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Comparison of the Kato-Katz method and ether-concentration technique for the diagnosis of soil-transmitted helminth infections in the framework of a randomised controlled trial

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Abstract Soil-transmitted helminth infections are a major public health problem. An accurate diagnosis is important in order to identify individuals and communities in need of intervention, and for monitoring drug efficacy and potential emergence of resistance. We compared the accuracy of the Kato-Katz method and ether-concentration technique for the diagnosis of soil-transmitted helminth infections within a randomised controlled trial. Quadruplicate Kato-Katz thick smears (duplicate Kato-Katz from two stool samples each) were examined before (baseline) and 3 weeks after treatment (follow-up). Additionally, at baseline and follow-up, the first stool sample was subjected to an ether-concentration method. We determined the prevalence, sensitivity, negative predictive value, diagnostic agreement and cure rates for single and duplicate Kato-Katz thick smears from the first stool sample, quadruplicate Kato-Katz thick smears produced from two stool samples and single ether-concentration as compared to our 'gold' standard (i.e. quadruplicate Kato-Katz plus ether-concentration). Quadruplicate Kato-Katz revealed a higher sensitivity than single ether-concentration for *Trichuris trichiura* at baseline (94.3 % vs. 88.5 %, $p = 0.002$) and follow-up (93.8 % vs. 83.5 %, $p < 0.001$). In contrary, at follow-up, ether-concentration showed a higher sensitivity than quadruplicate Kato-Katz for *Ascaris lumbricoides* diagnosis (86.7 % vs. 46.7 %, $p = 0.012$). The ether-concentration method showed similar or slightly higher sensitivity than the Kato-Katz technique based on a single stool sample for all soil-transmitted helminth infections. The estimated cure rates were heavily dependent on the diagnostic technique and sampling effort. In conclusion, data on the prevalence of soil-transmitted helminth infections and the efficacy of anthelminthics are greatly influenced by the diagnostic method and sampling effort. The ether-concentration technique is a valuable alternative to the Kato-Katz method for helminth diagnosis.

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Introduction

Chronic infections with one or several of the common soil-transmitted helminths (i.e. *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) cause an estimated global burden of 5.2 million disability-adjusted life years (DALYs) [1]. Preventive chemotherapy, that is, the large-scale administration of anthelminthic drugs (mainly the two benzimidazoles albendazole and mebendazole) to at-risk populations to avert morbidity, is the mainstay of control [2–4]. Accurate diagnostic tools are of pivotal importance for the identification of infected individuals, assessing endemicity in a given epidemiological setting and monitoring of drug efficacy and response to interventions, including resistance development [5, 6]. The most widely used technique to diagnose intestinal helminth infections (i.e. soil-transmitted helminths, *Schistosoma*

japonicum, *S. mansoni* and *S. mekongi*) is the Kato-Katz method [7–11]. A single Kato-Katz thick smear examines, on average, 41.7 mg of stool on a microscopic slide for the detection and quantification of helminth eggs. The diagnostic sensitivity of the Kato-Katz method can be improved by examining multiple thick smears from a single stool sample or by examining multiple stool samples [8, 12–17]. Nevertheless, the Kato-Katz technique has limitations, in particular, when the prevalence of soil-transmitted helminth infections is lower than 20 % or when infection intensities are low [5, 18].

The ether-concentration method is a diagnostic approach which can detect soil-transmitted helminth eggs within a large amount of a fixed stool sample [19]. For example, a recent study conducted in the People's Republic of China revealed that the ether-concentration method based on one single stool sample is nearly as sensitive as the Kato-Katz method based on two to three stool samples for the diagnosis of soil-transmitted helminth infections [14].

The aim of the present study was to assess the sensitivity of single and multiple Kato-Katz thick smears in comparison to the ether-concentration method for the diagnosis of soil-transmitted helminths before and after a treatment intervention. Stool samples were collected in the frame of a randomised controlled trial, carried out among school-aged children on Pemba Island, Tanzania, which evaluated the efficacy and safety of nitazoxanide, albendazole and nitazoxanide–albendazole against *T. trichiura* infections [20]. We assessed the influence of the diagnostic technique and sampling effort on the prevalence of soil-transmitted helminth infections before and after treatment, as well as on observed cure rates (CRs).

