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A single FLOTAC is more sensitive than triplicate Kato—Katz for the diagnosis of low-intensity soil-transmitted helminth infections

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Summary Accurate diagnostic tools are pivotal for patient management and surveillance of helminth control programmes, particularly in the current era of preventive chemotherapy. Three consecutive stool samples were obtained from 279 schoolchildren from Zanzibar, an island where anthelminthic drugs have been administered on a large scale for more than a decade. All stool samples were examined with the Kato—Katz method. Additionally, one sample per child was preserved in sodium acetate—acetic acid-formalin solution, and examined with the FLOTAC technique. Considering the pooled results of both methods as diagnostic 'gold' standard, the observed prevalences of *Trichuris trichiura*, hookworm and *Ascaris lumbricoides* were 63.4, 35.8 and 22.9%, respectively. The sensitivity of examining a single stool sample by FLOTAC for diagnosing *T. trichiura*, hookworm and *A. lumbricoides* was 88.7, 83.0 and 82.8%, respectively. Lower sensitivities were observed for Kato—Katz even after examining three stool samples: 71.8, 46.0 and 70.3%, respectively. Kato—Katz revealed considerably higher infection intensities than FLOTAC. The κ agreement between a single FLOTAC and triplicate Kato—Katz was 0.63 for diagnosing *A. lumbricoides* and 0.50 for *T. trichiura*, but only 0.30 for hookworm.

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The high sensitivity of FLOTAC holds promise for patient management, monitoring soil-transmitted helminth transmission and endpoint(s) of control at the population level.

1. Introduction

The most common soil-transmitted helminths are *Ascaris lumbricoides*, the hookworms (*Ancylostoma duodenale* and *Necator americanus*) and *Trichuris trichiura*. Globally, more than one billion people are infected with one or several of these intestinal nematodes.[1–3] Infections can cause chronic and debilitating disease with a global burden that might be as high as 39 million disability-adjusted life years.[4]

The regular administration of anthelminthic drugs to high-risk groups without prior diagnosis has become the strategy of choice for the control of helminth infections, and the term 'preventive chemotherapy' is now widely used.[5–7] There is growing emphasis on co-administering anthelminthic drugs to populations living in areas where multiple helminths co-exist.[3,4,8,9] Such large-scale interventions are usually school-based, as school-aged children generally are at highest risk of soil-transmitted helminth infection and constitute a readily accessible group within the community. Moreover, the beneficial impact of treatment is believed to be greatest during childhood development.[1,6]

The evidence base that repeated mass drug administration significantly reduces the prevalence and morbidity associated with soil-transmitted helminths is compelling.[5,10–12] On the Zanzibar islands, periodic school-based drug distribution has been implemented since 1994 using single-dose oral mebendazole (500 mg) and, from 2003 onwards, single-dose oral albendazole (400 mg).[13,14] Within the frame of the Global Programme to Eliminate Lymphatic Filariasis (GPELF), single-dose oral albendazole (400 mg) plus single-dose oral ivermectin (200 μg/kg) have been administered once a year to the whole eligible population in Zanzibar since 2001. These chemotherapy-based control efforts have significantly reduced the prevalence and intensity of soil-transmitted helminth infections, and have led to a decline in the burden caused by lymphatic filariasis.[9]

As low-intensity helminth infections are often missed if a single stool sample is examined by the widely applied Kato–Katz method,[15] there is a pressing need for the development and validation of alternative diagnostic tools. These tools should be accurate and user-friendly so that they can be utilized for surveillance of helminth control programmes. Recently, it has been shown that the FLOTAC technique (a non-invasive multivalent faecal egg-count technique)[16] holds promise for the accurate diagnosis of hookworm infections.[17] As FLOTAC uses a much larger amount of stool for the detection of helminth eggs than does the Kato–Katz method (up to 1 g vs. 41.7 mg) there is a greater chance of detecting helminth eggs, especially if contained in the sample at low density.

The aim of our study was to compare single and multiple Kato–Katz thick smears with a single FLOTAC examination for diagnosing *A. lumbricoides*, hookworm and *T. trichiura* in schoolchildren on the island of Unguja, Zanzibar, an area that has been targeted for annual anthelminthic treatment since 2003.

2. Materials and methods

2.1. Study area and population

The study was carried out in the primary schools of Chaani and Kinyasini, located in the North of Unguja, the main island of Zanzibar, United Republic of Tanzania, in June and July 2007. A total of 401 schoolchildren were randomly selected and invited to participate in the study.

