Kato Katz and Lumbreras rapid sedimentation test to evaluate helminth prevalence in the setting of a school based deworming program

Martha Lopez[1], Maria Luisa Morales[1], Monisha Konana[2], Paige Hoyer[2], Roberto Pineda-Reyes[1], Arthur Clinton White Jr[1,3], Hector Hugo Garcia[4,5,6], Andres Guillermo Lescano[7,8], Eduardo Gotuzzo[9], Miguel Mauricio Cabada[1,3,9]

[1]Universidad Peruana Cayetano Heredia and University of Texas Medical Branch Collaborative Research Center, Cusco, Peru, [2]School of Medicine, University of Texas Medical Branch, Galveston, TX, USA, [3]Infectious Disease Division, Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX, USA, [4]Cysticercosis Working Group in Peru, Lima, Peru, [5]Faculty of Science and Philosophy, Infectious Diseases Laboratory Research-LID, Alberto Cazorla Talleri, Universidad Peruana Cayetano Heredia, Lima, Peru, [6]Cysticercosis Unit, Instituto de Ciencias Neurologicas, Lima, Peru, [7]School of Public Health and Administration, Universidad Peruana Cayetano Heredia, Lima, Peru, [8]US Naval Medical Research Unit No. 6 (NAMRU-6), Lima, Peru, [9]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru

The sensitivity of the Kato-Katz test is suboptimal for the evaluation of intestinal helminth prevalence. Moreover, during mass deworming, as helminth egg burden decreases, the sensitivity is likely to decrease. The Lumbreras rapid sedimentation (Lumbreras) is a low-cost non-quantitative test, but may provide useful information in low burden areas. We compared the prevalence of intestinal helminth infections assessed by the Kato-Katz and the Lumbreras rapid sedimentation test on 3 stool specimens from each of 1083 children. The sensitivities were compared using the McNemar paired test. Using the combined outcome of the 3 different stool tests as the standard, Kato-Katz had lower sensitivity than Lumbreras rapid sedimentation tests for *Ascaris lumbricoides* (85.1% vs. 95.1%, $p = 0.03$), *Hymenolepis nana* (77.7% vs. 97.9%, $p < 0.01$), *Trichuris trichura* (41.7% vs. 100%, $p = 0.01$), hookworm (0% vs. 100%, $p = 0.01$), and *Strongyloides stercoralis* (0% vs. 88%, $p < 0.01$). Kato-Katz demonstrated significantly lower sensitivity, missing most *T. trichiura*, hookworm, and *S. stercoralis* infections. The combination of Kato-Katz and Lumbreras rapid sedimentation tests enables the detection of more intestinal helminths infections in post-deworming low prevalence areas.

Keywords: Kato-Katz, Lumbreras rapid sedimentation, Soil-transmitted helminths, Sensitivity, Screening tests

Introduction

Soil-transmitted helminth (STH) infections affect poor populations in low resource countries all over the world.[1] In 2013, the World Health Organization (WHO) estimated that over 266 million pre-school aged children and over 609 million school-aged children were at high risk for complications associated with STH.[2,3] High burden STH infections are associated with anemia, malnutrition, and diarrhea in children. In turn, anemia and malnutrition are associated with decreased quality of life and productivity later in life.[4,5] Periodic mass chemotherapy is recommended to decrease parasite burden among children and avoid the development of complications.[4,5] Mass chemotherapy is recommended twice a year in high-risk areas (STH prevalence > 50%), but only once a year in lower risk areas (STH prevalence from 20 to 50%).[6]

The WHO recommends performing Kato-Katz test on single stool samples to evaluate the prevalence of STH infections.[7] However, the sensitivity of a single Kato-Katz test ranges between 14 and 85% for different STH.[8,9] It is likely that the sensitivity is lower in the setting of ongoing control programs with decreased worm burdens.[10,11] Thus, policy decisions and resource allocations are often based on suboptimal epidemiologic information. Furthermore, the Kato-Katz test is labor-intensive and requires additional training and reagents. Other methods like FLOTAC have demonstrated high sensitivity for the diagnosis of STH.[9,12,13] Nonetheless, the cost of supplies and training may decrease their usefulness in resource-limited settings. The Lumbreras rapid sedimentation test is a low cost non-quantitative test that uses tap water and reusable

Correspondence to: Miguel Mauricio Cabada. Universidad Peruana Cayetano Heredia and University of Texas Medical Branch Collaborative Research Center, Cusco, Peru. Email: micabada@utmb.edu

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cups.[14] This test was developed for the diagnosis of fascioliasis.[14] Its usefulness for STH has not been systematically evaluated. The goal of this study was to compare the sensitivity of the Lumbreras rapid sedimentation test with the qualitative results of the Kato-Katz test for the diagnosis of STH infection in the setting of an ongoing school-based albendazole mass chemotherapy program.