Materials and methods

Ethics statement

The data presented here stem from a randomised controlled trial carried out among school-aged children on Pemba Island, Tanzania. The ethics statement has been presented previously [20]. In short, ethical clearance was obtained from the Ministry of Health and Social Welfare in Zanzibar (ZAMREC, reference no. 0001/010) and from the ethics committee of Basel, Switzerland (EKBB, reference no. 225/10). The study is registered at Current Controlled Trials (ISRCTN08336605). Written informed consent was acquired from the children's parents or legal guardians to participate in this trial. Children provided assent. At the end of the study, all children remaining positive for soil-transmitted helminths were offered standard treatment (i.e. 400 mg albendazole).

Collection and processing of stool samples

Stool samples were collected from school-aged children participating in the trial, which assessed the safety and efficacy of the following four treatments: (i) nitazoxanide–albendazole combination (1,000 mg–400 mg), with each drug given separately on two consecutive days; (ii) single albendazole (400 mg); (iii) single nitazoxanide (1,000 mg); and (iv) placebo. The clinical trial took place from June to July 2011. The study setting and trial procedures have been described elsewhere [20].

Two stool samples collected before (baseline) and 3 weeks after treatment (follow-up) were available from 657 children. From each stool sample, duplicate Kato-Katz thick smears were prepared according to guidelines put forward by the World Health Organization (WHO) [18]. Within 30 min, Kato-Katz thick smears were examined quantitatively for hookworm eggs. Subsequently, the slides were re-read for *A. lumbricoides* and *T. trichiura* eggs that were counted and recorded separately. Additionally, from 550 children, approximately 2 g of stool from the first specimen before and after treatment were fixed in 10 ml of 5 % formalin in 15-ml Falcon tubes. Formalin-fixed samples were transferred to the Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, Switzerland, and semi-quantitatively analysed for soil-transmitted helminth eggs and intestinal protozoan cysts within 10 months, using an ether-concentration method [21, 22]. Results pertaining to intestinal protozoa infections have been described elsewhere [23]. Complete datasets were available from 528 individuals (i.e. quadruplicate Kato-Katz thick smears before and after treatment, as well as a single formalin-fixed stool sample subjected to an ether-concentration method before and after treatment) and considered for the present work.

Statistical analysis

All data were double-entered into an Excel file (Microsoft 2010) and cross-checked. For statistical analysis, Stata version 10.1 was used (StataCorp., College Station, TX, USA).

The prevalence at baseline and treatment follow-up was calculated as the percentage of individuals diagnosed positive for a specific soil-transmitted helminth species, considering the following diagnostic approaches: (i) the first Kato-Katz thick smear from the first stool sample; (ii) duplicate Kato-Katz thick smears from the first stool sample; (iii) quadruplicate Kato-Katz thick smears (two stool samples, each subjected to duplicate Kato-Katz); and (iv) single ether-concentration test from the first stool sample. As the diagnostic 'gold' standard, we considered the combined results from quadruplicate Kato-Katz thick smears plus single ether-concentration. Each sample found positive with either method was considered as "true positive". The sensitivity and negative predictive value (NPV) were calculated for the different diagnostic

approaches and sampling efforts before and after treatment, assuming that the 'gold' standard had a sensitivity of 100 %. Differences in prevalence were determined under the assumption that non-overlapping 95 % confidence intervals (CIs) indicate statistical significance. Cohen's kappa measure was used to assess agreement between the two methods and between different sampling efforts for the Kato-Katz technique, as follows: κ<0, no agreement; κ=0–0.20, poor agreement; κ=0.21–0.40, fair agreement; κ=0.41–0.60, moderate agreement; κ=0.61–0.80, substantial agreement; and κ=0.81–1.00, nearly perfect agreement [24, 25]. The McNemar test was used to examine differences in sensitivity between diagnostic methods. Therefore, only individuals who were positive according to the 'gold' standard were included. For a sample size of 20 or fewer individuals in the discordant pair, the exact McNemar value was taken ($p*$), otherwise, we used the McNemar χ^2 value (p) [26].

