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Microscopic diagnosis of sodium acetate-acetic acid-formalin-fixed stool samples for helminths and intestinal protozoa: a comparison among European reference laboratories

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Abstract

The present study aimed to compare the diagnostic performance of different European reference laboratories in diagnosing helminths and intestinal protozoa, using an ether-concentration method applied to sodium acetate-acetic acid-formalin (SAF)-preserved faecal samples. In total, 102 stool specimens were analysed during a cross-sectional parasitological survey in urban farming communities in Côte d'Ivoire. Five SAF-preserved faecal samples were prepared from each specimen and forwarded to the participating reference laboratories, processed and examined under a microscope adhering to a standard operating procedure (SOP). *Schistosoma mansoni* (cumulative prevalence: 51.0%) and hookworm (cumulative prevalence: 39.2%) were the predominant helminths. There was excellent agreement ($\kappa > 0.8$; $p < 0.001$) among the reference laboratories for the diagnosis of *S. mansoni*, hookworm, *Trichuris trichiura* and *Ascaris lumbricoides*. Moderate agreement ($\kappa = 0.54$) was found for *Hymenolepis nana*, and lesser agreement was observed for other, less prevalent helminths. The predominant intestinal protozoa were *Entamoeba coli* (median prevalence: 67.6%), *Blastocystis hominis* (median prevalence: 55.9%) and *Entamoeba histolytica/Entamoeba dispar* (median prevalence: 47.1%). Substantial agreement among reference laboratories was found for *E. coli* ($\kappa = 0.69$), but only fair or moderate agreement was found for other *Entamoeba* species, *Giardia intestinalis* and *Chilomastix mesnili*. There was only poor agreement for *B. hominis*, *Isospora belli* and *Trichomonas intestinalis*. In conclusion, although common helminths were reliably diagnosed by European reference laboratories, there was only moderate agreement between centres for pathogenic intestinal protozoa. Continued external quality assessment and the establishment of a formal network of reference laboratories is necessary to further enhance both accuracy and uniformity in parasite diagnosis.

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Introduction

International travel, facilitated by rapid air transport, continues to grow [1]. It has been paralleled by an exponential increase in tourism, which includes many destinations in developing countries [2]. Returning travellers are recognized as an importation mode for introducing parasitic diseases into industrialized countries. Sick returning travellers must be diagnosed promptly and accurately, in order to guide treatment plans and minimize the risk of patient morbidity or mortality [3]. Accurate diagnoses are also important for epidemiological surveillance, in order to understand where and how infectious agents are likely to be contracted and spread.

Specialized laboratories with state-of-the-art equipment and trained personnel are essential for accurate diagnoses. Although many laboratories examine a range of biological

specimens (e.g. blood, faeces and urine), only a few are designated reference laboratories. The latter often serve as national reference centres for other diagnostic laboratories and are contacted for expert opinion, particularly when diagnostic problems arise. Given the status of a reference laboratory, diagnoses made at such centres are less likely to be challenged than at other less specialized laboratories. However, previous studies have brought to light diagnostic discrepancies among laboratories. For example, microscopic examination of thick and thin blood films for malaria diagnosis among ten laboratories on the Thai–Myanmar border showed considerable interlaboratory differences [4]. A comparison between two European laboratories and between a European and a West African centre regarding faecal samples submitted for helminth and intestinal protozoa diagnosis revealed poor, slight or only fair agreement for most parasites [5].

Five European reference laboratories were invited to participate in the diagnosis of parasitic infections and the extent of agreement was assessed for the diagnosis of helminths and intestinal protozoa. Faecal samples, obtained from an African setting, were preserved in a sodium acetate-acetic acid-formalin (SAF) solution and processed with an ether-concentration method according to the same standard operating procedure (SOP) in each participating laboratory [6]. The results were compared and a workshop was held to discuss the findings, and to issue recommendations on how to enhance the reliability of helminth and intestinal protozoa diagnosis.

