International Scholars Journals

African Journal of Parasitology Research ISSN 2343-6549 Vol. 4 (6), pp. 227-233, June, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Assessment of diagnostic performances of parasitological stool examinations performed in public sector medical laboratories of Abidjan (Côte d'Ivoire)

Konaté A[1*], Kiki-Barro PCM[1], Kassi KF[1,3], Djohan V[1], Angora KE[1], Bosson-Vanga H[1], Doukouré CY[1], Yavo W[1] and Menan EIH[1,3]

[1]Department of Parasitology, Mycology and Zoology, Félix Houphouët-Boigny University, BPV 34, Abidjan, Côte d'Ivoire.
[2]Laboratory of Parasitology and Mycology, Yopougon Teaching Hospital, 21 BP 632 Abidjan 21, Côte d'Ivoire.
[3]Laboratory of Parasitology and Mycology, Center for Diagnosis and Research on AIDS and other infectious diseases, 01 BPV 13, Abidjan, Côte d'Ivoire.

Accepted 08 June, 2017

Parasitological stool examinations allows to identify intestinal parasites in order to achieve a better management. Their importance implies the need to ensure high reliability and to seek perfection in their performance. We therefore carried out this study in order to update data on the diagnostic performance of public sector medical laboratories of Abidjan with regard to the research and identification of helminths and protozoa contained in stools. Our study was led from April to November 2011. Each participating laboratory received a survey sheet for the collection of information and two suspensions of stools to be analyzed. Our results showed a participation rate of 78.4 %. The responsibility for laboratories was entrusted to technicians and engineers in 62.1 % of cases. The written operating procedures were absent in 34.5 % of cases. We obtained for a correct identification estimated at 10.3 % and 89.7 % respectively for samples No.1 and No.2. *Strongyloides Stercoralis* and *Entamoeba coli* were the most recognized (69%). Confusion was noted between the various species of parasites in both suspensions. This study shows the importance of the application of existing texts on quality, in particular on the external quality assessment which must be compulsory at the national level.

Keywords: Quality control, intestinal parasites, medical laboratory.

INTRODUCTION

Parasitic infections caused by helminths and protozoa are the major causes of human diseases in most tropical countries (G/hiwot Y et *al.,* 2014). The number of cases of intestinal parasitic infections is estimated at 3.5 billion worldwide with 450 million cases of morbidity (Keiser and Utzinger, 2010; Brooker et *al.,* 2009) and children are most affected (Brooker et *al.,* 2009). Effective management of these diseases requires prompt and accurate biological diagnosis through the implementation of different techniques and analysis in order to reduce morbidity and mortality. These analysis, named parasitological stool examinations aim to highlight and identify intestinal parasites. It is therefore

Corresponding author. E-mail: abykonate@yahoo.fr
Tel: +22507571931;+22540239134

an act of medical biology that is part of a preventive, diagnostic, prognostic and therapeutic approach whose results contribute to the diagnosis and prescription of care (France, 2002). Its importance therefore implies the need to ensure a high degree of security and to seek perfection in their performance. In Côte d'Ivoire, a previous study about assessment of laboratories performance with regard to parasitological stool examinations showed a discrepancy between the results obtained by the laboratories participating in these studies (Menan et *al.,* 2007; Bogoch et *al.,* 2006). We therefore carried out this study five years after the first one in order to update these data at the level of public sector medical laboratories of Abidjan.

MATERIAL AND METHODOLOGY

Type and study period

This is a cross-sectional study that was carried out from April to November 2011.

Selection of laboratories

The study concerned public health structures of Abidjan, which have a medical laboratory and where parasitological stool examinations are performed. An invitation letter to participate to the study was submitted to each laboratory identified with a decision card (acceptance or refusal). The laboratories selected for the study are those which agreed to participate.

Samples collection

It consisted of stool specimens obtained from subjects living in the village of ABOBOTE (Abidjan), irrespective of gender or age and having given their informed consent for the collection of their feces. These samples were collected directly in plastic pots and sent as soon as possible to the parasitology laboratory of the Center for Diagnosis and Research on AIDS and Other Infectious Diseases (CeDReS) for analysis.