The CR was calculated as the percentage of individuals who were diagnosed negative at the 3-week post-treatment follow-up, but had a positive diagnostic test before drug administration. CRs were calculated for each diagnostic approach and for each treatment arm individually. Differences between CRs among the two diagnostic methods and varying sampling efforts were compared with a two-sample test of proportion.

Results

Baseline data

The overall prevalence in school-aged children in the study setting before treatment based on our 'gold' standard was 87.1 % (95 % CI, 84.3–90.0 %) for T. trichiura, 11.2 % (95 % CI, 8.5–13.9 %) for hookworm and 6.8 % (95 % CI, 4.7–9.0 %) for A. lumbricoides (Table 1). For all three soil-transmitted helminth species, statistically significantly lower prevalences were calculated when relying on the Kato-Katz method from a single stool sample compared to the 'gold' standard, regardless of whether stool samples were subjected to single or duplicate Kato-Katz thick smears.

Duplicate Kato-Katz thick smears identified a larger number of children with T. trichiura and A. lumbricoides infection compared to the ether-concentration method, while the ether-concentration method detected slightly more hookworm cases. However, these differences lacked statistical significance, as indicated by overlapping 95 % CIs.

Our study revealed a high sensitivity for all diagnostic methods for the detection of T. trichiura eggs (Table 1). On the contrary, the sensitivity for hookworm and A. lumbricoides was considerably lower for all diagnostic methods. Quadruplicate Kato-Katz thick smears showed the highest sensitivity for T. trichiura and A. lumbricoides

diagnosis compared to less intensive sampling and the ether-concentration method. The McNemar test revealed a statistically significantly higher sensitivity at baseline using quadruplicate Kato-Katz thick smears compared to the ether-concentration method for diagnosing T. trichiura (94.3 % vs. 88.5 %; $p=0.002$; Table 2). The ether-concentration method had the highest sensitivity for hookworm. We observed a significant difference for hookworm diagnosis with the ether-concentration method compared to a single Kato-Katz (69.5 % vs. 39.0 %; $p=0.004$).

NPVs were high for both methods and different sampling efforts of the Kato-Katz technique for hookworm and A. lumbricoides diagnosis (92.9–98.2 %), while lower NPVs were observed for T. trichiura, ranging from 49.6 % (single Kato-Katz) to 72.3 % (quadruplicate Kato-Katz).

The agreement between the ether-concentration method and quadruplicate Kato-Katz thick smears was moderate for all helminth species before treatment (T. trichiura, κ=0.54; A. lumbricoides, κ=0.48; hookworm, κ=0.47) (Table 2).

Treatment follow-up

The overall prevalence 3 weeks after treatment according to our 'gold' standard was 84.9 % (95 % CI, 81.8−87.9 %) for T. trichiura, 7.2 % (95 % CI, 5.0−9.4 %) for hookworm and 5.7 % (95 % CI, 3.7−7.7 %) for A. lumbricoides (Table 3). Single stool examination with the Kato-Katz and ether-concentration methods resulted in a significantly lower prevalence for T. trichiura compared to the 'gold' standard. Duplicate Kato-Katz thick smears revealed a significantly higher number of T. trichiura compared to the ether-concentration method. Duplicate Kato-Katz showed a better diagnostic performance than the ether-concentration method with regard to detecting hookworm eggs. On the other hand, the ether-concentration method detected more A. lumbricoides cases compared to quadruplicate Kato-Katz thick smears. However, these differences lacked statistical significance.