Methods

Study Setting

Data were obtained from an ongoing study among children being evaluated for *Fasciola hepatica* and intestinal helminth infections in the Ancahuasi, Zurite, and Anta districts of the Anta province in the Cusco region of Peru. This region is at an elevation of approximately 3350 meters and the minimum and maximum average temperatures range between 1–8 °C and 20–21 °C respectively. The average monthly rainfall is 77–152 mm during the rainy season (November–March) and 8–49 mm during the dry season (April–October). Most homes have a single room with dirt floor and improved latrines. Access to piped running water is seasonal and depends on agricultural cycles. Children receive school-based albendazole mass treatment twice a year with coverage above 90%.

Study Procedures

Children ranging from 3 to 16 years old were invited to participate in the study and were enrolled in pre-schools and schools between July 2013 and May 2015. Prior to enrollment, informed consent was obtained from the parents and assent from children. Participants were asked to provide three stool samples on consecutive days for analysis. Each sample was tested by the Lumbreras rapid sedimentation and Kato-Katz tests using 10% formalin preserved stools and fresh stools, respectively.[15,16] Sedimentation of stools was performed as previously described in the Lumbreras rapid sedimentation method.[14,17,18] One drop from the sediment was examined using microscope slides at 100× and 400× magnification (slide test). The rest of the sediment was poured into Petri dishes and examined at 100× magnification as described in the Lumbreras rapid sedimentation method (Lumbreras test). Kato-Katz tests were processed within 48 h of sample collection. The Lumbreras and slide tests were processed on subsequent days. Three experienced microscopists performed all stool tests blinded to the results obtained by other methods.

Data Analysis

Data were analyzed using the Statistical Package for the Social Sciences v.18 (SPSS Inc., Chicago, IL). Only subjects that provided three stool samples were included in the study. A combined variable using the results of the Kato-Katz, Lumbreras, and slide tests on three stools samples was created for each helminth parasite. A positive result in any of the tests or samples was used to define positivity. The arithmetic mean of the three Kato-Katz test results for each parasite was calculated to create a variable containing an egg count for each subject. The overall frequencies and geometric mean of egg counts for each STH and *H. nana* were calculated to describe the epidemiologic situation in the area. As none of the tests used in this study is 100% sensitive, the sensitivity of the tests was calculated comparing them to a "pseudo-gold standard" defined by combining the positive results. The sensitivity of each tests performed in one and three stool samples was calculated for each parasite. The McNemar Chi test was used to compare the sensitivities of the tests for each of the helminths. The Kappa statistic was used to evaluate the agreement between the Lumbreras test and the Kato-Katz test for different parasites. Differences were considered statistically significant if p was less than 0.05.

Ethical Statement

The study protocol was approved by the Institutional Ethics Committee of Universidad Peruana Cayetano Heredia (# 60574) and by the Institutional Review Board of University of Texas Medical Branch (# 13-080).

Results

A total of 1290 children were enrolled during the study period and 1083 (83.9%) provided 3 stool specimens for analysis. The overall prevalence of intestinal helminths using the combined results of the 3 stool samples and tests was 25.6% (278/1083) and the overall prevalence of STH was 7.3% (79/1083). *H. nana* infection was the most common infection affecting 18.3% (198/1083) of the children followed by *A. lumbricoides* (6.2%, 67/1083), *S. stercoralis* (2.3%, 25/1083), *T. trichiura* (1.1%, 12/1083), and hookworm (0.6%, 7/1083). The geometric mean for the egg counts was 210.8 eggs/g of stool for *H. nana*, 220.6 eggs/g of stool for *A. lumbricoides*, and 52.6 eggs/g of stool for *T. trichiura*. No *Strongyloides* larvae or hookworm eggs were detected by Kato-Katz.