Materials and Methods

Subjects and faecal samples

Faecal samples were collected in May and June 2005, during two cross-sectional parasitological surveys carried out in two urban farming communities: (i) a poor neighbourhood in Abidjan (economic capital of Côte d'Ivoire) and (ii) the town of Man (regional capital city in west Côte d'Ivoire). The data presented stem from a larger study aiming to investigate risk factors for helminth infections and malaria among urban farming communities, and to develop locally-adapted strategies for prevention and control [7,8].

The study was approved by the institutional research commissions of the Swiss Tropical Institute (Basel, Switzerland) and the Centre Suisse de Recherches Scientifiques (Abidjan, Côte d'Ivoire). Ethical clearance was granted by the Ministry of Health in Côte d'Ivoire. Details of the study area, population surveyed and field procedures are provided elsewhere [7,8]. In brief, households were visited, the purpose of the study explained and participants were invited to provide a lemon-sized portion (~50 g) of their next morning stool. Filled stool containers were collected in the early morning, labelled with unique identifiers and transferred to nearby laboratories.

Laboratory procedures

Within 4–8 h after the stool specimens were produced, a single Kato-Katz thick smear was prepared from each sample and examined for helminths [9]. Participants infected with *Schistosoma mansoni* were given a single oral dose of praziquantel (40 mg/kg), and those infected with other helminths were administered a single oral dose of albendazole (400 mg) [10].

From a sub-sample of 103 stool specimens, selected according to a random number list, five hazelnut-sized portions (~1–2 g) of stool were put in separate tubes filled with 10 mL of SAF. Each sample was homogenized with a wooden spatula and vigorously shaken. Sets of the same clinical specimens were forwarded to the five participating European reference laboratories. An SOP, as used at the Swiss Tropical Institute, was included and the reference laboratories were instructed to adhere to a five-step procedure. First, re-suspension of SAF-fixed stool samples and straining through a gauze into a centrifuge tube. Second, centrifuging the tube for 1 min at 500 ***g***. Third, decanting the supernatant; if the final sediment contained more than 1 mL, the first two steps were repeated or the sediment was re-suspended in 0.85% NaCl and part of the suspension was removed. Fourth, adding 7 mL of 0.85% NaCl plus 2–3 mL diethyl ether to the remaining sediment. Closing the tube with a rubber stopper, shaking for ~30 s and centrifuging for 5 min at 500 ***g***. Finally, from the four layers formed, the three top layers were discarded and the resulting sediment was examined microscopically for helminths and intestinal protozoa. It was emphasized that the entire sediment should be examined: The number of helminth eggs was counted and recorded for each species separately. If more than 100 eggs were found for a specific helminth, the microscopist terminated the reading and recorded '100+ eggs'. For intestinal protozoa, the following semi-quantitative scheme was adopted: (i) negative (no cysts or trophozoites in the entire sediment); (ii) rare (one to five cysts or trophozoites per slide); (iii) frequent (one cyst or trophozoite per observation field of ×400 or 500); and (iv) very frequent (more than one cyst or trophozoite per observation field of ×400 or 500).

European reference laboratories

Five European reference laboratories participated in the study, namely Bernhard Nocht Institute for Tropical Medicine (Hamburg, Germany), Hospital for Tropical Diseases (London, UK), Institute for Infectious and Tropical Diseases

(Brescia, Italy), Swedish Institute for Infectious Disease Control (Solna, Sweden), and the Swiss Tropical Institute (Basel, Switzerland). Four months after collection of stool specimens in Côte d'Ivoire, the five sets of the same SAF-preserved stool samples were forwarded to the participating centres. The heads of the laboratories were contacted and the accompanying SOP discussed further to ascertain consistency in the methodology from sample preparation to data recording.

Statistical analysis

Data were double-entered and checked for consistency using EpiData, version 3.1 (EpiData Association, Odense, Denmark). For statistical analysis, we employed STATA software, version 9 (StataCorp, College Station, TX, USA). The analysis used results from those individuals who had complete data records (i.e. results from all five reference laboratories). The agreement between the five centres for species-specific diagnosis of helminth and intestinal protozoa was assessed using the kappa (κ) statistic [11], with the cut-offs: $\kappa < 0$, no agreement; $\kappa = 0.01$–0.2, poor agreement; $\kappa = 0.21$–0.4, fair agreement; $\kappa = 0.41$–0.6, moderate agreement; $\kappa = 0.61$–0.8, substantial agreement; and $\kappa = 0.81$–1.0, almost perfect agreement [12]. The magnitude of κ depends on the prevalence of a specific parasite, and hence the obtained value of κ should be reported alongside the prevalence [13].

