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Comparison of Kato-Katz, ethyl-acetate sedimentation, and Midi Parasep® in the diagnosis of hookworm, *Ascaris* and *Trichuris* infections in the context of an evaluation of rural sanitation in India

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The Kato-Katz, conventional ethyl-acetate sedimentation, and Midi Parasep® methods for diagnosing infection with soil-transmitted helminths were compared. The Kato-Katz technique gave the best overall diagnostic performance with the highest results in all measures (prevalence, faecal egg count, sensitivity) followed by the conventional ethyl-acetate and then the Midi Parasep® technique. The Kato-Katz technique showed a significantly higher faecal egg count and sensitivity for both hookworm and *Trichuris* as compared to the Midi Parasep® technique. The conventional ethyl-acetate technique produced smaller pellets and showed lower pellet mobility as compared to the Midi Parasep®.

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1. Introduction

Soil-transmitted helminths (STH) cause a large global health burden, with 807, 604, and 576 million persons infected with *Ascaris lumbricoides* (*Ascaris*), *Trichuris trichuria* (*Trichuris*) and the human hookworms respectively (Bethony et al., 2006). Lack of access to improved sanitation is associated with STH infection (Ziegelbauer et al., 2012). However, there is uncertainty about the effectiveness of large-scale sanitation interventions to prevent STH, particularly in rural, low-income settings with potential faecal exposure from multiple transmission pathways (Ziegelbauer et al., 2012). This uncertainty may be partially responsible for the lack of progress in reaching the estimated 2.5 billion who lack improved sanitation (UNICEF and WHO, 2012). In order to address this knowledge gap, a randomized controlled trial (RCT) with latrines as intervention is being conducted among 100 villages in the state of Odisha, India. STH re-infection is one of the outcome measures of this RCT.

The Kato-Katz technique is recommended by the WHO for helminth diagnosis (Montressor et al., 1998). However, limited reproducibility, a low sensitivity for light intensity infections, and the fact that it needs to be performed on fresh samples means that it is not always the ideal method (Knopp et al., 2008). This may be particularly true in the context of evaluating large-scale scale interventions aimed at STHs. The formalin ethyl(-acetate) concentration technique is commonly used in laboratories due to its ability to isolate a large variety of faecal parasites from fresh and preserved samples (Utzinger et al., 2010). In addition the technique is more sensitive for the detection of light infections, and it lessens the risk of infection with faecal pathogens (Cheesbrough, 2006). The Midi-Parasep® is a disposable, closed, single use device in which the concentration technique is contained, which has been suggested as an ideal technique for STH diagnostic in large trials (Zeeshan et al., 2011). However, an increasing number of studies have reported a low sensitivity (Lier et al., 2009).

The distance to the central laboratory, combined with limited staff availability meant that samples needed to be fixed, and led to the choice of a concentration technique over the Kato-Katz technique. This paper presents the results of a study in which the diagnostic performance of the regular concentration, and Midi Parasep® technique were compared to the Kato-Katz technique.

2. Materials and methods

2.1. Study area and ethical considerations

The study was undertaken in Puri district, Odisha, India. Ethical approval was obtained from the LSHTM (010/395 & 5561) and the

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Table 1
Prevalence and mean infection intensity (EPG) of hookworm, *Ascaris*, and *Trichuris*, as detected by each of the three diagnostic techniques.

	Kato-Katz (K)	Conventional concentration (*C*)	Midi-Parasep® (P)	*p* values		
				K vs. *C*	*K* vs. *P*	*C* vs. *P*
Hookworm						
Prevalence (%)[a]	28.2 (19.6–36.7)	23.6 (15.6–31.7)	20.0 (12.4–27.6)	0.332	0.1078	0.4807
Intensity *epg*	62.1	14.0	13.6	0.0000	0.0000	0.2777
Ascaris lumbricoides						
Prevalence (%)[a]	0.9 (0.0–2.7)	4.5 (0.6–8.5)	1.8 (0.0–4.4)	0.21875	1	0.4531
Intensity *epg*	0.4	1.1	0.1	0.228	.8937	0.0993
Trichuris trichuria						
Prevalence (%)[a]	6.4 (1.7–11.0)	4.5 (0.6–8.5)	2.7 (0.0–5.8)	0.6875	0.125	0.5
Intensity *epg*	7.0	0.5	4.1	0.0226	0.1391	0.4583

[a] Values in parentheses are 95% Confidence Interval.

Xavier Institute of Management, in Bhubaneswar, Orissa (310510). All study participants with a positive stool sample were provided with albendazole following Indian Ministry of Health guidelines.

