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BRIEF REPORT

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Differences in microscopic diagnosis of helminths and intestinal protozoa among diagnostic centres

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Sub-specialized tropical medicine laboratories are essential for the accurate diagnosis of many tropical infections that may not be correctly diagnosed in conventional laboratories. However, processing and examination of 'identical' biofluid specimens such as blood, stool and urine at different laboratories often results in considerable diagnostic discrepancies. For example, interlaboratory differences have been documented in the microscopic examination of malaria slides [1], and discrepancies have been observed when comparing different rapid tests for malaria diagnosis [2]. The aim of this study was to compare the results obtained at sub-specialized tropical medicine laboratories for the microscopic diagnosis of helminths and intestinal protozoa in faecal samples obtained from a rural community in an endemic area of sub-Saharan Africa. The study compared two European tropical disease diagnostic centres with each other and one of the two European centres with a West African centre.

Our study was designed as a cross-sectional community-based survey and was carried out in May 2002 in the village of Zouatta II, western Côte d'Ivoire. This village is endemic for *Schistosoma mansoni*, soil-transmitted helminths (*Ascaris lumbricoides*, hookworms and *Trichuris trichiura*) and intestinal protozoa [3]. Polyparasitism is extremely common [3, 4]. Details of the study area, as well as field and laboratory procedures, have been presented elsewhere [3]. In brief, approval of the study was obtained from the institutional review boards of the Centre Suisse de Recherches Scientifiques (Abidjan, Côte d'Ivoire) and the Swiss Tropical Institute (Basel, Switzerland). The study received ethical clearance from the Ministry of Public Health in Côte d'Ivoire and permission was also granted to us by local village authorities.

Three consecutive morning stool specimens were collected over a 3-day period from 561 randomly selected community members aged 5 days to 91 years. Specimens were transferred to a laboratory in the nearby district town and processed the same day. From each specimen, a 42 mg Kato–Katz thick smear was prepared according to a standard procedure [5]. The slides were allowed to clear for 30–45 min prior to examination under a light microscope by experienced laboratory technicians. Specimens were analysed for *S. mansoni* and soil-transmitted helminths, with the numbers of eggs counted and recorded for each species separately. Individuals who tested positive for *S. mansoni* and/or soil-transmitted helminths were treated with a single oral dose of 40 mg/kg praziquantel and/or 400 mg albendazole, respectively. In addition, 1–2 g of each stool specimen was conserved in a sodium acetate-acetic acid-formalin (SAF) solution on the same day of collection for future processing 6–12 months later at the other diagnostic centres.

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Two interlaboratory comparisons were performed. The first comparison was made between two European diagnostic centres sub-specialized in tropical diseases (A and B). Fifty randomly selected SAF-conserved stool specimens from collection day 2 were shaken and then split into two equal parts; one half was examined by European centre A and the other half was examined by European centre B. Two specimens were discarded because the participants had provided less than three stool samples for Kato–Katz processing, resulting in a sample size of 48.

The second comparison was made between European centre A and a West African diagnostic centre. A total of 78 randomly selected SAF-preserved samples were compared; centre A analysed samples obtained from participants on collection day 1 or 2, while the West African diagnostic centre analysed corresponding stool samples obtained from the same participants on collection day 3. In these three laboratories, stool specimens were processed using a formol-ether concentrate, according to the procedure outlined by Allen and Ridley [6], and analysed for the presence of helminth eggs and intestinal protozoa under a light microscope by experienced laboratory technicians.

Data were first entered into Microsoft Excel and then transferred to EpiInfo version 6.04 (Centre for Disease Control and Prevention, Atlanta, GA, USA) for statistical analysis. The results obtained for the individual SAF-preserved stool specimens examined at European centre A and European centre B were compared against the laboratory gold standard, which was the results of the three locally performed Kato–Katz thick smears for the corresponding participants plus the collective results obtained by European centers A and B for the 48 SAF-preserved specimens. This laboratory gold standard was devised to maximize sensitivity values, with the underlying rationale being that an egg detected at any level of processing (e.g. 'on the spot' by the Kato–Katz technique or in specialized European laboratories) should count as a true-positive result. Negative predictive values (NPV) were also calculated; however, specificity and

Table 1 Interlaboratory differences in microscopic examination of faecal samples for helminths and intestinal protozoa between two European tropical disease diagnostic centres A and B (n=48) and between European centre A and a West African centre (n=78)

Parasite	Number (%)		Kappa coefficient			Number (%)		Kappa coefficient		
	European centre A	European centre B	Difference	κ (SE)	p value	European centre A	West African centre	Difference	κ (SE)	p value
Schistosoma mansoni	19 (38)	5 (10)	14 (28)	0.21 (0.10)	0.02	24 (31)	9 (12)	15 (19)	0.16 (0.09)	0.04
Soil-transmitted helminths										
Hookworm	18 (36)	12 (24)	6 (12)	0.43 (0.14)	<0.01	31 (40)	15 (19)	16 (21)	0.18 (1.10)	0.03
Ascaris lumbricoides	0	0	0	NA		0	0	0	NA	
Trichuris trichiura	0	0	0	NA		0	1 (1)	1 (1)	0.00 (0.07)	0.50
Intestinal protozoa										
Blastocystis hominis	29 (58)	38 (76)	9 (18)	−0.00 (0.13)	0.51	38 (49)	7 (9)	31 (40)	0.08 (0.07)	0.10
Chilomastix mesnili	7 (14)	0	7 (14)	NA		9 (12)	7 (9)	2 (3)	0.44 (0.11)	<0.01
Endolimax nana	10 (20)	2 (4)	8 (16)	0.29 (0.10)	<0.01	12 (15)	15 (19)	3 (4)	−0.03 (0.11)	0.60
Entamoeba coli	35 (70)	34 (68)	1 (2)	0.76 (0.14)	<0.01	50 (64)	34 (44)	16 (20)	0.21 (0.10)	0.02
Entamoeba hartmanni	16 (32)	0	16 (32)	NA		20 (26)	2 (3)	18 (23)	−0.05 (0.06)	0.80
Entamoeba histolytica/ E. dispar	18 (36)	2 (4)	16 (32)	0.13 (0.72)	0.03	25 (33)	10 (13)	15 (20)	0.33 (0.10)	<0.01
Giardia duodenalis	6 (12)	5 (10)	1 (2)	0.69 (0.14)	<0.01	7 (9)	7 (9)	0	0.37 (0.11)	<0.01
Iodamoeba bütschlii	9 (18)	8 (16)	1 (2)	0.36 (0.14)	<0.01	16 (21)	2 (3)	14 (18)	−0.05 (0.07)	0.77