Laboratory procedures

Samples was macroscopically examined for consistency, color and presence of blood, mucus or adult parasitic form. Then, microscopic examinations followed by a direct examination in physiological saline then a centrifugation-sedimentation by the formalin-ether method named simplified Ritchie technique previously described (Ritchie, 1948). For this technique, about 1-2 g of faeces is thoroughly emulsified with about 7 mL of 10% formalin (1 volume of formaldehyde diluted with 9 volumes of distilled water), and strained through wire gauze into a 15 mL centrifuge tube. Three milliliters of ether, is added (1 volume of ether for 2 volumes of suspension) and the mixture shaken vigorously for one minute. It is then centrifuged, accelerating slowly and gradually over a period of three minutes to a speed of 1,500 r.p.m., and then allowed to come to rest. Finally, from the four layers formed, the three top layers were discarded with a stick. The upper part of the tube is wiped clear of fatty debris. The entire resulting sediment is shaken up and poured on to a slide for microscopic examination for helminths and intestinal protozoa. Each suspension of stool was observed by two different biologists to estimate the approximate number of parasites per field observed. Stools containing parasites were then collected and concentrated several times by the same technique. All resulting sediments collected were brought together in order to constitute a stool suspension rich in parasites. At the end, the number of helminth eggs, and larvaes and, protozoa cysts and, oocycts was counted and recorded for each species separately. The number of these parasites varied from 1 to 2 elements per microscopic field observed and per species. In the same context, stools containing no parasitic elements were also made using the same methods.

At the end of these analysis, two types of stool suspensions were constituted: the first one called "Stool No.1" containing *Strongyloides stercoralis* rhabditiform larvae, *Entamoeba coli* cysts, *Endolimax nana* cysts, *Giardia intestinalis* cysts, *Isospora belli* oocysts and Hookworm eggs; totaling 6 species of parasites to be identified. The second named "Stool No.2" contained no parasites. Both suspensions were distributed in labeled cryotubes and handed over to the participating laboratories under a triple package.

Investigation procedures

A questionnaire was administrated to laboratory managers in order to collect data about the organization of the laboratory. At the same time, an individual result sheet identified by a unique code and stool suspensions ("Stool No.1" and "Stool No. 2") were handed over to each participating laboratory. Results should be gave backs in the form of a free text by simply recording the names of the parasites found in spaces reserved for this purpose. For each suspensions, ten different parasite species could be listed. Then, laboratories had two days to give back the results. All the sheets were collected on the 3rd day.

Data processing and analysis

The results were recorded and the responses of the survey organizers as well as the overall results of the participants were returned to each laboratory under cover to ensure confidentiality. The data were analyzed using Epi Info 6.04 software.

RESULTS

Out of the 37 public laboratories contacted, 29 agreed to participate in the study (78.4 %). The teaching hospitals and urban health units were the most represented (Table 1). Laboratory management accountability was at 62.1 % for biologist technicians and engineers of laboratory techniques. All laboratories had at least one microscope. Most of them were limited to a direct examination (macroscopy and microscopy) for the diagnosis of intestinal parasitic infections (37.9 %). Table 2 presents data on the organization, equipment and functioning of participating laboratories. Regarding the assessment itself, only 10.3 % of the laboratories were able to identify the 6 parasites present in stool No.1. *Strongyloides Stercoralis* rhabditiform larvae and *Entamoeba coli* cyst were the most recognized (69%). For stool No.2, 89.7 % gave a correct response which corresponded to the absence of parasites. Confusions were also noted about some species (Tables 3 and 4).

DISCUSSION

This study allowed to assess the diagnostic performance of public sector laboratories with regard to parasitological stool examinations and their internal organization. The overall participation rate obtained was higher than that reported in 2007 (Menan et *al.,* 2007). This could reflect an increasing interest in the implementation of quality in public services. However, as all the laboratories contacted should be able to give a positive answer for a participation to an external evaluation, this rate remains low.

The certification of analysis results must be entrusted firstly to a pharmacist biologist or a physician biologist as stipulated by law (Côte d'Ivoire, 1987). However, in our study, this activity was mainly done by biologist technicians and laboratory engineers. This situation could lead to dysfunctions in the internal organization of the laboratory and, consequently, in diagnostic activities.