For each parasite, the extent of total agreement was determined among the centres (i.e. whether none or all of the centres diagnosed the parasite). Where disagreement occurred, there was further investigation into how often only one centre disagreed (i.e. one centre found a specific parasite, whereas the remaining four centres failed to diagnose the parasite), and how often a specific parasite was diagnosed by four of the five centres. The actual helminth egg counts recorded by the centres were also considered. If, for example, only one or two hookworm eggs were recorded by one centre, whereas the other centres recorded no hookworm eggs, such a result was considered to be less problematic than four centres recording high hookworm egg counts, whereas the remaining centre failed to diagnose any hookworm eggs.

Results

Study cohort

Of the five sets of 103 SAF-preserved stool samples forwarded to the participating European reference laboratories, complete data were available for 102 subjects (99.0%); 71 (69.6%) individuals were sampled during the cross-sectional survey in Man, and 31 individuals (30.4%) were sampled in Abidjan. There were more males than females (65 vs. 37). The mean age of the participants was 23.3 years (standard deviation: 15.6 years, range: 2–61 years).

Prevalence and interlaboratory agreement

Table 1 shows the individual, median and cumulative results from the five participating European reference laboratories

TABLE 1. Number (%) of faecal samples found positive for different helminths and intestinal protozoa in five European reference laboratories (centres 1–5)[a], including cumulative results and kappa statistics (n = 102)

Parasite	Cumulative	Median	Centre 1	Centre 2	Centre 3	Centre 4	Centre 5	Kappa	p
Helminths									
Schistosoma mansoni	52 (51.0)	42 (41.2)	41 (40.2)	40 (39.2)	44 (43.1)	45 (44.1)	42 (41.2)	0.83	<0.001
Hookworm	40 (39.2)	32 (31.4)	32 (31.4)	30 (29.4)	33 (32.4)	33 (32.4)	31 (30.4)	0.87	<0.001
Trichuris trichiura	17 (16.7)	15 (14.7)	11 (10.8)	14 (13.7)	15 (14.7)	15 (14.7)	16 (15.7)	0.89	<0.001
Strongyloides stercoralis	7 (6.9)	2 (2.0)	2 (2.0)	0	3 (2.9)	4 (3.9)	0	0.10	0.001
Ascaris lumbricoides	5 (4.9)	3 (2.9)	2 (2.0)	3 (2.9)	3 (2.9)	4 (3.9)	4 (3.9)	0.81	<0.001
Hymenolepis nana	5 (4.9)	2 (2.0)	1 (1.0)	1 (1.0)	3 (2.9)	3 (2.9)	2 (2.0)	0.54	<0.001
Enterobius vermicularis	3 (2.9)	1 (1.0)	1 (1.0)	0	2 (2.0)	2 (2.0)	0	0.19	<0.001
Dicrocoelium dendriticum	2 (2.0)	0	0	0	1 (1.0)	0	1 (1.0)	0.00	0.55
Fasciola hepatica	1 (1.0)	0	1 (1.0)	1 (1.0)	0	0	0	0.00	0.55
Capillaria spp.	1 (1.0)	0	0	1 (1.0)	1 (1.0)	0	0	0.25	<0.001
Clonorchis sinensis	1 (1.0)	0	0	0	0	0	1 (1.0)	0.00	0.53
Intestinal protozoa									
Blastocystis hominis	96 (94.1)	57 (55.9)	34 (33.3)	58 (56.9)	57 (55.9)	90 (88.2)	25 (24.5)	0.12	<0.001
Entamoeba coli	84 (82.4)	69 (67.6)	68 (66.7)	68 (66.7)	69 (67.6)	76 (74.5)	71 (69.6)	0.69	<0.001
Entamoeba hartmanni	72 (70.6)	33 (32.4)	17 (16.7)	38 (37.3)	23 (22.5)	59 (57.8)	33 (32.4)	0.27	<0.001
Entamoeba histolytica/E. dispar	70 (68.6)	48 (47.1)	53 (52.0)	49 (48.0)	48 (47.1)	46 (45.1)	15 (14.7)	0.46	<0.001
Endolimax nana	56 (54.9)	22 (21.6)	22 (21.6)	26 (25.5)	15 (14.7)	49 (48.0)	16 (15.7)	0.33	<0.001
Iodamoeba bütschlii	50 (49.0)	20 (19.6)	27 (26.5)	20 (19.6)	17 (16.7)	23 (22.5)	20 (19.6)	0.38	<0.001
Giardia intestinalis	22 (21.6)	10 (9.8)	5 (4.9)	10 (9.8)	12 (11.8)	20 (19.6)	3 (2.9)	0.45	<0.001
Chilomastix mesnili	18 (17.6)	11 (10.8)	2 (2.0)	11 (10.8)	11 (10.8)	11 (10.8)	7 (6.9)	0.51	<0.001
Sarcocystis hominis	1 (1.0)	0	0	1 (1.0)	0	1 (1.0)	0	0.25	<0.001
Isospora belli	1 (1.0)	0	0	0	0	1 (1.0)	0	0.00	0.53
Trichomonas intestinalis	2 (2.0)	0	0	0	0	2 (2.0)	0	0.00	0.55