2.2. Ethical considerations and anthelminthic treatment

This study was embedded in the 2007 parasitological school survey carried out annually by the Helminth Control Laboratory of Unguja (HCLU; Stone Town, Zanzibar). The study protocol was cleared by the World Health Organization (WHO; OD/TS-07-00331) and the Ministry of Health and Social Welfare (MoHSW) of Unguja (Stone Town, Zanzibar). Written informed consent to parasitological surveys and all related medical interventions at school level in Unguja is routinely obtained from the parents and/or legal guardians at the time children are registered for schooling. School directors were informed about the specific aims of the present study. After obtaining their consent, the purpose and procedure of the study were explained to the participating children. At study completion, all children were given a single 400 mg oral dose of albendazole regardless of their infection status in the frame of the annual mass drug administration conducted by the HCLU.

2.3. Field and laboratory procedures

The schoolchildren were invited to submit three early morning stool samples, collected over consecutive days. Filled containers were transferred to HCLU. Processing of stool samples was as follows. First, a single 41.7 mg Kato–Katz thick smear was prepared.[18] Kato–Katz thick smears were allowed to clear for 40–60 min before examination under a microscope by experienced laboratory technicians. The number of helminth eggs was counted and recorded for each helminth species separately. A random sample of 5% of the slides was re-examined by a senior technician for quality control. Second, ~1–2 g of stool from one out of the three consecutively submitted stool samples was transferred to a 20 ml plastic tube and conserved in 10 ml of a sodium acetate–acetic acid-formalin (SAF) solution[19] for subsequent FLOTAC examination.

The SAF-preserved stool samples were forwarded to the Department of Pathology and Animal Health, University of

Naples 'Federico II' (Naples, Italy). After ~6 months, the SAF-preserved samples were processed with the FLOTAC technique.[16] In brief, each sample was passed through a screen with an aperture of 350 μm in order to remove large fibres, and an additional 10 ml of SAF was added. Equal amounts of this stool-SAF suspension were pipetted into two pre-weighed 15 ml Falcon tubes and centrifuged for 3 min at 170 g using a Hettich centrifuge (Tuttlingen, Germany). The supernatant was discarded and the pellets weighed to the nearest milligram using a Gibertini balance (Milan, Italy). Each tube was then filled to the 6 ml mark with one of two flotation solutions, namely: (1) flotation solution no. 4 (sodium nitrate: $NaNO_3$ 315 g plus 685 ml H_2O; specific density = 1.20; designated 'S4'); and (2) flotation solution no. 13 (zinc sulphate and mercury II iodine and potassium iodide: $ZnSO_4 \times 7\ H_2O$ 600 g plus 600 ml H_2O and KI 78 g plus HgI_2 100 g plus 63 ml H_2O; specific density = 1.45; designated 'S13').[20] The pellets in the respective solutions were suspended, and 5 ml of the suspension transferred into one of the two chambers of the FLOTAC apparatus, each holding a volume of 5 ml. The apparatus was then centrifuged for 5 min at 120 g. Finally, after the translation of the top portion of the flotation chambers with the FLOTAC apparatus, microscopy of both observation grids at 100× magnification commenced. Helminth eggs were counted and separately recorded for each species according to the flotation solution used.

2.4. Statistical analysis

Data were entered twice in a Microsoft Excel 2002 spreadsheet (Microsoft Corp., Redmond, WA, USA). The consistency of the two files was validated using EpiInfo version 6.04d (Centers for Disease Control and Prevention; Atlanta, GA, USA).

Statistical analyses were carried out using JMP version 5.0.1 (SAS Institute, Cary, NC, USA) and EpiInfo. Only children with complete data records (i.e. three Kato–Katz thick smears and one FLOTAC) were included in the final analysis. We considered the pooled results from the FLOTAC (single stool sample) and the Kato–Katz thick smears (three consecutive stool samples) as diagnostic 'gold' standard. The prevalence of helminth infections, the sensitivity and the negative predictive value (NPV), including 95% confidence intervals (CIs), were calculated for a single FLOTAC examination, and the respective or all three Kato–Katz thick smears. The agreement between the FLOTAC and the triplicate Kato–Katz thick smear readings for the diagnosis of *A. lumbricoides*, hookworm and *T. trichiura* was assessed using κ statistics.[21]

The number of helminth eggs per gram of faeces (epg) was obtained by multiplying the number of helminth eggs recorded in the Kato–Katz thick smear by a factor of 24. The epg for the FLOTAC technique was estimated for each chamber separately, as follows: epg = (helminth-specific egg count × 1.2)/(weight of stool pellet and tube − weight of tube). The classification into low, medium and high infection intensity was based on the arithmetic mean epg derived from the three Kato–Katz readings, considering thresholds set forth by WHO.[22]

The arithmetic mean epg and standard error of the mean (SEM) for the whole study cohort as well as the 25, 50, 75 and 90% percentiles of the epg were calculated considering the results of the triplicate Kato–Katz and the single FLOTAC (both flotation solutions separately). Differences between the three mean epg values (i.e. FLOTAC according to the two different flotation solutions and the triplicate Kato–Katz results) were analysed using the Kruskal–Wallis test. Finally, pair-wise comparisons were made and analysed using the Wilcoxon test. Statistical significance was considered at a significance level of 0.05.

