sensitive with percentages of 100% in identifying *A. lumbricoides* and *T. trichiura*. They were also very specific with the exception of the KOP colour in

identifying *A. lumbricoides* which showed specificity at 50% (95% CI 0–100) (Table 2).

After concentration, the microscopic examination of the preparation was used to compare the amount of debris observed and the morphology of parasites identified for each technique. No significant difference was observed about the amount of debris between the techniques as shown in Table 3 ($p = 0.60$). In terms of morphology, the parasites were more altered with the kit techniques compared to conventional methods. Indeed, it was observed that 11.6% of parasites were altered with Bailenger Fumouze and 21.4% with the Iodesine Fumouze technique as against 7, 13.7 and 11.9%, respectively, with the conventional Bailenger, conventional MIF and modified Ritchie. The differences observed were significant with $p = 0.009$ (Table 3).

In the comparison of concentration methods, the Fumouze kit techniques proved to be as effective as the conventional methods in detecting intestinal protozoa especially flagellates and helminths with sensitivities

Table 1 Performance of direct techniques in identifying intestinal protozoa

Species	Direct examination	Sensitivity	Specificity	PPV	NPV
<i>Endolimax nana</i>	Water	100% [100–100]	95.7% [87.3–100]	96.9% [90.7–99.1]	100% [100–100]
	KOP color	93.3% [84.4–100]	95.2% [86.1–100]	96.5% [90.1–98.9]	90.9% [83–95.4]
	Lugol	100% [100–100]	94.4% [83.9–100]	96.5% [90.1–98.9]	100% [100–100]
<i>Giardia intestinalis</i>	Water	100% [100–100]	77.8% [50.6–100]	95.7% [89.1–98.5]	100% [100–100]
	KOP color	92.9% [85.1–100]	100% [100–100]	100% [100–100]	75% [50.5–99.5]
	Lugol	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
<i>Blastocystis hominis</i>	Water	93.3% [80.7–100]	94.9% [87.9–100]	87.5% [79–93]	97.4% [91.4–99.4]
	KOP color	86.7% [69.5–100]	100% [100–100]	100% [100–100]	94.7% [87.7–97.9]
	Lugol	100% [100–100]	97.1% [91.4–100]	92.3% [84.7–96.4]	100% [100–100]

PPV positive predictive value, NPV negative predictive value

Table 2 Performance of direct techniques in identifying helminths

Species	Direct examination	Sensitivity	Specificity	PPV	NPV
<i>Ascaris lumbricoides</i>	Water	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
	KOP color	100% [100–100]	50% [0–100]	98% [94.1–100]	100% [100–100]
	Lugol	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
<i>Trichuris trichiura</i>	Water	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
	KOP color	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
	Lugol	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]

PPV positive predictive value, NPV negative predictive value

Table 3 Comparison of quality of preparations after concentration

	Aspect of debris N = 117			
	Little		Substantial	
	Number	(%)	Number	(%)
Bailenger Fumouze	102	87.1	15	12.9
Iodesine Fumouze	103	88	14	12
Conventional Bailenger	103	88	14	12
Conventional MIF	97	82.9	20	17.1
Modified Ritchie	97	82.9	20	17.1
<i>p</i> value	0.55		0.60	
	Morphology of parasites			
	Normal		Altered	
	Number	(%)	Number	(%)
Bailenger Fumouze N = 171	151	88	20	11.6
Iodésine Fumouze N = 149	117	78.5	32	21.4
Conventional Bailenger N = 141	131	92.9	10	7
Conventional MIF N = 132	114	86.3	18	13.7
Modified Ritchie N = 118	104	88.1	14	11.9
<i>p</i> value	0.016		0.009	

MIF merthiolate-iodine-formalin

ranging from 97 to 100%. However, some differences have been observed in terms of sensitivity and specificity in the identification of *E. nana* and *B. hominis* as shown in Tables 4 and 5.

Table 6 summarizes the level of correlation between the Fumouze Kit techniques and the modified Ritchie method routinely used in our laboratory. In identifying helminths as *A. lumbricoides*, the correlation between the Bailenger Fumouze and modified Ritchie method was high ($k = 0.66$), whereas it was almost perfect ($k = 1$) between the Iodesine Fumouze and the modified Ritchie methods. The same goes for the diagnosis of such flagellates as *G. intestinalis* where the comparison showed an almost perfect correlation ($k = 0.83$) between the Fumouze kit

techniques and those of the modified Ritchie. However, the correlation between these different techniques was just acceptable in identifying protozoa as *E. nana* ($k = 0.37$ for Bailenger Fumouze vs modified Ritchie and $k = 0.40$ for Iodesine Fumouze vs modified Ritchie) and *B. hominis* ($k = 0.28$ for Bailenger Fumouze vs modified Ritchie and $k = 0.27$ for Iodesine Fumouze vs modified Ritchie).