As the number of stools treated per day is most from 1 to 2, the number of staff dedicated for this activity seems satisfactory. The deadline for reporting results is also satisfactory. However, routine parasitological examinations of stools are not the only activity of laboratories; thus, an advocacy for an increase in staff is always useful and must take into account all activities carried out by the structure.

The non-computerization of most laboratories raises the risk incurred in any other type of recording, in particular manual one. But, although this rate is still very low, we note a greater importance of introducing the IT tools into the daily management of laboratories because in 2007 it was reported that 76 % of them were not equipped with computers (Menan et *al.,* 2007).

Considering the list of the minimum equipment available to laboratories (France, 2002), only one in our series had all of the said material. All, the others had at least one microscope, which is an essential tool in the implementation of parasitological stool examinations. As a suitable technical platform in a laboratory augurs well for the quality of the services provided in this laboratory, the lack of equipment should be brought to the attention of managers of health facilities. In addition, device maintenance remains essential to avoid frequent breakdowns and improve their service life.

Most of the laboratories surveyed were limited to performing a direct examination. This trend was previously observed in Abidjan (Menan et *al.,* 2007). Indeed, direct examination is a procedure which alone, does not allow a correct result of stool examinations (Kassi et *al.,* 2004). In addition to direct examination, concentration techniques must be performed which is not routinely performed in our context due to the lack of adequate equipment. In fact, only 34.5 % of the laboratories used the Ritchie concentration technique; which is an inexpensive analysis relatively simple to carry out.

The results of the medical biology tests could only be communicated to the patient himself, to a third party duly mandated by the patient, to the prescribing practitioner and to any other practitioner designated by the patient, unless there is a derogation or specific rules laid down by law and the regulations in force (France, 2002). In our assessment, the confidentiality of the results was generally respected.

The archiving of test results is carried out in most laboratories with a variable shelf life. The guide of good practice of medical biology analysis recommends that archives should be preserved over a period of at least 5 years (France, 2002). However, only about one out of three laboratories meet this standard.

Like archiving, the mode of waste disposal is important. About half of the participating laboratories did not have regulatory texts or waste disposal procedures. This rate is low when it is known that the risk of infection in the medical laboratory has been clearly established (Meite et *al.,* 2007). The waste produced by sampling activity and analysis must be separated into hazardous wastes and the others must be assimilated to household waste. For each group of waste, an elimination procedure must be established with specific conditioning, storage, transport, treatment and pre-treatment modalities (France, 2002).

Procedures are operations to be performed, precautions to be taken and measures to be applied appearing on documents specific to each laboratory (France, 2002). They are essential to the implementation of a quality system in a laboratory.

Table 1. Distribution of participating laboratories.

Structures	Contacted Laboratories N	Participating Laboratories n (%)
Specialized institute	3	1 (33.3)
Teaching hospital	3	3 (100)
General hospital	7	6 (85.7)
Urban health unit	10	9 (90)
Urban health center	14	10 (71.4)
Total	**37**	**29 (78.4)**

Table 2. Organization, equipment and functioning of participating laboratories.

	N (%)		N (%)
Staff number		**Number of stools tests/day**	
1-5	19 (65.5)	1-2	22 (75.9)
6-10	10 (34.5)	3-4	3 (10.3)
Qualification of the manager		≥ 5	4 (13.8)
Physician	10 (34.5)	**Techniques performed**	
Pharmacist	1 (3.4)	Direct examination	29 (100)
Technical engineer	2 (6.9)	Ritchie Technique	10 (34.5)
Biologist technician	16 (55.2)	Stool culture	6 (20.7)
		Baermann Technique	2 (6.9)
Laboratory computerization		**Results delivery delay**	
Yes	10 (34.5)	The same day	17 (58.6)
No	19 (65.5)	The following day	12 (41.4)
Equipment maintenance		**Qualification of staff validating results**	
Yes	21 (72.4)	Biologist	7 (24.1)
No	8 (27.6)	Technician	24 (82.7)
Frequency of maintenance		Engineer	5 (17.2)
Weekly	2 (9.5)	**Results reporting mode**	
Monthly	16 (76.2)	To the patient	26 (89.7)
Annual	3 (14.3)	By mail to the physician	3 (10.3)
Number of microscopes		**Presence of texts regulating waste disposal**	
1	25 (86.2)	Yes	14 (48.3)
2	3 (10.3)	No	15 (51.7)
≥3	1 (3.5)	**Disposal of waste (equipment)**	
Others equipments		Incinerated	25 (86.2)
Centrifuge	28 (96.5)	Trash can	3 (10.3)
Refrigerator	28 (96.5)	Recycled	2 (6.9)
Glassware	27 (93.1)	**Archiving duration**	
Freezer	15 (51.7)	Yes	28 (96.5)
Oven	12 (41.4)	No	1 (3.5)
Water bath	7 (24.1)	**Archiving time**	
Distiller	6 (20.7)	<5 ans	8 (27.6)
Precision Balance	3 (10.3)	≥ 5 ans	20 (69)
		Written operating Procedures	
		Present	19 (65.5)
		Absent	10 (34.5)