2.2. Stool sample collection, preparation and analysis

Stool samples were collected in July 2011. A convenience sample (n = 110) was selected from the total amount of samples processed by the laboratory over a period of 4 weeks. The first 6 stool samples collected each day that contained more than 3 g of stool were selected for inclusion. All stool samples were mixed thoroughly, and divided into three portions of approximately 1 g. Two 1 g portions were dissolved in pre-weighed tubes containing 6 ml of 10% formalin. Following the addition of the stool sample, the tubes were weighted in order to determine the weight of the stool sample analyzed, so that upon examination an intensity of infection (eggs/gram of stool) could be determined. The unfixed portions were processed immediately using the Kato-Katz technique following standard methods (Cheesbrough, 2006). For the conventional concentration technique the previously weighted stool sample was sieved using a 360 μm sieve, following which standard procedures were applied (Cheesbrough, 2006). Samples were processed with the Midi Parasep® according to the manufacturer's (Diasys Ltd., Berkshire, England) instructions. To the sediment generated by both methods, 3–4 drops of saline were added and suspension was measured and noted. Three slides were prepared following which the remaining quantity of the suspension was measured and noted. The number of eggs per slide was recorded. For the Kato-Katz technique the number of eggs found per slide was multiplied by 24 in order to obtain eggs/gram for each helminth. For both concentration techniques, the average number of eggs found per slide was multiplied by the remaining volume left and divided by the original amount of stool dissolved in the 6 ml of formalin.

2.3. Pellet size and stability observations

Observations were made on the size and the stability of the sediment pellet. The size of the pellet was estimated with the use of a graduated centrifuge tube or with the Midi Parasep® tube itself, while the stability of the pellet was assessed when the excess grease and fat layers were removed. Sediment was as not mobile (N), mobile (Y) when the top of the pellet appeared to be leaking off and moving down the side of the tube, or fully mobile (YF) when the pellet was completely dislodged and moved freely about the tube upon inversion.

2.4. Statistical analysis

Data was analyzed used STATA 11.2 (StataCorp LP, College Station, USA). McNemar's test for paired data was used to do pair-wise comparisons of the diagnostic methods. Wilcoxon's signed rank test was used to compare the means. The 'gold standard' was defined as a sample positive by any one of the three diagnostic techniques. Single, duplicate, and triplicate slide sensitivity and negative predictive values (NPVs) of each diagnostic method were calculated for each helminth species, using the above defined 'gold standard'. Two way contingency tables to compare frequencies were used to analyze pellet stability observations.

3. Results

3.1. Prevalence and intensity of helminth infections

The highest prevalence for hookworm was detected by Kato-Katz, followed by the conventional concentration, and the Midi Parasep (Table 1). The same trend was observed for *Trichuris*, while the conventional concentration technique detected the highest prevalence of *Ascaris*. The differences in prevalence between the methods were not statistically significant. Kato-Katz detected a significantly higher intensity of infection for hookworm when compared to both concentration techniques.

3.2. Sensitivities and negative predictive values for hookworm

The 'gold' standard for hookworm was n = 40 (36.3%) (Table 2). Single, duplicate and triplicate slide sensitivity and NPV for hookworm were highest with the Kato-Katz technique (Fig. 1). Kato-Katz triplicate slide sensitivity was significantly higher than that of the Midi Parasep®. There were no significant differences between any of the methods in terms of NPV (Fig. 1).

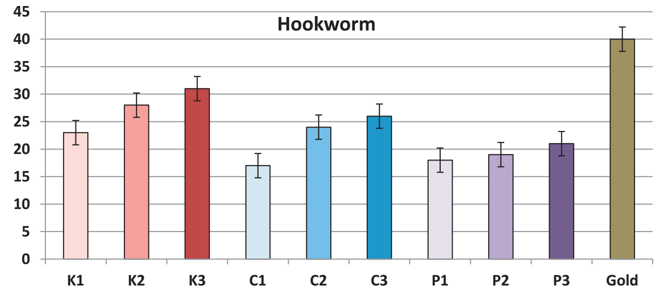

Fig. 1. Cumulative numbers of positive samples for hookworm for each processing method, as assessed by single, duplicate, and triplicate slides. K: Kato-Katz; *C*: conventional concentration; P: Midi Parasep ®; Gold: gold standard; 1,2,3- indicates single, duplicate, and triplicate slides respectively.

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Table 2
Cumulative number of samples positive with cumulative sensitivity and negative predictive values (NPV) for single, duplicate, and triplicate slides using each of the three candidate diagnostic techniques for hookworm.