Discussion

Intestinal parasitic diseases have a significant impact on populations and constitute an obstacle to socio-economic development in our regions. Thus, accurate diagnosis is

Table 4 Performance of concentration techniques in identifying intestinal protozoa

Species	Techniques	Sensitivity	Specificity	PPV	NPV
<i>Endolimax nana</i>	Bailenger Fumouze	88.8% [82.5–95]	89.5% [75.7–100]	97.7% [91.8–99.5]	60.7% [50.4–70.1]
	Iodesine Fumouze	92.9% [87.8–98]	84.2% [67.8–100]	96.8% [90.5–99.1]	69.6% [59.4–78.1]
	Conventional Bailenger	94.9% [90.5–99.3]	84.2% [67.8–100]	96.8% [90.5–99.1]	76.2% [66.4–83.9]
	Conventional MIF	99% [97–100]	89.5% [75.7–100]	98% [94.1–100]	94.5% [87.5–97.8]
	Modified Ritchie	94.9% [90.5–99.3]	63.2% [41.5–84.8]	93% [85.6–96.8]	70.6% [60.5–79.1]
<i>Giardia intestinalis</i>	Bailenger Fumouze	100% [100–100]	97.2% [91.9–100]	98.7% [93.2–99.8]	100% [100–100]
	Iodesine Fumouze	98.8% [96.4–100]	94.4% [87–100]	97.5% [91.5–99.4]	97.1% [90.9–99.2]
	Conventional Bailenger	98.8% [96.4–100]	97.2% [91.9–100]	98.8% [96.4–100]	97.2% [91.9–100]
	Conventional MIF	100% [100–100]	91.7% [82.6–100]	96.4% [90–98.9]	100% [100–100]
	Modified Ritchie	97.5% [94.2–100]	86.1% [74.8–97.4]	94% [86.8–97.5]	93.9% [86.7–97.4]
<i>Blastocystis hominis</i>	Bailenger Fumouze	82.9% [75.6–90.1]	75% [50.5–99.5]	96.7% [90.4–99.1]	33.3% [24.3–43.5]
	Iodesine Fumouze	88.6% [82.5–94.7]	83.3% [62.2–100]	97.9% [92.1–99.6]	45.4% [35.5–55.6]
	Conventional Bailenger	97.1% [94–100]	75% [50.5–99.5]	97.1% [94–100]	75% [50.5–99.5]
	Conventional MIF	98.1% [95.5–100]	75% [50.5–99.5]	97.1% [94–100]	81.8% [72.5–88.5]
	Modified Ritchie	100% [100–100]	58.3% [30.4–86.2]	95.4% [88.6–98.3]	100% [100–100]

PPV positive predictive value, NPV negative predictive value, MIF merthiolate-iodine-formalin

Table 5 Performance of concentration techniques in identifying helminths

Species	Techniques	Sensitivity	Specificity	PPV	NPV
<i>Ascaris lumbricoides</i>	Bailenger Fumouze	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
	Iodesine Fumouze	100% [100–100]	50% [0–100]	99.1% [97.4–100]	100% [100–100]
	Conventional Bailenger	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
	Conventional MIF	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
	Modified Ritchie	100% [100–100]	50% [0–100]	99.1% [97.4–100]	100% [100–100]
<i>Trichuris trichiura</i>	Bailenger Fumouze	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
	Iodesine Fumouze	100% [100–100]	50% [0–100]	99.1% [97.4–100]	100% [100–100]
	Conventional Bailenger	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
	Conventional MIF	100% [100–100]	100% [100–100]	100% [100–100]	100% [100–100]
	Modified Ritchie	100% [100–100]	50% [0–100]	99.1% [97.4–100]	100% [100–100]

PPV positive predictive value, NPV negative predictive value, MIF merthiolate-iodine-formalin

essential for patient management and to guide the design, implementation and monitoring of programmes for community control of infectious diseases. This study was conducted to compare the efficacy of new kits in detecting intestinal parasites in the faeces with conventional techniques.