[a]Note: centres 1–5 does not correspond to the listing in the Materials and Methods section.

for the diagnosis of helminths and intestinal protozoa, including interlaboratory agreement and κ statistics. The predominant helminth was *S. mansoni*, for which a cumulative prevalence of 51.0% (n = 52) was found. Although centre 2 diagnosed 40 cases of *S. mansoni* (39.2%), centre 4 recorded *S. mansoni* eggs in 45 stool samples (44.1%). Hookworm was the second most prevalent helminth; the cumulative prevalence was 39.2% (n = 40). Individual centres diagnosed a hookworm infection at least 30 times (29.4%) and, at maximum, in 33 cases (32.4%). Eggs of *Trichuris trichiura* were found in a minimum of 11 (10.8%) and a maximum of 16 stool samples (15.7%) with a cumulative prevalence of 16.7% (n = 17). A low cumulative prevalence was found for *Ascaris lumbricoides* (4.9%, n = 5). There was almost perfect agreement among reference laboratories in the diagnosis of these four helminths (all $\kappa > 0.8$, $p < 0.001$).

Although there was moderate agreement (κ = 0.54) for *Hymenolepis nana* (cumulative prevalence: 4.9%, range among centres: 1.0–2.9%), less agreement was observed for all of the other helminth species discovered. For example, although centres 2 and 5 failed to diagnose larvae of *Strongyloides stercoralis* altogether, centres 1, 3 and 4 reported them (in three stool samples in the case of centre 3); the cumulative prevalence was 6.9%. There was one case of *Capillaria* spp. diagnosed both by centres 2 and 3 and documented by photography, whereas the remaining centres reported no *Capillaria* spp.

Regarding intestinal protozoa, the predominant species were *Entamoeba coli* (median prevalence: 67.6%), *Blastocystis hominis* (median prevalence: 55.9%), *Entamoeba histolytica/Entamoeba dispar* (median prevalence: 47.1%) and *Entamoeba hartmanni* (median prevalence: 32.4%). The number of stool samples diagnosed positive for *E. coli* was fairly comparable among centres (range: 68–76). However, large discrepancies were found for *B. hominis* (centre 5: 25 cases, centre 4: 90 cases), *E. hartmanni* (centre 1: 17 cases, centre 4: 59 cases) and *E. histolytica/E. dispar* (centre 5: 15 cases, centre 1: 53 cases). On average, the pathogenic intestinal protozoan *Giardia intestinalis* was found in ten out of 102 participants (9.8%), with considerable variation among centres (centre 5: 3 cases, centre 4: 20 cases). The highest level of agreement among the five reference laboratories was found for *E. coli* (κ = 0.69). A moderate agreement was observed for *E. histolytica/E. dispar* (κ = 0.46), *G. intestinalis* (κ = 0.45) and *Chilomastix mesnili* (κ = 0.51). Poor or only fair agreement was observed for *B. hominis*, *E. hartmanni*, *Endolimax nana* and *Iodamoeba bütschlii*. *Sarcocystis hominis* was found by two centres in only one sample, whereas *Isospora belli* and *Trichomonas intestinalis* was only reported by centre 4 (all $\kappa < 0.4$).