The McNemar test revealed that quadruplicate Kato-Katz thick smears were more sensitive for diagnosing T. trichiura than the ether-concentration method (93.8 % vs. 83.5 %; $p < 0.001$; Table 2). On the other hand, the ether-concentration method showed a significantly higher sensitivity for A. lumbricoides compared to the Kato-Katz method based on one stool sample (86.7 vs. 40.0; $p=0.003$), but also compared to quadruplicate Kato-Katz thick smears (86.7 vs. 46.7; $p=0.012$).

The NPVs calculated at the 3-week post-treatment follow-up for T. trichiura ranged from 55.2 % (single Kato-Katz) to 74.1 % (quadruplicate Kato-Katz), while the NPVs for hookworm and A. lumbricoides diagnosis were above 95 % for both techniques, regardless of the sampling effort. The agreement between quadruplicate Kato-Katz thick smears and the ether-concentration method was moderate at follow-up

Table 1 Prevalence of soil-transmitted helminth infections on Pemba Island, Tanzania, in mid-2011 according to different diagnostic methods and their corresponding sensitivities and negative predictive values at baseline

Parasite	Diagnostic technique	Number of infected children			Sensitivity in % (95 % CI)	NPV (95 % CI)
		n	%	95 % CI		
T. trichiura	'Gold' standard	460	87.1	84.3–90.0	100.0	100.0
	Single Kato-Katz	391	74.1	70.3–77.8	85.0 (81.4–88.1)	49.6 (41.0–58.3)
	Duplicate Kato-Katz	406	76.9	74.4–80.5	88.3 (85.0–91.1)	55.7 (46.5–64.6)
	Quadruplicate Kato-Katz	434	82.2	78.9–85.5	94.3 (91.8–96.3)	72.3 (62.0–80.8)
	Ether-concentration	407	77.1	73.5–80.7	88.5 (85.2–91.2)	56.2 (46.9–65.1)
Hookworm	'Gold' standard	59	11.2	8.5–13.9	100.0	100.0
	Single Kato-Katz	23	4.4	2.6–6.1	39.0 (26.5–52.6)	92.9 (90.2–94.9)
	Duplicate Kato-Katz	24	4.6	2.8–6.3	40.7 (28.1–54.3)	93.1 (90.4–95.0)
	Quadruplicate Kato-Katz	38	7.2	5.0–9.4	64.4 (50.9–76.4)	95.7 (93.4–97.3)
	Ether-concentration	41	7.8	5.5–10.1	69.5 (56.1–80.8)	96.3 (94.1–97.7)
A. lumbricoides	'Gold' standard	36	6.8	4.7–9.0	100.0	100.0
	Single Kato-Katz	14	2.7	1.3–4.0	38.9 (23.1–56.5)	95.7 (93.5–97.2)
	Duplicate Kato-Katz	16	3.0	1.6–4.5	44.4 (27.9–61.9)	96.1 (93.9–97.5)
	Quadruplicate Kato-Katz	27	5.1	3.2–7.0	75.0 (57.8–87.9)	98.2 (96.5–99.1)
	Ether-concentration	21	4.0	2.3–5.7	58.3 (40.8–74.5)	97.0 (95.1–98.3)

(*T. trichiura*, κ=0.49; *A. lumbricoides*, κ=0.48; hookworm, κ=0.42) (Table 2).

Estimated CRs

CRs calculated for each diagnostic approach for the three soil-transmitted helminths, stratified by treatment arm, are presented in Table 4. According to our 'gold' standard, CRs for *T. trichiura* were 11.7 % (95 % CI, 5.3–18.0 %) for the nitazoxanide–albendazole combination, 9.1 % (95 % CI, 3.6–14.5 %) for single albendazole and 1.6 % (95 % CI, 0.0–3.9 %) for single nitazoxanide. Overall CRs (including the placebo treatment arm), according to our 'gold' standard, were significantly lower compared to all diagnostic tests relying on a single stool sample ('gold' 7.2 %; single Kato-Katz, 16.4 %; duplicate Kato-Katz, 15.8 %; ether-concentration, 17.2 %; 'gold' vs. single Kato Katz *p* <0.001; 'gold' vs. duplicate Kato-Katz, *p* <0.001; 'gold' vs. ether-concentration *p* <0.001). Borderline significance was observed for quadruplicate Kato-Katz thick smears (CR: 10.8 %) compared to the 'gold' standard (*p* = 0.056). CRs based on quadruplicate Kato-Katz thick smears were significantly lower compared to diagnostic approaches based on a single stool sample (quadruplicate Kato-Katz vs. single Kato-Katz, *p* =0.012; duplicate Kato-Katz vs. single Kato-Katz, *p* =0.035; quadruplicate Kato-Katz vs. ether-concentration, *p* =0.008).