This study conducted in rural areas has shown that children living in the area and particularly those attending Koranic schools are highly parasitized. Indeed, the overall prevalence (87.1%) noted in this study is very high. Our results are quite similar to those obtained by Dancesco et al. (2005) who reported a prevalence of intestinal parasites at 84.8% in a village located in Ivory Coast in school and preschool-aged children. However, these

figures are more important than those described by several authors who have reported prevalence rates ranging between 30 and 50% in the same target (Diouf et al. 2000; Buchy 2003). Indeed, Fall in 2006 (Fall et al. Unpublished data) and Faye et al. (2008) had found during cross-sectional surveys in rural and peri-urban areas in Senegal prevalence rates of 36.44 and 44.6%, respectively. The same trends were observed in the rest of the continent with the example of Hamit et al. (2008) in Chad and Cisse et al. (2011) in Burkina who reported a prevalence of around 50%. Therefore, the results obtained in this study show the persistence of these tropical diseases in Koranic schools as authors had reported 20 years ago prevalence rates at 84.09% in a Koranic school in

Table 6 Correlation between the Kit Fumouze techniques and those of modified Ritchie

Species	Technique	Results			Kappa coefficient	P value
		Modified Ritchie				
		Positive	Negative			
<i>Endolimax nana</i>	Bailenger Fumouze	Positive	11	17	0.37	0.99
		Negative	6	83		
	Iodesine Fumouze	Positive	10	13	0.4	0.98
		Negative	7	87		
<i>Blastocystis hominis</i>	Bailenger Fumouze	Positive	6	21	0.28	1
		Negative	1	89		
	Iodesine Fumouze	Positive	5	17	0.27	1
		Negative	2	93		
<i>Giardia intestinalis</i>	Bailenger Fumouze	Positive	30	5	0.83	0.004
		Negative	3	79		
	Iodesine Fumouze	Positive	30	5	0.83	0.004
		Negative	3	79		
<i>Ascaris lumbricoides</i>	Bailenger Fumouze	Positive	1	1	0.66	0.42
		Negative	0	115		
	Iodesine Fumouze	Positive	1	0	1	0.20
		Negative	0	116		

Dakar (Kane A. Thesis for a doctorate in pharmacy, unpublished data).

Parasites isolated through this study were essentially dominated by intestinal protozoa. Compared to previous studies in Senegal (Dieng et al. 1999; Ndir et al. 2002; Faye et al. 2008), a significant decrease or even a scarcity of helminths among children with predominantly intestinal protozoa was noted. Regular administration, twice a year (June and December) of mebendazole to children could explain the scarcity of helminths portage in the study population.

However, while providing effective protection against helminths, mebendazole is ineffective against protozoa and therefore intestinal protozoa remained highly prevalent in the study area. It is therefore important to refocus the mass deworming strategy. Indeed, the replacement of mebendazole with albendazole, which would act against both protozoa and helminths, could make the strategy more efficient and operative, by targeting both the two classes of parasitic diseases.

In order to facilitate the diagnosis of these parasitic diseases, this study was conducted to test a new tool for detecting intestinal parasites. Our main objective was to evaluate the performance of the Fumouze kit techniques

compared to conventional microscopic methods in identifying intestinal parasites. Examination of the preparation after concentration didn't show any significant difference between the techniques about the amount of the faecal debris. However, the density of this debris in the pellet prevented us from fully reading when using the Ritchie technique and conventional MIF technique than the Fumouze kit techniques. This observation could be related to the presence of formaldehyde in these two methods, unlike the techniques of kits using either an acetoacetic buffer or an Iodesine solution as dilution liquid. American authors had already revealed several years ago that the modified Ritchie technique had among others the disadvantage of having a concentration of granular materials which tended to densify the base (Truant et al. 1981). Using a concentration technique that facilitates the reading of the pellet may therefore significantly improve the identification of eggs and cysts especially when the parasite burden is low.

Morphologically, it was noted that the parasites examined were more frequently altered with the Fumouze kit techniques. Indeed, the Bailenger and MIF Fumouze techniques were more likely to alter or even detach altogether the wall of the observed species. Such a situation

may be problematic because the microscopist may easily misdiagnose. This could therefore explain the low sensitivity of a kit in identifying some parasites whose cysts would probably not have been correctly identified. The detachment of the wall of parasites may therefore be a consequence of the chemical constituents of the kit that might be aggressive on some eggs or cysts. This assumption had already been made by some authors who had suspected the role of chemical constituents of Flotac 400 in the deformation of cysts when compared to other microscopic methods, namely the modified Ritchie and kato katz methods (Glinz et al. 2010; Becker et al. 2011). However, it is still premature to issue an opinion on the true role of chemical constituents of the Fumouze kit in the deformation of cysts. Further studies should be carried out in order to yield better information on this matter.

However, when the parasite was intact, the very beautiful colour of the constituents of the kit allowed us to identify the parasite easily. Unfortunately, we were not able to compare our observations because very few data are currently available in the literature.

Overall, 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. Direct examination with physiological water and after Lugol staining proved to be more sensitive in identifying intestinal protozoa compared to direct examination after staining with KOP colour. This is probably due to the alteration of the wall of some cysts by one of the KOP colour components that prevented identification at times. As for helminths, we did not observe any differences in the sensitivity of both staining techniques. One of the reasons could be the very low number of helminths found in the stools.