3. Results

3.1. Operational results

Figure 1 shows that of 401 randomly selected schoolchildren in Chaani and Kinyasini, 279 had complete data records, i.e. three stool samples examined with the Kato–Katz method and one SAF-preserved sample additionally examined with the FLOTAC technique, using two different flotation solutions. Hence, the overall compliance was 69.6%. The final study cohort comprised 164 (58.8%) girls and 115 boys (41.2%). The median age was 12 years (range: 7 to 20 years). Reasons for non-compliance were absence during collection days ($n = 25$), submission of only one or two stool samples ($n = 36$) and insufficient quantity of stool for SAF-conservation ($n = 61$).

The mean weight of the SAF-preserved stool pellets examined with S4 and S13 was 0.84 g (range: 0.19 to 2.61 g) and 0.85 g (range: 0.21 to 2.60 g), respectively.

3.2. Comparison of methods

Considering the pooled results from three Kato–Katz thick smears and a single FLOTAC as diagnostic 'gold' standard, the prevalence was 63.4, 35.8 and 22.9% for *T. trichiura*, hookworm and *A. lumbricoides*, respectively. The observed prevalence of soil-transmitted helminths increased as a function of higher sampling effort (Figure 2). The examination of three instead of only one Kato–Katz thick smear increased the observed prevalence of hookworm from 5.0 to 16.5% (an increase of 230%), that of *T. trichiura* from 25.8 to 45.5% (+76%) and that of *A. lumbricoides* from 12.9 to 16.1% (+25%). Prevalence estimates based on a single FLOTAC examination (combined results of both flotation solutions) were 56.3% for *T. trichiura*, 29.8% for hookworm and 19.0% for *A. lumbricoides*. These estimates are 81% (for hookworm), 24% (for *T. trichiura*) and 18% (for *A. lumbricoides*) higher than triplicate Kato–Katz results. Differences were observed in diagnosing *T. trichiura* and hookworm according to the flotation solution used in the FLOTAC apparatus. There were slightly more *T. trichiura* infections discovered using S13 (+13%), whereas S4 revealed 5.4-fold more hookworm infections than S13. No difference was observed for *A. lumbricoides* diagnosis with regard to the flotation solution used.

According to the Kato–Katz results and infection intensity thresholds set forth by WHO, all children infected with hookworm, 99.2% of the children infected with *T. trichiura* and 95.6% of those with an *A. lumbricoides* infection were of light intensity. The remainder were categorized as

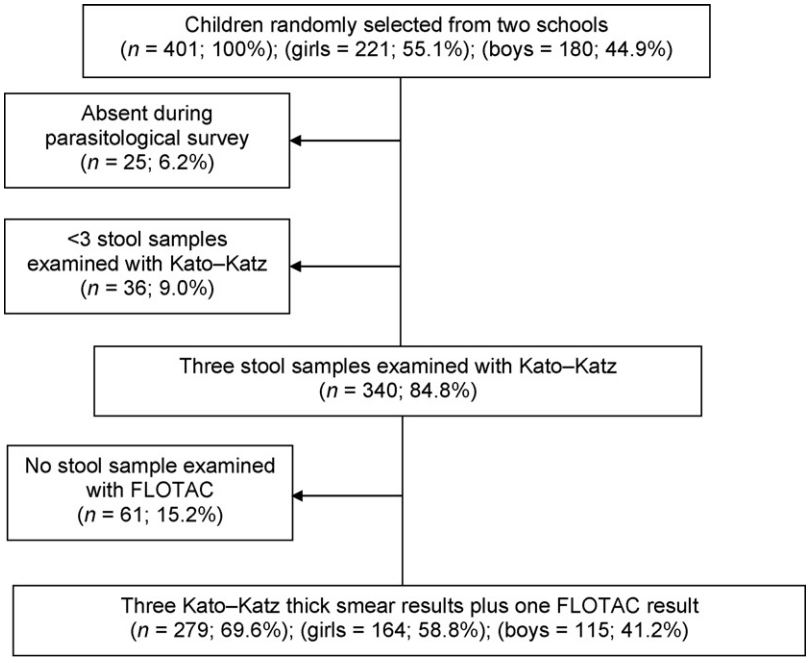

Figure 1 Diagram detailing study participation and stool sample submission compliance of randomly selected children attending Chaani and Kinyasini schools, Zanzibar, in mid-2007. All children who had three stool samples examined using the Kato–Katz method and one stool sample also examined with the FLOTAC method were included in the final analyses.

moderate infections. No child was found to be heavily infected with any of the considered intestinal nematodes.