The overall CR according to the 'gold' standard against hookworm was 59.3 % (95 % CI, 46.4−72.2 %). The CR for the individual diagnostic approaches were higher (Table 4); however, the differences lacked statistical significance. For

A. lumbricoides, we determined an overall CR of 63.9 % (95 % CI, 47.4−80.4 %) using the 'gold' standard. Using single or duplicate Kato-Katz from a single stool sample and the ether-concentration technique resulted in lower overall CRs than the 'gold' standard, while higher CRs were recorded when analysing quadruplicate Kato-Katz (all *p* >0.05).

Discussion

Accurate diagnostic methods are mandatory for assessing soil-transmitted helminth infections, drug efficacies and the possible development of drug resistance [3, 5, 6, 27]. The most widely used technique for diagnosing soil-transmitted helminth infections is the Kato-Katz method [18]. Because of the low sensitivity of a single Kato-Katz thick smear, it is recommended to collect consecutive stool samples that are subjected to multiple Kato-Katz thick smears to enhance the sensitivity [12, 14–16]. We assessed the diagnostic performance of single, duplicate (from a single stool sample) and quadruplicate Kato-Katz thick smears (from two samples) in the frame of a randomised controlled trial and compared the results with an ether-concentration technique using formalin-fixed stool samples. The latter method is rarely used in settings where soil-transmitted helminths are endemic, although it is often used in reference laboratories in Europe [22].

Our results demonstrate that the ether-concentration method has a similar or even higher sensitivity for the diagnosis of all three soil-transmitted helminths than single or duplicate Kato-Katz prepared from a single stool sample. Hence, we

Table 2 Agreement between ether-concentration and Kato-Katz (different sampling efforts) for the diagnosis of soil-transmitted helminths among school-aged children on Pemba Island, Tanzania, in mid-2011

Before treatment		Ether-concentration		Total	Kappa	McNemar	After treatment		Ether-concentration		Total	Kappa	McNemar
		−	+						−	+			
T. trichiura													
Single Kato-Katz	−	83	54	137	0.53	p=0.095	Single Kato-Katz	−	101	44	145	0.55	p=0.361
	+	38	353	391				+	53	330	383		
	Total	121	407	528				Total	154	374	528		
Duplicate Kato-Katz	−	74	48	122	0.49	p=0.918	Duplicate Kato-Katz	−	99	43	142	0.54	p=0.225
	+	47	359	406				+	55	331	386		
	Total	121	407	528				Total	154	374	528		
Quadruplicate Kato-Katz	−	68	26	94	0.54	p=0.002	Quadruplicate Kato-Katz	−	80	28	108	0.49	p<0.001
	+	53	381	434				+	74	346	420		
	Total	121	407	528				Total	154	374	528		
Hookworm													
Single Kato-Katz	−	476	29	505	0.34	p=0.004	Single Kato-Katz	−	495	14	509	0.37	p=0.549
	+	11	12	23				+	11	8	19		
	Total	487	41	528				Total	506	22	528		
Duplicate Kato-Katz	−	476	28	504	0.36	p=0.007	Duplicate Kato-Katz	−	495	14	509	0.31	p=0.549
	+	11	13	24				+	11	8	19		
	Total	487	41	528				Total	506	22	528		
Quadruplicate Kato-Katz	−	469	21	490	0.47	p=0.631	Quadruplicate Kato-Katz	−	490	11	501	0.43	p=0.336
	+	18	20	38				+	16	11	27		
	Total	487	41	528				Total	506	22	528		
A. lumbricoides													
Single Kato-Katz	−	504	10	514	0.62	p=0.092*	Single Kato-Katz	−	499	17	516	0.46	p=0.003*
	+	3	11	14				+	3	9	12		
	Total	507	21	528				Total	502	26	528		
Duplicate Kato-Katz	−	502	10	512	0.58	p=0.302*	Duplicate Kato-Katz	−	499	17	516	0.46	p=0.003*
	+	5	11	16				+	3	9	12		
	Total	507	21	528				Total	502	26	528		
Quadruplicate Kato-Katz	−	492	9	501	0.48	p=0.221	Quadruplicate Kato-Katz	−	498	16	514	0.48	p=0.012*
	+	15	12	27				+	4	10	14		
	Total	507	21	528				Total	502	26	528		