Table 2 shows how often there was total agreement among the five European reference laboratories, stratified by helminths and intestinal protozoa (either all or none of the centres diagnosed a particular parasite) or disagreement by one or more centres. Complete agreement in the diagnosis of the two predominant helminths (i.e. *S. mansoni* and hookworm) was achieved in 81.4% and 87.3%, respectively. In 11 out of 14 individuals where a single centre disagreed in the diagnosis of *S. mansoni*, consistent low egg counts were reported by the other centres (median *S. mansoni* egg count: 2; range: 1–14). There were three cases where one centre failed to report *S. mansoni*, whereas the other four centres found, on average, nine eggs (range: 3–31). Regarding hookworm diagnosis, in ten out of the 11 cases where a single centre disagreed, there were low hookworm egg counts reported by the other centres (median: 3; range: 1–13). For one subject, one centre reported no hookworms, whereas high egg counts were recorded by the remaining centres (i.e. 20–25). The majority of the *S. stercoralis* cases were diagnosed by one centre only (four of the seven cases).

Regarding intestinal protozoa, among those found with a median prevalence above 30%, a complete agreement of

TABLE 2. Different levels of agreement among five European reference laboratories in the diagnosis of helminths and intestinal protozoa

Parasite	Agreement in all five centres (%)	Agreement in four of the five centres (%)	Agreement in two or three of the centres (%)
Helminths			
Schistosoma mansoni	83 (81.4)	14 (13.7)	5 (4.9)
Hookworm	89 (87.3)	11 (10.8)	1 (1.0)
Trichuris trichiura	96 (94.1)	5 (4.9)	1 (1.0)
Strongyloides stercoralis	95 (93.1)	5 (4.9)	2 (2.0)
Ascaris lumbricoides	99 (97.1)	3 (2.9)	0
Hymenolepis nana[a]	98 (96.1)	3 (2.9)	1 (1.0)
Enterobius vermicularis[a]	99 (97.1)	1 (1.0)	2 (2.0)
Dicrocoelium dendriticum[a]	100 (98.0)	2 (2.0)	0
Fasciola hepatica[a]	100 (98.0)	2 (2.0)	0
Capillaria spp.[a]	101 (99.0)	0	1 (1.0)
Clonorchis sinensis[a,b]	101 (99.0)	1 (1.0)	0
Intestinal protozoa			
Blastocystis hominis	12 (11.8)	46 (45.1)	44 (43.1)
Entamoeba coli	73 (71.6)	19 (18.6)	10 (9.8)
Entamoeba hartmanni	34 (33.3)	38 (37.3)	30 (29.4)
Entamoeba histolytica/E. dispar	45 (44.1)	38 (37.3)	19 (18.6)
Endolimax nana	49 (48.0)	30 (29.4)	23 (22.5)
Iodamoeba bütschlii	56 (54.9)	33 (32.4)	13 (12.7)
Giardia intestinalis	81 (79.4)	13 (12.7)	8 (7.8)
Chilomastix mesnili	86 (84.3)	10 (9.8)	4 (3.9)
Sarcocystis hominis[a]	101 (99.0)	0	1 (1.0)
Isospora belli[a]	101 (99.0)	1 (1.0)	0
Trichomonas intestinalis[a]	100 (98.0)	2 (2.0)	0

[a]Very low point-prevalence was observed for these parasites; hence, care is needed in the interpretation of these data.
[b]*Clonorchis sinensis* is not usually found in African settings; hence, the one centre reporting a one case of this liver fluke probably comprised a false-positive result.