According to our diagnostic 'gold' standard, the sensitivity of FLOTAC based on a single stool sample was higher than three Kato–Katz thick smears prepared from three consecutive stool samples for the detection of common soil-transmitted helminths (Table 1). The sensitivity of a single FLOTAC for diagnosing *T. trichiura*, hookworm and *A. lumbricoides* was 88.7, 83.0 and 82.8%, respectively. Triplicate Kato–Katz resulted in respective sensitivities of 71.8, 46.0 and 70.3%. Considering a single Kato–Katz, the sensitivity of hookworm diagnosis was only 14.0%. The highest NPV was observed for diagnosing *A. lumbricoides* using the FLOTAC method (95.1%), whereas the lowest NPV was found for diagnosing *T. trichiura* with a single Kato–Katz thick smear (49.3%).

Table 2 shows the results in two-way contingency table format, comparing FLOTAC from a single stool sample with Kato–Katz thick smears from three consecutive stool samples. While both a single FLOTAC examination and triplicate Kato-Katz thick smears identified 107 cases of *T. trichiura*, an additional 50 cases of *T. trichiura* were detected by FLOTAC only and 20 cases of *T. trichiura* were found only after examining three Kato–Katz thick smears. The κ agreement between the two methods for the diagnosis of *T. trichiura* was moderate ($\kappa = 0.50$). Higher additional numbers of hookworm and *A. lumbricoides* were discovered by a single FLOTAC rather than triplicate Kato–Katz (54 vs. 17 for hookworm, 19 vs. 11 for *A. lumbricoides*). The κ agreement between the two methods for diagnosing *A. lumbricoides* was substantial ($\kappa = 0.63$) but only fair for hookworm diagnosis ($\kappa = 0.30$). The κ agreement between the two methods for each helminth showed high statistical significance ($P < 0.001$).

Table 3 shows that the Kato–Katz method yielded considerably higher mean epg estimates than FLOTAC (regardless of the flotation solution) for each helminth species investigated. Indeed, the mean epgs derived from FLOTAC and triplicate Kato–Katz showed a highly statistically significant difference for *T. trichiura* ($P < 0.001$) and hookworm ($P < 0.001$). A significantly higher mean epg count for hookworm was obtained with S4 compared with S13 ($P < 0.001$). No significant differences in the mean epg for *A. lumbricoides* were found between FLOTAC and Kato–Katz and between the two flotation solutions used by FLOTAC, respectively.

In addition to the three common soil-transmitted helminths, eggs of *Enterobius vermicularis* and *Hymenolepis nana* were also diagnosed both by FLOTAC and the Kato–Katz technique. The κ agreement between a single FLOTAC and triple Kato–Katz for the diagnosis of *E. vermicularis* was fair ($n = 5$; $\kappa = 0.30$; $P < 0.001$), derived from one case detected with both methods and two cases each detected by either method. There was a single case of *H. nana*, and it was identified by both methods. In four stool samples the FLOTAC revealed larvae that were most likely *Strongyloides stercoralis*. No *S. stercoralis* larvae were detected by the Kato–Katz method.

4. Discussion

With the further scaling up of preventive chemotherapy targeting common helminthic diseases, the prevalence and intensity of infection and morbidity are expected to decline in many places. As the widely used Kato–Katz method lacks sensitivity to detect low-intensity soil-transmitted helminth infections,[15,23] there is a pressing need for more accurate,

Table 1 Sensitivity and negative predictive value (NPV) including 95% confidence intervals (95% CIs) of the first and all three Kato–Katz thick smears and single FLOTAC examinations for the diagnosis of soil-transmitted helminths among 279 schoolchildren from Zanzibar[a]

	First Kato–Katz thick smear		All three Kato–Katz thick smears		Single FLOTAC (both flotation solutions)	
	Sensitivity (%) [95% CI]	NPV (%) [95% CI]	Sensitivity (%) [95% CI]	NPV (%) [95% CI]	Sensitivity (%) [95% CI]	NPV (%) [95% CI]
A. lumbricoides	56.3 [43.3–68.4]	88.5 [83.6–92.1]	70.3 [57.4–80.8]	91.9 [87.4–94.9]	82.8 [70.9–90.7]	95.1 [91.2–97.4]
T. trichiura	40.7 [33.4–48.3]	49.3 [42.3–56.3]	71.8 [64.4–78.1]	67.1 [59.0–74.4]	88.7 [82.9–92.8]	83.6 [75.6–89.5]
Hookworm	14.0 [8.1–22.7]	67.5 [61.5–73.1]	46.0 [36.1–56.2]	76.8 [70.8–82.0]	83.0 [73.9–89.5]	91.3 [86.3–94.7]

[a] The pooled results of triplicate Kato–Katz plus one FLOTAC test were considered as diagnostic 'gold' standard.