Sensitivity Analysis

The sensitivity of three Lumbreras, three slide, and three Kato-Katz tests for STH and *H. nana* infections is shown in Table 1. Three Kato-Katz were less sensitive than three Lumbreras tests for each of the intestinal helminths evaluated. However, there were no significant differences between the three Kato-Katz and three slide tests. In addition, we compared the sensitivity of Lumbreras test with the sensitivity of Kato-Katz on the first stool sample collected from each subject. The Lumbreras test was significantly more sensitive than Kato-Katz for overall STH infections, *Ascaris*, *H. nana*, and *Strongyloides* (Table 2).

The sensitivity comparison of the Lumbreras test on the first sample and the Kato-Katz on three samples showed that the Kato-Katz test was significantly more sensitive only for *Ascaris* eggs. There were no significant differences in the sensitivities for the detection of *Trichuris*, hookworm, or *H. nana* eggs. In addition, Lumbreras

Table 1 Sensitivity of Lumbreras and slide tests compared to the Kato-Katz tests for intestinal helminths in three stool samples

Helminth	Kato-Katz % (*n*)	Lumbreras % (*n*)	*p*	Slide % (*n*)	*p*
A. lumbricoides (*n* = 67)	85.1 (57)	95.5 (64)	0.039	83.6% (56)	1.0
T. trichiura (*n* = 12)	41.7 (5)	100 (12)	0.016	0 (0)	0.063
Hookworm (*n* = 7)	0 (0)	100 (7)	0.016	57.1 (4)	0.125
All STH (*n* = 79)	77.2 (61)	96.2 (76)	< 0.001	74.7 (59)	0.791
H. nana (*n* = 198)	77.7 (154)	97.9 (194)	< 0.001	81.8 (162)	0.216
S. stercoralis (*n* = 25)	0 (0)	88 (22)	< 0.001	16 (4)	0.125

Table 2 Sensitivity and McNemar Chi test comparing the Lumbreras test with Kato-Katz for intestinal helminthes in the first stool sample

Helminth	One Kato-Katz % (*n*)	One Lumbreras % (*n*)	*p* value
A. lumbricoides (*n* = 67)	56.7 (38)	70.1 (47)	0.012
T. trichiura (*n* = 12)	8.3 (1)	50 (6)	0.063
Hookworm (*n* = 7)	0 (0)	57.1 (4)	0.125
All STH (*n* = 79)	49.4 (39)	69.6 (55)	<0.001
H. nana (*n* = 198)	52.0 (103)	76.8 (152)	<0.001
S. stercoralis (*n* = 25)	0 (0)	48 (12)	<0.001

Table 3 Sensitivity and McNemmar Chi test comparing the sensitivity of a single Lumbreras test and three Kato-Katz tests for intestinal helminths

Helminth	One Lumbreras % (*n*)	Three Kato-Katz % (*n*)	*p* value
A. lumbricoides (*n* = 67)	70.1 (47)	85 (57)	0.031
T. trichiura (*n* = 12)	50 (6)	41.7 (5)	1.00
Hookworm (*n* = 7)	57.1 (4)	0 (0)	0.125
All STH (*n* = 79)	69.6 (55)	77.2 (61)	0.286
H. nana (*n* = 198)	76.8 (152)	77.7 (154)	0.894
S. stercoralis (*n* = 25)	48 (12)	0 (0)	<0.001

Table 4 Kappa statistic comparing the Lumbreras test with three Kato-Katz tests for different helminth parasites

	3 Lumbreras tests*		1 Lumbreras test*	
	Kappa statistic	*p*	Kappa statistic	*p*
A. lumbricoides	0.92	<0.001	0.81	<0.001
T. trichura	0.58	<0.001	0.72	<0.001
H. nana	0.84	<0.001	0.78	<0.001
All STH	0.86	<0.001	0.80	<0.001

*Compared to 3 Kato-Katz tests. Comparisons for hookworm and *Strongyloides* were not possible due to negative Kato-Katz tests.

test was significantly more sensitive than Kato-Katz for the detection of *Strongyloides* larvae (Table 3). Finally, Lumbreras tests showed substantial agreement with Kato-Katz regardless of the number of stool samples examined. Table 4 shows the Kappa statistic for one and three Lumbreras tests compared with three Kato-Katz.