*Instead of the McNemar χ^2 value, the exact McNemar value was taken because a sample size of 20 individuals or less was in the discordant pair

speculate that a diagnostic examination based on two stool samples using the ether-concentration technique reveals a higher sensitivity than quadruplicate Kato-Katz thick smears. For diagnosing *A. lumbricoides*, the ether-concentration method showed a significantly higher sensitivity compared to quadruplicate Kato-Katz thick smears at the 3-week post-treatment follow-up. This might be explained by the large amount of stool (~2 g) examined with the ether-concentration method, which should allow the detection of low infection intensities. The ether-concentration method offers the advantage of preservation. Stool samples are maintained in low-concentration formalin or within sodium acetate-acetic acid formalin (SAF), and, hence, can be kept for several weeks or months prior to microscopic examination for soil-transmitted helminth eggs as well as for intestinal protozoan cysts at a later time point [21, 28]. This diagnostic technique is, therefore, a useful alternative to the Kato-Katz method, particularly in remote resource-constrained settings [29]. It is interesting to note that mobile phone microscopy might be an alternative promising diagnostic method in underserviced rural areas. The proof-of-concept of a 'first-generation' mobile phone light microscope was recently demonstrated in our study setting on Pemba Island [30].

The diagnostic accuracy was the same if either single or duplicate Kato-Katz thick smears were examined (single Kato-Katz vs. duplicate Kato-Katz). This result is in contrast to a recent study, which reported that multiple Kato-Katz thick smears from a single stool sample enhances the overall sensitivity for hookworm and *T. trichiura* [16]. Setting-specific differences in prevalence and infection intensities might

Table 3 Prevalence of soil-transmitted helminth infections on Pemba Island, Tanzania, in mid-2011, according to different diagnostic methods and their corresponding sensitivities and negative predictive values 3 weeks post-treatment

Parasite	Diagnostic technique	Number of infected school children			Sensitivity in % (95 % CI)	NPV (95 % CI)
		n	%	95 % CI		
T. trichiura	'Gold' standard	448	84.9	(81.8−87.9)	100	100
	Single Kato-Katz	383	72.5	(68.7−76.4)	85.5 (81.9−88.6)	55.2 (46.7−63.4)
	Duplicate Kato-Katz	386	73.1	(69.3−76.9)	86.2 (82.6−89.2)	56.3 (47.8−64.6)
	Quadruplicate Kato-Katz	420	79.6	(76.1−83.0)	93.8 (91.1−95.8)	74.1 (64.6−81.8)
	Ether-concentration	374	70.8	(66.9−74.7)	83.5 (79.7−86.8)	51.9 (43.8−60.0)
Hookworm	'Gold' standard	38	7.2	(5.0-9.4)	100	100
	Single Kato-Katz	19	3.6	(2.0−5.2)	50.0 (33.4−66.6)	96.3 (94.1−97.7)
	Duplicate Kato-Katz	19	3.6	(2.0−5.2)	50.0 (33.4−66.6)	96.3 (94.1−97.7)
	Quadruplicate Kato-Katz	27	5.1	(3.2−7.0)	71.1 (54.1−84.6)	97.8 (96.0−98.8)
	Ether-concentration	22	4.2	(2.5−5.9)	57.9 (40.8−73.7)	96.8 (94.8−98.1)
A. lumbricoides	'Gold' standard	30	5.7	(3.7−7.7)	100	100
	Single Kato-Katz	12	2.3	(1.0−3.6)	40.0 (22.7−59.4)	96.5 (94.4−97.9)
	Duplicate Kato-Katz	12	2.3	(1.0−3.6)	40.0 (22.7−59.4)	96.5 (94.4−97.9)
	Quadruplicate Kato-Katz	14	2.7	(1.3−4.0)	46.7 (28.3−65.7)	96.9 (94.9−98.1)
	Ether-concentration	26	4.9	(3.1−6.8)	86.7 (69.3−96.2)	99.2 (97.8−99.7)