71.6% was found for *E. coli*, whereas low percentages resulted for the other intestinal protozoa; the worst for *B. hominis* (11.8%).

Discussion

Comparison between five European reference laboratories for the diagnosis of parasitic infections when adhering to the same SOP of an ether-concentration method of SAF-preserved stool samples revealed excellent agreement for *S. mansoni* and the three most common soil-transmitted helminths (i.e. *A. lumbricoides*, hookworm and *T. trichiura*) [14] (all $\kappa > 0.8$). On the other hand, there was far less agreement for *Capillaria* spp., *Dicrocoelium dendriticum* and *Fasciola hepatica* ($\kappa < 0.4$). It should be noted, however, that the prevalence of these helminths was very low; indeed, these parasites were diagnosed only in one or two patients (prevalence < 2%). With the exception of *Capillaria* spp., where two centres found six and nine eggs in SAF-preserved stool samples from the same individual, the other reports showed that in each case only a single egg of the respective parasite species was found. At low prevalence, only low κ values can be obtained and, hence, the scale of agreement is not independent of infection prevalence, which is the main disadvantage of the κ index [15,16]. Furthermore, it should be noted that the SAF concentration method is not ideal for sensitive diagnosis of either *S. stercoralis* or *Enterobious vermicularis*. For *S. stercoralis*, either the Baermann technique [17] or a culture method such as the Koga-agar plate [18], or both techniques combined [19] should be used in addition to the formalin ether-concentration method. With regard to *E. vermicularis*, the adhesive tape method is recommended [20]. For *E. histolytica*/*E. dispar* and *G. intestinalis*, there was moderate agreement between the participating centres; the respective κ values were 0.46 and 0.45. With the exception of *E. coli*, where substantial agreement was found ($\kappa = 0.69$), all other intestinal protozoa were diagnosed with poor, slight or only fair agreement ($\kappa < 0.2$). The very low prevalence of *S. hominis*, *I. belli* and *T. intestinalis* partially explains this issue [13,15,16].

Strengths and limitations

Five sets of the same SAF-preserved stool samples were processed and microscopically examined by different European reference laboratories adhering to the same SOP. Efforts were made to clarify the various steps in the SOP and emphasis was placed on examining the entire stool sediment. All helminth eggs were counted and recorded separately; whereas a semi-quantitative scoring system was adopted for intestinal protozoa. The participating centres were blinded, but, once the results were available from all centres, they were analysed by a statistician and subsequently discussed during a joint workshop.

The present study has a number of limitations. First, not all of the participating centres were equally familiar with the SOP and some of the centres use different methods and procedures in their routine helminth and intestinal protozoa diagnostic work. In Sweden, for example, because ether is no longer allowed in laboratories, diethyl acetate, which has very similar chemical properties but is less inflammable, was used instead. Second, the five sets of 'identical' stool samples were prepared on the spot in Côte d'Ivoire by putting hazelnut-sized portions (~1–2 g) of stool from a single specimen into different tubes containing 10 mL of SAF. It is conceivable that the amount of stool varied from one tube to another, as observed in a recent study comparing the Kato-Katz method with an ether-concentration and the FLOTAC method for hookworm diagnosis [21]. Because parasite elements were not necessarily evenly distributed between the tubes that were sent to the participating laboratories, this might explain the observed differences, particularly in low level infections, for which it is difficult to exclude variation as a result of chance as opposed to individual laboratory performance. It would have been preferable to homogenize the faecal sample prior to aliquoting, as carried out during the preparation of test samples in some external quality assessment (EQA) services, such as the UK national EQA service (UKNEQAS) [22], or the German Society for Promotion of Quality Assurance in the Medical Laboratories e.V. (Instand e.V.; http://www.instandev.de), but this was not a feasible proposition in the field and, even with homogenized samples, cysts or ova present in very low numbers cannot be guaranteed to be present in every aliquot. Third, the stool samples were analysed several months after SAF fixation, which might have raised problems, particularly in identifying the internal structure of small protozoan cysts (e.g. *E. nana* and *E. hartmanni*), although samples preserved in SAF for teaching purposes can remain intact for many years and each laboratory received samples that were preserved in SAF for the same time. Finally, the absence of a diagnostic 'gold' standard made it impossible to rate a discrepancy in the result from any of the centres as either true or false. Confirmation of a positive finding in the same sample by another centre made it likely that the individual from whom the sample came was indeed infected with that parasite, but, if only a single centre reported an infection in a specific sample, there was no way to achieve confirmation because taking photographs of all findings in all individuals would have been impractical. Of note, the single case of *Capillaria* spp. was diagnosed by two centres, with one taking a picture of the egg, which was later confirmed