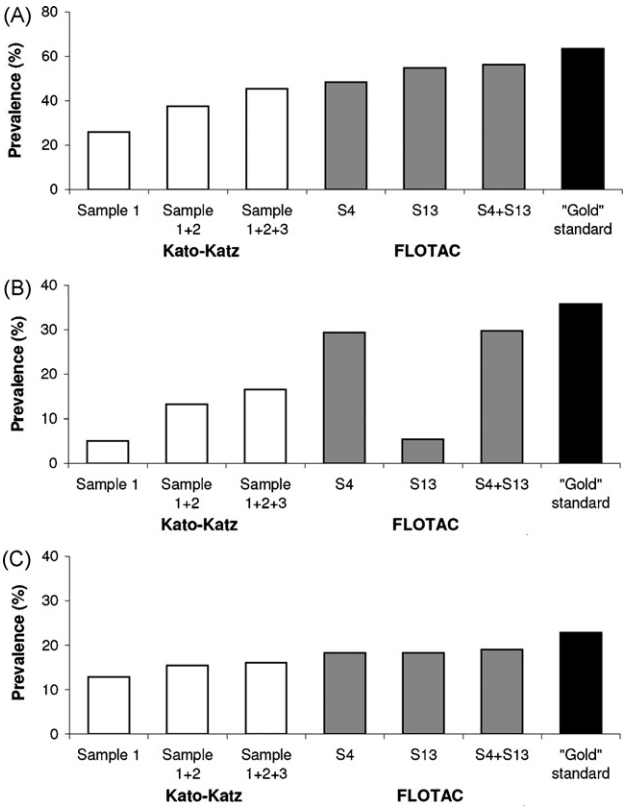

Figure 2 Prevalence of soil-transmitted helminth infections in children from Chaani and Kinyasini schools, Zanzibar, in mid-2007. Results are shown for the first, the first plus the second, and all three Kato–Katz thick smears separately, and for S4, S13 and the combination of both flotation solutions of a single FLOTAC examination. The pooled results of triplicate Kato–Katz plus one FLOTAC test were considered as diagnostic 'gold' standard ($n = 279$). (A) Kato–Katz and FLOTAC for *Trichuris trichiura*; (B) Kato–Katz and FLOTAC for hookworm; (C) Kato–Katz and FLOTAC for *Ascaris lumbricoides*.

yet simple, user-friendly and non-invasive diagnostic tools.[24] Here, we have shown that in a random sample of schoolchildren from Zanzibar, the examination of a single stool sample with the FLOTAC method more accurately diagnosed infections with the three main soil-transmitted helminths than the examination of three consecutive stool samples with the Kato–Katz technique. Our study further underscores the low sensitivity of a single Kato–Katz thick smear in detecting low-intensity hookworm infections and, to a lesser extent, also that of *T. trichiura* and *A. lumbricoides*. However, neither of the employed diagnostic tools was able to detect all helminth infections.

Combining the results of a single FLOTAC and triplicate Kato-Katz thick smears, the observed prevalence of *T. trichiura*, hookworm and *A. lumbricoides* were 63.4, 35.8 and 22.9%, respectively. The sensitivity of a single FLOTAC ranged between 82.8% (for *A. lumbricoides* diagnosis) and 88.7% (for *T. trichiura* diagnosis), and was considerably higher than the sensitivity of triplicate Kato–Katz examinations (range: from 46.0% for hookworm diagnosis to 71.8% for *T. trichiura* diagnosis).

Table 2 Two-way contingency tables showing the agreement between triplicate Kato–Katz thick smears and a single FLOTAC examination (both flotation solutions) for the diagnosis of soil-transmitted helminths in stool samples from 279 schoolchildren from Zanzibar

	Single FLOTAC	Triplicate Kato–Katz		Total
		Positive	Negative	
A. lumbricoides[a]	Positive	34	19	53
	Negative	11	215	226
	Total	45	234	279
T. trichiura[b]	Positive	107	50	157
	Negative	20	102	122
	Total	127	152	279
Hookworm[c]	Positive	29	54	83
	Negative	17	179	196
	Total	46	233	279

[a] κ agreement = 0.63 ($P < 0.001$), indicating a substantial agreement.
[b] κ agreement = 0.50 ($P < 0.001$), indicating a moderate agreement.
[c] κ agreement = 0.30 ($P < 0.001$), indicating a fair agreement.