However, our study found that one-third of the laboratories did not have written procedures. These procedures should not be fixed in time, but should be adapted to the evolution of knowledge and technical data, as was the case in our study in some laboratories, where procedures underwent modifications during reagent or device changes. Let's note that any modification of a procedure must be written, approved by the manager biologist and be the subject of information and training of the personnel.

Table 3. Results for stool suspension "No.1"

	Number of laboratories (N)	Percentage (%)
Number of parasites identified		
0	3	10.3%
1-2	10	34.5%
3-4	12	41.4%
5-6	4	13.8%
Parasites correctly recognized in stool No.1		
Strongyloides Stercoralis rhabditiform larvae	20	69%
Entamoeba coli cyst	20	69%
Hookworm egg	16	55.2%
Isospora belli oocyst	9	31%
Endolimax nana cyst	8	27.6%
Giardia intestinalis cyst	5	17.2%
Errors reported after observation of stool No.1		
Hookworm rhabditiform larvae	2	6.9%
Entamoeba hartmanni cyst	2	6.9%
Chilomatix mesnilii cyst	2	6.9%
Entamoeba histolytica cyst	1	3.4%
Enteromonas hominis cyst	1	3.4%
Various larvaes	1	3.4%

Our study reports relatively low rates of laboratories that have correctly identified at least 5 parasites.

Rhabditiform larvae of *Strongyloides stercoralis*, *Entamoeba coli* cysts and hookworm eggs were the most correctly identified parasites. There is a slight improvement in the diagnosis of these 3 species compared to 2007's study (Menan et *al.,* 2007), with respective rates of 65 %, 59 % and 41%. In France (AFSSAPS, 2007) in 2007, in their annual external quality assessment, the French Agency for the Safety of Health Products reported a recognition of rhabditiform larvae by 9 out of 10 participants; a high rate compared to ours. Strongyloidiasis is a tropical or subtropical disease caused by *Strongyloides stercoralis*; It induces various syndromes up to the observation of severe forms in immunocompromised patients (Adle-Biassette, 2007). It is the only helminth responsible of disseminated infections (Adle-Biassette, 2007). The rate of recognition of the hookworm eggs by 55.2 % of the participants in our study is not satisfactory because the richness of the stools was 2 eggs per microscopic field (x40). In France (AFSSAPS, 2007), the correct recognition of hookworm eggs was reported in 96.9% participants. In Côte d'Ivoire, previous studies have shown *Necator americanus* as the only hookworm species responsible for ancylostomiasis cases named necatoriasis in the country (Kassi et *al.,* 2008; Menan et *al.,* 1997; Oga et *al.*, 2004; Penali et *al.,* 1993). This disease constitute a serious danger for children infected because of anemia that it causes, which can be fatal in

Table 4. Results of stool suspension "No.2"