SE standard error, *NA* not applicable

positive predictive values (PPV) could not be determined with this approach. Sensitivity and NPV were also estimated using the mathematical model of Marti and Koella [7]. This model relates the number of stool samples found to be positive for ova and parasites to false-negative results and is an accurate method of predicting sensitivity based on the number of stool specimens provided. Positivity rates were calculated as the number of positive diagnoses per number of samples analysed and compared between the two European centres and between European centre A and the West African centre. A kappa coefficient was calculated to determine the degree of agreement between diagnoses at different centres.

Frank diagnostic discrepancies became apparent when comparing the results from the three Kato–Katz thick smears plus the SAF-preserved specimens analysed at European centres A and B (our laboratory gold standard) with those obtained for each individual SAF-preserved specimen processed at centre A and at centre B. For example, the diagnostic sensitivity values for *S. mansoni* at centres A and B were 71% (95% confidence interval [CI] 49–87%) and 17% (95% CI 6–38%), respectively. For hookworm, the respective sensitivity values for centres A and B were 75% (95% CI 53–98%) and 50% (95% CI 30–70%). Using a mathematical model to evaluate the results of microscopic examination of the three Kato–Katz thick smears [7], the overall diagnostic sensitivity and NPV for hookworms was 44 and 43%, respectively, while the diagnostic sensitivity and NPV for *S. mansoni* was 89 and 91%, respectively.

Major interlaboratory differences became apparent when comparing the rates of detection of *S. mansoni*, soil-transmitted helminths and intestinal protozoa in SAF-preserved specimens between European centres A and B and between European centre A and the West African centre. Table 1 summarizes the positivity rates and kappa agreement coefficients for the different helminths and intestinal protozoa investigated.

This study revealed considerable diagnostic discrepancies among laboratories that specialize in the diagnosis of tropical infections. In particular, European diagnostic centre B performed significantly worse than centre A, which warrants urgent attention. However, the tests employed in our study do not reflect the entire spectrum of diagnostic approaches that are used for returning travelers. Many travelers returning from endemic areas will be screened with serologic tests such as the enzyme-linked immunosorbent assay (ELISA) or the Falcon assay screening test (FAST) [8, 9], the Kato–Katz thick smear of fresh stool [5], or a combination of different stool-examination methods [10]. Such combinations of tests enhance the diagnostic performance and result in a higher sensitivity and specificity. Newer diagnostic tests, such as the polymerase chain reaction (PCR) for *S. mansoni*, also hold promise for improving future diagnoses [11].

Although many laboratories use SAF-preserved stool specimens, they will often examine multiple specimens from each patient in order to increase the diagnostic sensitivity. Microscopic examination of one SAF-preserved stool specimen is not the optimal diagnostic approach due to the irregular excretion pattern of most intestinal parasites and the difficulty of detecting large eggs, such as *S. mansoni*, which are frequently missed [12]. This approach consequently leads to false-negative results and an underestimation of the true prevalence. Still, microscopic examination of SAF-preserved stool samples is more sensitive than direct examination of fresh faecal smears in the diagnosis of intestinal protozoa [13]. Moreover, SAF concentration is used by many laboratories as a screening method for intestinal parasites. In case of clinical suspicion of a specific helminth infection, more sensitive methods should be employed (e.g. culture for *Strongyloides stercoralis*) [14].

In this study, we were surprised to see such a large degree of variance in the positivity rates reported by laboratories analysing the same SAF-preserved stool specimens. However, the discrepancy in results obtained by the West African diagnostic centre and European centre A could be explained by the fact that these centres tested stool samples collected from the same individuals on different days, since day-to-day sample variation has been documented for *S. mansoni* and hookworms [15]. In general, diagnostic discrepancies among laboratories specialized in tropical parasitology is likely multifactorial, involving such aspects as the level of training of laboratory personnel, malfunctioning or dated laboratory equipment, and human error. A multifaceted approach is thus necessary to decrease the incidence of interlaboratory discrepancies and it typically involves three general initiatives. (a) Institutions that train laboratory personnel must be up-to-date and adhere to the highest standards of care, although this may be difficult in resource-poor settings, such as sub-Saharan Africa. (b) Laboratories should utilize a series of standardized methodologies designed to reduce errors and to facilitate communication and collaboration among diagnostic centres. (c) Systems that reduce human error should be introduced to the laboratory environment [16]. Such error-reducing systems are often technologically oriented, but they can also be as simple as having multiple people confirm that transcribed information is correct, or using a series of standardized laboratory procedures to reduce the number of false-positive and false-negative results. Such a combined approach will decrease the high level of discrepancies in laboratory analyses.

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