The remarkably low sensitivity of a single (and even triplicate) Kato–Katz for the detection of hookworm infections is alarming. The use of glycerol (which destroys hookworm eggs over time) in the Kato–Katz method might, at least partially, explain this observation. However, bench aids edited by WHO suggest reading of Kato–Katz thick smears within 30–60 min after preparation to remedy this issue,[25] and our examinations were within this time frame. Interestingly, the hookworm prevalence obtained in this setting of Unguja was even higher than our expectations based on previous findings.[14] The sensitivity of a single FLOTAC for hookworm diagnosis was considerably higher than the sensitivities of single and triple Kato–Katz smears. Compared to a recent investigation employing the FLOTAC technique for the first time in human parasitology, which revealed a sensitivity of 88.2%, a slightly lower sensitivity was noted in the present investigation (83.0%).[17] As the overall hookworm infection intensity according to multiple Kato–Katz thick smears was 155.8 epg in Côte d'Ivoire, but only 12.2 epg in Zanzibar, it is conceivable that the somewhat lower sensitivity of FLOTAC for hookworm diagnosis in the present study is mainly due to the lower mean infection intensity. Indeed, the sensitivity of the Kato–Katz and other coprological tools is also influenced by the intensity of infection.[26,27]

Although the FLOTAC had superior diagnostic sensitivity compared with multiple Kato–Katz thick smears, it failed to detect some cases. As these were diagnosed in the same or consecutive stool samples by the Kato–Katz method only, the κ coefficient indicated only substantial to fair agreement between techniques. In view of this observation, the Kato–Katz method might be considered an important complement to the FLOTAC method, for example in drug-efficacy trials or sentinel surveillance of helminth control programmes.

In our study, the advantage of examining several-fold larger amounts of stool with the FLOTAC apparatus than with the widely used Kato–Katz method was most evident for the diagnosis of light-intensity infections with *T. trichiura* and hookworm. Since these helminths produce fewer eggs than *A. lumbricoides*, the probability of detecting their eggs in a larger amount of stool is higher than in a smaller one. However, the reasons for a single FLOTAC resulting in several-fold lower hookworm and *T. trichiura* epgs compared with the Kato–Katz must be investigated. This issue had been stressed already in our preceding work, focusing on hookworm diagnosis among schoolchildren in Côte d'Ivoire.[17] It is also worth mentioning that the two flotation

Table 3 The epg values (expressed as arithmetic mean [AM], standard error of the mean [SEM], percentiles and maximum) and their statistically significant differences as revealed by a single FLOTAC (two different flotation solutions, designated 'S4' and 'S13') and triplicate Kato–Katz thick smears for the diagnosis of soil-transmitted helminths in stool samples from 279 schoolchildren from Zanzibar

	AM	SEM	Percentile				Maximum	Kruskal–Wallis test
			25%	50%	75%	90%		*P*-value
A. lumbricoides								
FLOTAC-S4	40.9	55.7	0.0	0.0	0.0	108.1	2526.3	0.302
FLOTAC-S13	63.4	55.7	0.0	0.0	0.0	275.9	2087.0	
3 Kato–Katz	278.2	32.2	0.0	0.0	0.0	600.0	14760.0	
T.trichiura								
FLOTAC-S4	26.2	9.8	0.0	0.0	5.3	28.9	2016.0	0.001[a]
FLOTAC-S13	22.1	9.8	0.0	1.4	8.1	33.3	2143.3	
3 Kato–Katz	51.0	5.7	0.0	0.0	24.0	124.8	2880.0	
Hookworm								
FLOTAC-S4	3.4	2.7	0.0	0.0	1.8	5.3	273.5	0.001[a]
FLOTAC-S13	0.4	2.7	0.0	0.0	0.0	0.0	88.7	
3 Kato–Katz	12.2	1.6	0.0	0.0	0.0	0.0	720.0	

[a] Statistically significant difference ($P < 0.05$).

solutions used in the current study performed differently for the identification of hookworm infections. Specifically, S13 revealed a lower hookworm mean epg value than did flotation solution S4. Indeed, S13 underperformed in the diagnosis of hookworm, yielding only 15 cases compared with 82 infections detected with S4.