	Number of laboratories (N)	Percentage (%)
Results for stool No.2		
Correct response	26	89.7%
Wrong response	3	10.3%
Errors reported after observation of stool No.2		
Entamoeba coli cyst	2	6.9%
Strongyloides stercoralis larvae	1	3.4%

the long term in conditions of permanent re-infection (Lariviere, 1987). A correct identification of this species would therefore be essential for an early and effective management of this disease. The proportion of *Isospora belli* oocysts identified is lower than that of Menan *et al.* (2007) which was 53 % of good diagnoses concerning this parasite. The lack of performance for this parasite is a concern. First, because *Isospora belli* is responsible for isosporiasis, an opportunistic disease during HIV/AIDS infection (Guiguet et *al.,* 2007), and secondly, because most of cases of isosporiasis come from tropical, sub-tropical regions as Côte d'Ivoire and rarely from temperate areas (Morakoten et *al.,* 1987). In addition, with a national prevalence of HIV/AIDS infection estimated at 3.7% in 2012, Côte d'Ivoire is the most affected country of west Africa [ONUSIDA, 2015]. Regarding *Endolimax nana* cysts, participants had a great difficulty in recognizing them. The cyst of *Giardia intestinalis* constitutes one of the forms under which this protozoon is easy to diagnose but it remains unknown by the majority of the participants.

We have also recorded laboratories that have identified other parasites apart from those present in stool suspension No.1 with a confusion as regards helminth larvae. However, depending on the stage of larvae, the differential diagnosis between rhabditiform and filariform stages is relatively easy (Vaubourdolle, 2007). For flagellates, *Chilomastix mesnilii* cysts which were noted by 6.9 % of participants, could have been confused with *Giardia intestinalis* cysts. Also, some participants found *Enteromonas hominis* cysts. The low proportion of identification of amoeba cysts, could be due to the frequency of confusion between *Entamoeba histolytica* cysts and the others species of amoebae. In this context, only competent and trained coprologists can make the distinction (Leger and Danis, 1995).

In our series, most laboratories did not use the staining technique for the study of amoebas or flagellates. Certainly, some biologists specializing in parasitology, manage in these conditions to identify amoebae and flagellates, but it is necessary, for less trained people, to have a staining technique for these difficult diagnoses.

In addition, we noted that one in 10 participants did not identify any parasite among the 6 contained in the suspension. This poor performance certainly finds its explanation in the lack of experience and the lack of in-house training on techniques of identification of intestinal parasites.

The second sample of this control consisted of a suspension of non-parasitic formalized stools. Nearly one in 10 laboratories reported erroneously the presence of a parasite. These were usually *Entamoeba coli* cysts. This proportion of good response was satisfactory and close to that reported in the literature, which was 94% (Menan et *al.,* 2007).

CONCLUSION

From this study, it is clear that efforts remain to be made to ensure the reliability of the results from public sector medical laboratories in Abidjan. From this reliability, results a correct management of intestinal parasitic diseases. Five years after the first study, it is clear that the texts are still not applied. It therefore seems important to make apply the texts in favor of a mandatory introduction of inter-laboratory quality control at the national level, which will help laboratory professionals improve their performance through the implementation of corrective measures. In addition, capacity building specific to parasitology, particularly on techniques for the identification of intestinal parasites, should be instituted.

ACKNOWLEDGMENTS

Our thanks are addressed to the managers of the laboratories who have agreed to participate in this study.

REFERENCES

Adle-Biassette H (2007). Infections opportunistes chez le patient immunodéprimé. Bulletin de la Division Française de l'AIP. 46 : 26-40.

AFSSAPS (2007). Annales du Contrôle National de Qualité des Analyses de Biologie Médicale. Parasitologie 06PAR1. Saint-Denis, France, 38p.

Bogoch I, Raso G, N'Goran EK, Marti HP, Utzinger J (2006). Differences in microscopic diagnosis of helminths and intestinal protozoa among diagnostic centres. Eur J Clin Microbiol Infect Dis. 25: 344–347.

Brooker S, Kabatereine NB, Smith JL, Mupfasoni D, Mwanje MT, Ndayishimiye O, Lwambo NJS, Mbotha D, Karanja P, Mwandawiro C, Muchiri E, Clements ACA, Bundy DAP, Snow RW (2009). An updated atlas of human helminth infections: the example of East Africa. Int J Health Geogr. 8: 42.

Côte d'Ivoire (1987). Ministère de la Santé et de la Protection Sociale. Décret portant modification des statuts des laboratoires d'analyses de biologie médicale. Journal officiel de la République de Côte d'Ivoire. Abidjan, Côte d'Ivoire, 5p.