Discussion

Recommendations for program implementation of mass chemotherapy depend on the prevalence of soil-transmitted helminth infections.[6] We found that the Lumbreras rapid sedimentation test had significantly superior sensitivity compared to the qualitative Kato-Katz test recommended by the WHO for community diagnosis in moderate to high-risk areas (≥20% prevalence).[6,19] The Lumbreras test was superior to the Kato-Katz test for the detection of ova of STH and *H. nana*, as well as, larvae of *Strongyloides*. Our evaluation in the setting of a school-based twice a year albendazole treatment program indicates that the sensitivity of the Kato-Katz test may be less than ideal for decision-making in low burden areas or where programs are already implemented. Thus, as recommended by WHO in such areas alternative methods like Lumbreras test may be useful.[19]

The prevalence and egg burden of STH was low in the area studied, which probably decreased the sensitivity of the Kato-Katz test. Some studies have shown that the sensitivity of the Kato-Katz tests decreases after albendazole treatment compared to other tests like FLOTAC. Albonico et al.[10] demonstrated that the sensitivity of three Kato-Katz to detect *Ascaris* eggs decreased from 72 to 12% after albendazole treatment. In contrast, the sensitivity for *Trichuris* only decreased from 95 to 93%. In this study, the egg reduction for *Ascaris* was 99% and the prevalence decreased from 45 to 7%. In contrast, for *Trichuris* the egg reduction was only 50% and the prevalence decreased only slightly from 95 to 92%. This shows that the sensitivity of even three Kato-Katz tests is highly dependent on prevalence and egg counts.

A single Kato-Katz test to diagnose STH infections may outperform other egg concentration techniques in areas with high parasite burdens.[20] However, as we demonstrated, the sensitivity of one Kato-Katz test is noticeable low when the burden of STH infections is low.[10] In addition, the loss in sensitivity when performing a single test compared to performing three tests was more for the Kato-Katz than for the Lumbreras test in our low burden setting. The decrease in sensitivity of the Kato-Katz by testing 3 stool samples compared to 1 was 33 and 80% for *Ascaris* and *Trichuris*, respectively. In contrast, the decrease in sensitivity of the Lumbreras tests on three stool samples compared to a single stool was 26 and 50% for the same parasites. Importantly, the only significant differences of a single Lumbreras test compared to 3 Kato-Katz were a lower sensitivity for *Ascaris* and a higher sensitivity for *Strongyloides* diagnosis. However, a substantial agreement between the Lumbreras test results and the Kato-Katz was found for most helminths.

The Lumbreras test is a simple concentration method that may be performed with reusable plastic ware and tap water. No hazardous solutions are required and the only equipment needed is a microscope. Also, it can be performed on fresh or preserved stool samples with little personnel training. These characteristics make it suitable for field laboratories with limited equipment in resource-constrained areas. The lack of quantitative results is a limitation of the Lumbreras test. Quantitative tests provide information regarding burden of infection in the population, which is directly associated with adverse health effects on infected individuals. The Lumbreras test cannot replace the Kato-Katz test since it is not quantitative. However, it may be an excellent screening tool given its simplicity and high sensitivity even in low burden areas.

Despite the significant differences in detection rates, the very low prevalence of positive tests for hookworm, *Trichuris*, and *Strongyloides* limited our ability to draw more solid conclusions. The time between collection and processing of the samples may have decreased the sensitivity of the Kato-Katz and Lumbreras tests, especially for hookworm. Decombe et al. suggested that the time between collection and testing of the samples decreased the sensitivity of the Kato-Katz test on fresh stool and formol–ether concentration test on stool preserved in 10% formalin by 50%.[21] Similarly, stool for Lumbreras test was preserved in 10% formalin, which may have decreased our ability to detect hookworm eggs. However, the effect of time to process on the sensitivity of Lumbreras test has not been established. The lack of a gold standard for the diagnosis of intestinal parasites forced us to use a combination of test results as the best test possible.[22] This is a limitation that may have decreased the accuracy of the comparisons between the Lumbreras sedimentation and Kato-Katz tests.

In summary, we have demonstrated again that the WHO-recommended Kato-Katz test may not perform well in low burden areas. We have also demonstrated that the simple, highly deployable Lumbreras rapid sedimentation test use is not limited to *Fasciola* infection and is a good alternative to diagnose other helminth infections including STH. The sensitivity of this test is higher than Kato-Katz for most helminth eggs and larvae and may be a suitable complement for screening in resource constrained areas. Validation of these results in other low burden areas or post-intervention settings is needed before considering its application.

Conflict of interest

The authors have no conflicts of interest to declare.

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