The identification of a single *H. nana* infection both by the FLOTAC apparatus and the Kato–Katz method indicates the potential of FLOTAC also for the detection of tapeworm infections. Interestingly, *E. vermicularis* was identified by the FLOTAC apparatus in three children but only a poor κ agreement was found with the Kato–Katz test results. However, care is needed when using the κ statistics for comparison of diagnostic methods for rare parasitic infections. As *E. vermicularis* infections can only occasionally be detected in stool samples, the method of choice to diagnose *E. vermicularis* remains the Scotch cellophane tape method.[28] The helminth larvae found in four stool samples were probably *S. stercoralis* larvae, as this helminth is endemic on Unguja island.[23,29] However, the present configuration of the FLOTAC apparatus does not allow the differentiation of species-specific characteristics (e.g. large genital primordium and short buccal cavity of *S. stercoralis* larvae). A modified FLOTAC apparatus allowing parasite diagnosis at 400$\times$ magnification is currently under development.

Our results show that the FLOTAC method holds promise for more accurate helminth diagnosis than with the Kato–Katz technique, especially in epidemiological settings characterized by low-intensity infections. Hence, FLOTAC might emerge as a suitable method for the surveillance of helminth control programmes, monitoring of soil-transmitted helminth transmission and verification of local elimination. The higher sensitivity might reveal that the 'true' soil-transmitted helminth prevalences in certain low-infection intensity settings are considerably higher than currently estimated. This could have a direct impact on policies and control programmes based on proposed WHO treatment algorithms. Higher prevalence estimates derived with the FLOTAC method would, for example, lead to a higher fraction of communities arbitrarily defined as 'category II' (i.e. prevalence of infection above 50% and low infection intensities), for which mass drug administration is recommended.[22] Higher prevalences of low-intensity helminth infections could also have far-reaching consequences on burden estimates because recent studies point to significant morbidity associated with even light infection intensities.[30]

The encouraging results obtained with the FLOTAC method for diagnosis of common soil-transmitted helminths call for further development and sequential validation steps. An issue that warrants particular investigation is to elucidate reasons for the significant differences observed in mean hookworm and *T. trichiura* infection intensities when comparing FLOTAC with Kato–Katz. Once the technique is fully validated in different epidemiological settings, the hope is to transfer this method to more basic field laboratories.

In conclusion, our results underscore previous observations that unanimously point to a low sensitivity of traditional and widely used parasitological methods to detect low-intensity soil-transmitted helminth infections. A single FLOTAC identified a higher percentage of 'all' cases than three Kato–Katz thick smears from consecutive stool samples. Thus, after further steps of standardization and validation, the FLOTAC could become a supportive diagnostic tool for patient management, and particularly for the rigorous surveillance of helminth control programmes.

Authors' contributions: SK, JRS, PS, HM, GC and JU conceived and designed the experiments; SK, LR, ISK, DR, MPM and HM performed the experiments; SK analysed the data; SK, LR, JRS, DR, PS, HM, GC and JU wrote the paper. All authors read, revised and approved the final version of the manuscript. SK and JU are guarantors of the paper.

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Conflicts of interest: GC is the inventor and current patent holder of the FLOTAC apparatus. In case the currently ongoing development and validation of the FLOTAC apparatus is successful, the method will be licensed free of charge to WHO and interested public non-commercial research centres. All other authors have no conflicts of interest.

Ethical approval: The study was embedded in the annual school survey, including parasitological examinations of stool and urine samples, and anthelminthic treatment of Zanzibari schoolchildren, conducted by the HCLU. The study protocol was cleared by WHO (Geneva, Switzerland) and the MoHSW (Stone Town, Zanzibar) of Unguja. The institutional research commission of the Swiss Tropical Institute (Basel, Switzerland) and the institutional review board of the National Health Service Local Research Ethics Committee (application 03.36) of St. Mary's Hospital (London, UK) on behalf of the Natural History Museum/Imperial College London approved the study.

References

1. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* 2006;**367**:1521–32.
2. Albonico M, Allen H, Chitsulo L, Engels D, Gabrielli AF, Savioli L. Controlling soil-transmitted helminthiasis in pre-school-age children through preventive chemotherapy. *PLoS Negl Trop Dis* 2008;**2**:e126.

3. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. *J Clin Invest* 2008;**118**:1311–21.
4. Hotez PJ, Molyneux DH, Fenwick A, Ottesen E, Ehrlich Sachs S, Sachs JD. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. *PLoS Med* 2006;**3**:e102.
5. Savioli L, Albonico M, Engels D, Montresor A. Progress in the prevention and control of schistosomiasis and soil-transmitted helminthiasis. *Parasitol Int* 2004;**53**:103–13.
6. Brooker S, Clements ACA, Bundy DAP. Global epidemiology, ecology and control of soil-transmitted helminth infections. *Adv Parasitol* 2006;**62**:221–61.
7. 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.
8. Lammie PJ, Fenwick A, Utzinger J. A blueprint for success: integration of neglected tropical disease control programmes. *Trends Parasitol* 2006;**22**:313–21.
9. Mohammed KA, Haji HJ, Gabrielli A, Mubila L, Biswas G, Chitsulo L, et al. Triple co-administration of ivermectin, albendazole and praziquantel in Zanzibar: a safety study. *PLoS Negl Trop Dis* 2008;**2**:e171.
10. Kabatereine NB, Brooker S, Koukounari A, Kazibwe F, Tukahebwa EM, Fleming FM, et al. Impact of a national helminth control programme on infection and morbidity in Ugandan schoolchildren. *Bull World Health Organ* 2007;**85**:91–9.
11. Dickson R, Awasthi S, Demellweek C, Williamson P. Anthelmintic drugs for treating worms in children: effects on growth and cognitive performance. *Cochrane Database Syst Rev* 2000; 2:CD000371.
12. Taylor-Robinson DC, Jones AP, Garner P. Deworming drugs for treating soil-transmitted intestinal worms in children: effects on growth and school performance. *Cochrane Database Syst Rev* 2007; 4:CD000371.
13. Renganathan E, Ercole E, Albonico M, De Gregorio G, Alawi KS, Kisumku UM, et al. Evolution of operational research studies and development of a national control strategy against intestinal helminths in Pemba Island, 1988–92. *Bull World Health Organ* 1995;**73**:183–90.
14. Stothard JR, Imison E, French MD, Sousa-Figueiredo JC, Khamis IS, Rollinson D. Soil-transmitted helminthiasis among mothers and their pre-school children on Unguja Island. Zanzibar with emphasis upon ascariasis. *Parasitology* 2008;**135**:1447–55.
15. Booth M, Vounatsou P, N'Goran EK, Tanner M, Utzinger J. The influence of sampling effort and the performance of the Kato-Katz technique in diagnosing *Schistosoma mansoni* and hookworm co-infections in rural Côte d'Ivoire. *Parasitology* 2003;**127**:525–31.
16. Cringoli G. FLOTAC, a novel apparatus for a multivalent faecal egg count technique. *Parassitologia* 2006;**48**:381–4.
17. Utzinger J, Rinaldi L, Lohourignon LK, Rohner F, Zimmermann MB, Tschannen AB, 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.
18. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Rev Inst Med Trop São Paulo* 1972;**14**:397–400.
19. Marti H, Escher E. SAF — an alternative fixation solution for parasitological stool specimens. *Schweiz Med Wochenschr* 1990;**120**:1473–6.
20. Cringoli G, Rinaldi L, Veneziano V, Capelli G, Scala A. The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. *Vet Parasitol* 2004;**123**:121–31.
21. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;**33**:159–74.
22. Montresor A, Crompton DWT, Hall A, Bundy DAP, Savioli L. *Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. A guide for managers of control programmes*. Geneva: World Health Organization; 1998.
23. Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, et al. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis* 2008;**2**:e331.
24. Peeling RW, Smith PG, Bossuyt PM. A guide for diagnostic evaluations. *Nat Rev Microbiol* 2006;**4**(9 Suppl):S2–6.
25. WHO. *Bench aids for the diagnosis of intestinal parasites*. Geneva: World Health Organization; 1994.
26. Hall A. Intestinal helminths of man: the interpretation of egg counts. *Parasitology* 1982;**85**:605–13.
27. Lin DD, Liu JX, Liu YM, Hu F, Zhang YY, Xu JM, et al. Routine Kato-Katz technique underestimates the prevalence of *Schistosoma japonicum*: a case study in an endemic area of the People's Republic of China. *Parasitol Int* 2008;**57**:281–6.
28. Beaver PC. Methods of pinworm diagnosis. *Am J Trop Med Hyg* 1949;**29**:577–87.
29. Marti HP, Haji HJ, Savioli L, Chwaya HM, Mgeni AF, Ameir JS, et al. A comparative trial of a single-dose ivermectin versus three days of albendazole for treatment of *Strongyloides stercoralis* and other soil-transmitted helminth infections in children. *Am J Trop Med Hyg* 1996;**55**:477–81.
30. Ezeamama AE, Friedman JF, Olveda RM, Acosta LP, Kurtis JD, Mor V, et al. Functional significance of low-intensity polyparasite helminth infections in anemia. *J Infect Dis* 2005;**192**:2160–70.