explain this observation [8, 31–33]. On the other hand, collecting multiple stool samples had a significant effect on improving the diagnostic sensitivity, and this observation corroborates previous findings [12, 14–16]. Hence, collecting multiple stool samples is often the method of choice for diagnosing soil-transmitted helminths in intervention trials.

Table 4 Cure rates (CR) determined following treatment with nitazoxanide–albendazole, albendazole, nitazoxanide and placebo against soil-transmitted helminths among school-aged children on Pemba Island, Tanzania, according to different diagnostic techniques

	Cure rate				
	Overall	Nitazoxanide–albendazole	Albendazole	Nitazoxanide	Placebo
T. trichiura					
Single Kato-Katz (95 % CI)	16.4 (12.7−20.1)	19.8 (11.2−28.4)	20.4 (12.3−28.5)	12.9 (6.2−19.5)	13.2 (6.7−19.8)
Duplicate Kato-Katz (95 % CI)	15.8 (12.2−19.3)	19.3 (10.9−27.7)	19.8 (11.9−27.7)	11.2 (5.1−17.3)	13.6 (7.1−20.2)
Quadruplicate Kato-Katz (95 % CI)	10.8 (7.9−13.8)	14.6 (7.4−21.8)	15.2 (8.2−22.2)	5.3 (1.1−9.4)	9.2 (4.0−14.5)
Ether-concentration (95 % CI)	17.2 (13.5−20.9)	22.0 (13.3−30.6)	17.3 (9.7−25.0)	19.4 (11.9−27.0)	10.9 (5.0−16.8)
'Gold' standard	7.2 (4.8−9.5)	11.7 (5.3−18.0)	9.1 (3.6−14.5)	1.6 (0.0−3.9)	7.2 (2.6−11.8)
Hookworm					
Single Kato-Katz (95 % CI)	69.6 (49.2−89.9)	80.0 (24.5−100.0)	83.3 (40.5−100.0)	83.3 (40.5−100.0)	33.3 (0.0−87.5)
Duplicate Kato-Katz (95 % CI)	66.7 (46.3−87.0)	80.0 (24.5−100.0)	83.3 (40.5−100.0)	71.4 (26.3−100.0)	33.3 (0.0−87.5)
Quadruplicate Kato-Katz (95 % CI)	71.1 (55.9−86.2)	88.9 (63.3−100.0)	80.0 (49.8−100.0)	66.7 (28.2−100.0)	50.0 (12.3−87.7)
Ether-concentration (95 % CI)	73.2 (59.0−87.3)	83.3 (58.6−100.0)	100.0 (−)	33.3 (0.0−71.8)	75.0 (46.3−100.0)
'Gold' standard	59.3 (46.4−72.2)	75.0 (51.2−98.8)	75.0 (46.3−100.0)	38.5 (7.9−69.1)	50.0 (24.4−75.6)
A. lumbricoides					
Single Kato-Katz (95 % CI)	57.1 (27.5−86.8)	100.0 (−)	100.0 (−)	50.0 (0.0−100.0)	0.0 (−)
Duplicate Kato-Katz (95 % CI)	62.5 (35.9−89.1)	100.0 (−)	100.0 (−)	50.0 (0.0−100.0)	0.0 (−)
Quadruplicate Kato-Katz (95 % CI)	70.4 (52.0−88.8)	100.0 (−)	100.0 (−)	70.0 (35.4−100.0)	16.7 (0.0−59.5)
Ether-concentration (95 % CI)	52.4 (29.1−75.7)	100.0 (−)	100.0 (−)	50.0 (12.3−87.7)	0.0 (−)
'Gold' standard	63.9 (47.4−80.4)	100.0 (−)	87.5 (57.9−100.0)	60.0 (31.9−88.1)	14.3 (0.0−49.2)