to be *Capillaria*. Such rare cases could be utilized for subsequent training purposes and quality assurance. The so-called web-microscope might play a role in this respect [23] (http://www.webmicroscope.net/).

Implications for practice and a way forward

Despite these potential shortcomings, there are a number of lessons that can be learned from this study. First, the available evidence suggests almost perfect agreement between European reference laboratories in the diagnosis of common helminths. This finding is encouraging, particularly in view of previous observations of a much lower agreement between two European laboratories for the diagnosis of helminths based on stool samples also obtained from Côte d'Ivoire with similar helminth prevalence [5]. Second the well known shortcomings of an ether-concentration method for the diagnosis of *E. vermicularis* (for which an adhesive tape test or perianal swab are preferred) and *S. stercoralis* (for which culture is superior), plus the low number of samples containing these parasites, may explain the interlaboratory differences observed for these two helminths. It should be noted that no method exists that is equally suitable for all parasites and a standard method has to be chosen if no specific parasite infection is suspected for which a particular method can be employed. Third, considerable discrepancies were found in the diagnosis of intestinal protozoa, which warrant targeted and continued training of laboratory technicians. In the present study, particular difficulty was encountered with *B. hominis* (κ = 0.12) and to a lesser extent with *E. nana*, *I. bütschlii* and *C. mesnili*. For *B. hominis*, there were only 12 stool samples where none of the centres or all five centres found this protozoan, whereas, in the remaining 90 samples, one or several centres reported the parasite. Identification by light microscopy of small protozoan cysts measuring less than 10 μm is a well recognized issue influencing performance in established EQA services. For example, when cysts of *E. nana* or *C. mesnili* were distributed either alone or with another parasite by the UKNEQAS, 25–37% of participants failed to report them [22]. It should be noted, however that moderate agreement was still found between the participating laboratories in the diagnosis of two clinically important intestinal protozoa, *E. histolytica*/*E. dispar* and *G. intestinalis*. From a clinician's point of view, reliable diagnosis of helminths and pathogenic protozoa is clearly more important than correct identification of non-pathogenic species.

Strategies on how to improve further the inter-rater reliability of reference laboratories were critically discussed during a workshop consisting of all of the participating centres. Results from reference laboratories are seldom challenged for their interlaboratory agreement. The present study emphasizes the point that sustained quality improvement, internal quality control and external quality assessment are as essential for reference laboratories as they are for non-specialist facilities. With the exception of the Italian centre, all laboratories included in the present study participate and perform well in established EQA schemes (and the Italian laboratory is the reference centre of the Region Lombardy for imported diseases) and, furthermore, all operate a policy of continuous quality improvement. We recommend the development of a stronger international network of reference laboratories to help ensure even greater accuracy and uniformity in parasite diagnosis. Finally, there is a need for rigorous validation of alternative diagnostic approaches, such as PCR or antigen-detection ELISAs for the diagnosis of intestinal protozoa where light microscopy is found to perform inadequately [24,25].

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Contributors

J.U. and H.M. designed the study and wrote the first draft of the manuscript. B.M. and E.K.N. coordinated the field work, collected stool samples and prepared five sets of SAF-preserved samples for reference laboratories. S.B.K., F.C., P.L.C., H.E., M.G., M.L., M.M. and E.T. performed the laboratory work and assisted in the interpretation of the data. N.K. and P.V. analysed the data. All authors read and revised the manuscript and approved the current submission. J.U. and H.M. are the guarantors of the paper.