G/hiwot Y, Degarege A, Erko B (2014). Prevalence of Intestinal Parasitic Infections among Children under Five Years of Age with Emphasis on Schistosoma mansoni in Wonji Shoa Sugar Estate, Ethiopia. Plos one. 9(10) : 1-5.

Guiguet M, Furco A, Tattevin P, Costagliola D, Molina JM (2007). HIV-associated Isospora belli infection: incidence and risk factors in the French Hospital Database on HIV. HIV Med. 8 (2) : 124-130.

Journal Officiel de la République Française (2002). Arrêté du 26 novembre 1999 relatif à la bonne exécution des analyses de biologie médicale (Journal Officiel du 11 décembre 1999) modifié par Arrêté n° 104 du 04/05/2002 du 26 avril 2002 (Journal Officiel du 4 mai 2002. Paris, France : Journal Officiel de la République de France, pp. 8375-8382.

Kassi FK, Menan EIH, Yavo W, Oga SSA, Djohan V, Vanga H, Barro PCK, Adjetey TAK, Kone M (2008). Helminthoses intestinales chez les enfants d'âge scolaire de la zone rurale et urbaine de Divo (Côte d'Ivoire). Cah Santé Publique. 7 (1) : 51-60.

Kassi RR, Kouassi RA, Yavo W, Barro-Kiki CP, Bamba A, Menan HI, Kone M (2004). Cryptosporidiosis and isosporiasis in children suffering from diarrhoea in Abidjan. Bull Soc Pathol Exot. 97(4) : 280-282.

Keiser J, Utzinger J (2010). The drugs we have and the drugs we need against major helminth infections. Adv Parasitol. 73: 197–230.

Lariviere M (1987). Les helminthoses In : parasitologie médicale. Paris, France: Edition Marketing, pp. 85-179.

Leger N, Danis M (1995). Amibes et amibiases. Paris, France: Encyclo. Med. Chir, 14p.

Méité S, Boni-Cissé C, Guéi MC, Houedanon C, Faye-Kette H.(2007) Evaluation du risque infectieux au laboratoire d'analyses médicales: Exemple du laboratoire de bactériologie-virologie du CHU de Yopougon (Abidjan, Côte d'Ivoire) en 2006. Revue bio-africa. 4 : 16-22.

Menan EIH, Rouamba E, Ouhon J, Nebavi N, Adjetey TAK, Barro-Kiki PCM, Penali L, Kone M. (1997). Helminthiases intestinales : résultats de cinq années de coprologie parasitaire à l'Institut Pasteur de Cocody (Abidjan- Côte d'Ivoire). Méd d'Af Noire. 44 (7) : 415- 419.

Menan EIH, Yavo W, Kiki-Barro PC, Oga SSA, Djohan V, Vangah H, Gbobouo G, Kone M. (2007). Evaluation externe de la qualité des examens parasitologiques des selles réalisée dans 17 laboratoires du secteur public de la ville d'Abidjan (Côte d'Ivoire). J Sci Pharm Biol. 8 (1) : 52-62.

Morakote N, Muangyimpong Y, Somboon P, Khamboonruang C (1987). Acute human Isosporiasis in Thailand: a case report. South east Asian J Trop Med Pub Hlth. 18 : 107-111.

Oga AS, Yavo W, Menan EIH, Attey MA, Kouadio L, Koné M. (2004). Helminthoses intestinales chez les enfants d'âge scolaire : résultats préliminaire d'une étude prospective à Agboville dans le sud de la Côte d'Ivoire. Revue santé 2004. 14 (3) : 143-147.

ONUSIDA (2015). Suivi de la déclaration de politique sur le SIDA de juin 2011 : Rapport national de la Côte d'Ivoire 2014. Abidjan, Côte d'Ivoire: Ministère de la lutte contre le SIDA, 40 p.

Ritchie LS. (1948) An ether sedimentation technique for routine stool examination. Bull United State Army Medical Department. 8:326.

Penali LK, Broalet EY, Kone M. (1993). Helminthiases intestinales de la femme enceinte en Côte d'Ivoire. Méd d'Af Noire. 40 (5) : 354-356.

Vaubourdolle M. Infectiologie (2007). Pharmacie, biologie : concours de l'Internat, formation continue. 3rd edn. Paris, France : Wolters Kluwer, 1036 p.