We observed significantly lower CRs for *T. trichiura*, preparing duplicate Kato-Katz thick smears from two stool samples compared to all diagnostic methods relying on only one stool sample, which re-emphasises the importance of an accurate diagnostic test in clinical trials. Further, our 'gold' standard had lower CRs compared to quadruplicate Kato-Katz thick smears for *T. trichiura*. Even though this result showed borderline significance, it demonstrates that sensitive tools for diagnosing soil-transmitted helminth infections are warranted. On the other hand, it is interesting to note that duplicate Kato-Katz revealed higher CRs for *A. lumbricoides*. However, no statistical significance was observed, but this might be explained by the relatively small sample size. Several studies showed that the FLOTAC technique revealed higher sensitivities compared to multiple stool samples examined with the Kato-Katz method [15, 16, 32, 34–36] and also compared to the ether-concentration technique [16]. However, the FLOTAC technique, as well as the ether-concentration method, requires additional laboratory equipment, such as a centrifuge, and is also more expensive than the Kato-Katz method [10].

Our study has the following limitations. First and foremost, for the ether-concentration method, helminth eggs were only counted in a semi-quantitative manner. Therefore, it was only possible to calculate the prevalence and CR, but no data could be provided on infection intensities and egg reduction rates, an important parameter for the evaluation of anthelminthic drug efficacy [37]. Second, all positive results obtained by the different techniques were recorded as "true positive". Though this is a common standard in helminth diagnosis [33, 38], it is worth highlighting that false-positive results might be reported even by well-trained laboratory technicians due to artefacts in stool that are misinterpreted as helminth eggs or due to writing errors on the entry forms. To our knowledge, the effect of false-positive results for the diagnosis of soil-transmitted helminths has not yet been studied. Third, all diagnostic approaches revealed high CRs against hookworm in the placebo group (as high as 75 % for the ether-concentration technique), indicating a diagnostic problem for hookworm in general. We have previously attributed this finding to the low prevalence and intensity of hookworm infections [20]. Finally, as mentioned before, the sample size for hookworm as well as for *A. lumbricoides* was relatively small due to the low prevalence of these infections in our study setting (11.2 % and 6.8 % at baseline, respectively). Therefore, even though we did not find significant differences between the diagnostic techniques for these two helminth species, we should not assume that they do not exist. Note that, also, the sensitivities and NPVs are strongly influenced by prevalence [5]. Thus, these parameters should only be compared among different diagnostic methods and not among helminth species. A negative diagnostic result is more likely to be true (high NPV) when the prevalence is low, as was the case in our study for hookworm and *A. lumbricoides*.

To conclude, our study confirmed that a sensitive diagnostic method is crucial in order to reliably assess the prevalence of soil-transmitted helminth infections, as well as determine CRs in clinical trials, and that using insensitive diagnostic methods overestimates drug efficacy when CR is employed as the outcome measure. Our trial re-emphasises that collecting multiple stool samples is useful to enhance the sensitivity, especially in settings where infection intensities are low. The diagnostic accuracy of the ether-concentration method from a single formalin-fixed stool sample revealed moderate diagnostic agreement with quadruplicate Kato-Katz thick smears and a similar or even higher sensitivity for the diagnosis of all three soil-transmitted helminths than single or duplicate Kato-Katz thick smears prepared from a single stool sample. Hence, the ether-concentration method provides an alternative in settings where fresh stool samples cannot be directly examined.

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