Kop-Color Fumouze is a method of differential staining of parasitic elements using a mixture of colouring agents including Lugol. Its use facilitates the detection of parasitic elements that appear to be yellow, orange-yellow or yellow-brown on a more or less dark blue background.

After concentration, the performance of the different techniques varied depending on the parasite identified but there were no major differences. Moreover, the calculation of the kappa coefficient in comparing the Fumouze kit techniques and the Ritchie method used routinely in our laboratory had shown very good correlation in identifying flagellates and helminths and an acceptable correlation in identifying rhizopods and *Blastocystis*.

In view of our results, it seems that the modified Ritchie technique routinely used in our laboratory remains a good diagnostic tool for intestinal parasites despite the importance of the amount of debris obtained after concentration which at times interfere with reading. However, it should be noted that the use of kit technique was very interesting

when reading the pellet after concentration but especially that the KOP Colour staining was a considerable contribution to the diagnosis of vegetative forms.

At the end of this study, it seems that certain parameters must be considered in choosing a routine technique aside performance. In particular, this relates to cost. Indeed, the use of the modified Ritchie technique allows for a very affordable cost of a stool test. Therefore, it would be particularly interesting to determine the cost of a stool test using Fumouze kit techniques to provide the most cost effective way.

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References

- Alum A, Rubino JR, Ijaz MK (2010) The global war against intestinal parasites—should we use a holistic approach? *Int J Infect Dis* 14(9):732–738
- Becker SL, Lohourignon LK, Speich B, Rinaldi L, Knopp S, N'goran EK, Cringoli G, Utzinger J (2011) Comparison of the Flotac-400 dual technique and the formalin-ether concentration technique for diagnosis of human intestinal protozoan infection. *J Clin Microbiol* 49(6):2183–2190
- Buchy P (2003) Les parasitoses digestives dans la région de Mahajanga, côte Ouest de Madagascar. *Bull Soc Pathol Exot* 96(1):41–45
- Cisse M, Coulibaly SO, Guiguemde RT (2011) Aspects épidémiologiques des parasitoses intestinales rapportées au Burkina Faso de 1997 à 2007. *Med Trop* 71:257–260
- Dancesco P, Abeu J, Akakpo C, Iamandi I, Kacou E, Quenou E, Keusse-Assi J (2005) Intestinal parasitoses in a village of Côte d'Ivoire. I: control and prevention plan. *Sante* 15(1):5–10
- Dieng Y, Tandia AA, Wane AT, Gaye O, Diop EH, Diallo S (1999) Les parasitoses intestinales chez des habitants d'une zone péri-urbaine à nappe phréatique polluée par les nitrates d'origine fécale (Yeumbeul, Sénégal). *Sante* 9(6):351–356
- Diouf S, Diallo A, Camara B, Diagne I (2000) Parasitoses intestinales de l'enfant en zone rurale sénégalaise (Khombole). *Médecine d'Afrique Noire* 47(5):229–232
- Faye B, Ndiaye JL, Tine RC, Lo AC, Gaye O (2008) Interaction paludisme et helminthiases intestinales au Senegal: influence du portage de parasites intestinaux sur l'intensité de l'infection plasmodiale. *Bull Soc Pathol Exot* 101(5):391–394
- Gelaw A, Anagaw B, Nigussie B, Silesh B, Yirga A, Alem M, Endris M, Gelaw B (2013) Prevalence of intestinal parasitic infections and risk factors among schoolchildren at the University of Gondar Community School, Northwest Ethiopia: a cross-sectional study. *BMC Public Health* 13(13):304
- Glinz D, Silue D, Knopp S, Lohourignon K, Kouassi P, Steinmann P, Giuseppe C, Eliezer KN, Utzinger J (2010) Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. *Plos Negl Trop Dis* 4(7):e754
- Hamit MA, Tidjani MT, Bilong Bilong CF (2008) Recent data on the prevalence of intestinal parasites in N'Djamena, Chad Republic. *Afr J Environ Sci Technol* 2(12):407–411
- Ndir I, Gaye A, Sy M, Gaye O, Ndir O (2002) Prevalence of intestinal parasitosis at the King Baudouin health center of Guediawaye (Senegal). *Dakar Med* 47(2):168–171

- Okay P, Ertug S, Gultekin B, Onen O, Beser E (2004) Intestinal parasites prevalence and related factors in school children, a western city sample—Turkey. *BMC Public Health* 22(4):64
- OMS (2007) Rapport de la première réunion du Groupe consultatif scientifique et technique de l'OMS sur les maladies tropicales négligées. OMS, Genève, p 12
- Truant AL, Elliott SH, Kelly MT, Smith JH (1981) Comparison of formalin-ethyl ether sedimentation, formalin-ethyl acetate sedimentation, and zinc sulfate flotation techniques for detection of intestinal parasites. *J Clin Microbiol* 13(5):882–884