Processing method	Cumulative # positive	Cumulative sensitivity (95%CI)	Cumulative negative predictive value (95%CI)
Kato-Katz (1)	23	57.5 (48.3–66.7)	80.5 (73.1–87.9)
Kato-Katz (2)	28	70.0 (61.4–78.6)	85.4 (78.8–92.0)
Kato-Katz (3)	31	77.5 (69.7–85.3)	88.6 (82.7–94.5)
Conventional Concentration (1)	17	42.5 (33.3–51.7)	75.3 (67.2–83.3)
Conventional Concentration (2)	24	60.0 (50.8–69.2)	81.4 (74.1–88.7)
Conventional Concentration (3)	26	65.0 (56.1–73.9)	83.3 (76.4–90.3)
Parasep® (1)	18	45.0 (35.7–54.3)	76.1 (68.1–84.1)
Parasep® (2)	19	47.5 (38.2–56.8)	76.9 (69.0–84.8)
Parasep® (3)	21	52.5 (43.2–61.8)	78.7 (71.0–86.3)

3.3. Pellet size and mobility

All pellets for the conventional concentration technique were found to be stable, while only 59% of the pellets for the Midi Parasep® were, with 31% classified as mobile and 10% as very mobile. The Midi Parasep® produced the largest pellet (0.23 ml vs. 0.27 ml), though the difference was non-significant ($p = 0.19$).

4. Discussion

Diagnostically, the Kato-Katz technique performed best of the three methods, with the highest prevalence, intensity of infection, diagnostic sensitivity, and negative predictive value, for both hookworm and *Trichuris* reported. The Midi Parasep® technique performed poorest, and problems with the pellet following processing were reported.

4.1. Diagnostic performance differences

The significantly lower sensitivity for hookworm and *Trichuris* by the conventional ethyl-acetate and Midi Parasep® techniques, as compared to the Kato-Katz, could be due to loss of eggs during the sedimentation procedure (Lier et al., 2009). The fact that the majority of positive samples were of low intensity, in this study would support this. The fixing of samples in formalin together with ethyl-acetate have been suggested to destroy fragile hookworm eggs (Glinz et al., 2010), while there is also potential for interactions between various reagents and components (i.e. formalin, ethyl-acetate, sediment, debris), which may effect sensitivity of the techniques (Cringoli et al., 2010). Higher FEC seen in the Kato-Katz slides could potentially be explained by the scraping and sieving processes involved in the technique, which could concentrate helminth eggs at a level higher than what is accounted for by concentration calculations (Glinz et al., 2010). The calculation for FEC as obtained for the Kato-Katz technique is a multiplication of 24, meaning that the lowest egg count for a positive sample is 24 EPG; this multiplication factor is lower, for the conventional concentration and Midi-Parasep methods (Glinz et al., 2010).

4.2. Clarity and ease of use

The higher pellet size and mobility seen in the Midi Parasep® tubes could possibly be explained by the use of less ethyl-acetate reagent (2 ml vs. 4 ml), and a larger sieve (425 μm vs. 360 μm) as compared to the conventional concentration technique. Both of these factors may lead to more fats/debris being included in the pellet. Furthermore, the recommended centrifugation speed (rpm) is lower for the Midi Parasep® than typically used for the conventional technique (1700 vs. 2400 rpm), which could result in a less secured pellet. The larger diameter of the Midi Parasep® tubes (50 ml) as compared to the 15 ml conical centrifuge tubes may also affect the ability of a pellet to become secured in its tip. Pellet mobility could lead to loss of sample, while a larger pellet will mean more slides for analysis. A larger pellet is also likely to result in more debris and fats making identification of thin shelled eggs more difficult.

4.3. Field study feasibility

The large number of samples collected for the RCT and the long distance to the laboratory made the Kato-Katz technique unfeasible for the RCT. The Midi Parasep® tubes are a closed concentration system, meaning that they have increased ease of use compared to the conventional concentration method. A closed method reduces health and safety hazards. Fixing stool samples lowers the risk of infection to laboratory technicians, and allows for a delay in stool sample processing. In the Indian context where both Hinduism and Islam consider contact with faeces taboo a fixed sample is considered more acceptable to work with. Although the Midi Parasep® offers a safer and quicker opportunity to conduct the concentration technique, its higher price, possible lower performance, and concerns regarding pellet size and mobility, meant that the sanitation RCT opted for the conventional concentration technique.

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Conflict of interest

No reported conflict of interest

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