Transparency Declaration

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References

1. Tatem AJ, Rogers DJ, Hay SI. Global transport networks and infectious disease spread. *Adv Parasitol* 2006; 62: 293–343.
2. Steffen R, deBernardis C, Banos A. Travel epidemiology – a global perspective. *Int J Antimicrob Agents* 2003; 21: 89–95.
3. Mascie-Taylor CG, Karim E. The burden of chronic disease. *Science* 2003; 302: 1921–1922.
4. Hemme F, Gay F. Internal quality control of the malaria microscopy diagnosis for 10 laboratories on the Thai-Myanmar border. *Southeast Asian J Trop Med Public Health* 1998; 29: 529–536.
5. Bogoch II, Raso G, N'Goran EK, Marti HP, Utzinger J. Differences in microscopic diagnosis of helminths and intestinal protozoa among diagnostic centres. *Eur J Clin Microbiol Infect Dis* 2006; 25: 344–347.
6. Yang J, Scholten T. A fixative for intestinal parasites permitting the use of concentration and permanent staining procedures. *Am J Clin Pathol* 1977; 67: 300–304.
7. Matthys B, Adiko FA, Cissé G *et al.* Le réseau social des maraîchers à Abidjan agit sur la perception des préoccupations et des risques sanitaires liés à l'eau. *VertigO* 2006; (Hors Série 3); Chapitre 8: 1–8.
8. Matthys B, Tschannen AB, Tian-Bi NT *et al.* Risk factors for *Schistosoma mansoni* and hookworm in urban farming communities in western Côte d'Ivoire. *Trop Med Int Health* 2007; 12: 709–723.
9. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo* 1972; 14: 397–400.
10. WHO. Preventive chemotherapy in human helminthiasis. *Coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers*. Geneva: World Health Organization, 2006.
11. Cohen J. A coefficient of agreement for nominal scales. *Educ Psychol Meas* 1960; 20: 37–46.
12. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 159–174.
13. Sim J, Wright CC. The kappa statistic in reliability studies: use, interpretation, and sample size requirements. *Phys Ther* 2005; 85: 257–268.
14. Bethony J, Brooker S, Albonico M *et al.* Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* 2006; 367: 1521–1532.
15. Kundel HL, Polansky M. Measurement of observer agreement. *Radiology* 2003; 228: 303–308.
16. Mak HKF, Yau KKW, Chan BPL. Prevalence-adjusted bias-adjusted kappa values as additional indicators to measure observer agreement. *Radiology* 2004; 232: 302–303.
17. García LS. *Diagnostic medical parasitology*. Washington, DC: ASM Press, 2007.
18. Koga K, Kasuya S, Khamboonruang C *et al.* A modified agar plate method for detection of *Strongyloides stercoralis*. *Am J Trop Med Hyg* 1991; 45: 518–521.
19. Steinmann P, Zhou XN, Du ZW *et al.* Occurrence of *Strongyloides stercoralis* in Yunnan province, China, and comparison of diagnostic methods. *PLoS Negl Trop Dis* 2007; 1: e75.
20. WHO. *Basic laboratory methods in medical parasitology*. Geneva: World Health Organization, 1991.
21. Utzinger J, Rinaldi L, Lohourignon LK *et al.* FLOTAC: a new sensitive technique for the diagnosis of hookworm infections in humans. *Trans R Soc Trop Med Hyg* 2008; 102: 84–90.
22. Kettelhut MM, Chiodini PL, Edwards H, Moody A. External quality assessment schemes raise standards: evidence from the UKNEQAS parasitology subschemes. *J Clin Pathol* 2003; 56: 927–932.
23. Linder E, Lundin M, Thors C *et al.* Web-based virtual microscopy for parasitology: a novel tool for education and quality assurance. *PLoS Negl Trop Dis* 2008; 2: e315.
24. Fotedar R, Stark D, Beebe N *et al.* Laboratory diagnostic techniques for *Entamoeba* species. *Clin Microbiol Rev* 2007; 20: 511–532.
25. Khairnar K, Parija SC, Palaniappan R. Diagnosis of intestinal amoebiasis by using nested polymerase chain reaction-restriction fragment length polymorphism assay. *J Gastroenterol* 2007; 42: 631–640.