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Combination of five diagnostic tests to estimate the prevalence of hookworm infection among school-aged children from a rural area of colombia

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A B S T R A C T

Background: Public health programs for the control of soil-transmitted helminthiases require valid diagnostic tests for surveillance and parasitic control evaluation. However, there is currently no agreement about what test should be used as a gold standard for the diagnosis of hookworm infection. Still, in presence of concurrent data for multiple tests it is possible to use statistical models to estimate measures of test performance and prevalence. The aim of this study was to estimate the diagnostic accuracy of five parallel tests (direct microscopic examination, Kato-Katz, Harada-Mori, modified Ritchie-Frick, and culture in agar plate) to detect hookworm infections in a sample of school-aged children from a rural area in Colombia.

Methods and results: We used both, a frequentist approach, and Bayesian latent class models to estimate the sensitivity and specificity of five tests for hookworm detection, and to estimate the prevalence of hookworm infection in absence of a Gold Standard. The Kato-Katz and agar plate methods had an overall agreement of 95% and kappa coefficient of 0.76. Different models estimated a sensitivity between 76% and 92% for the agar plate technique, and 52% to 87% for the Kato-Katz technique. The other tests had lower sensitivity. All tests had specificity between 95% and 98%. The prevalence estimated by the Kato-Katz and Agar plate methods for different subpopulations varied between 10% and 14%, and was consistent with the prevalence estimated from the combination of all tests. The Harada-Mori, Ritchie-Frick and direct examination techniques resulted in lower and disparate prevalence estimates. Bayesian approaches assuming imperfect specificity resulted in lower prevalence estimates than the frequentist approach.

Conclusions Overall, the Kato-Katz and agar plate culture techniques performed better than other hookworm diagnostic tests. These results support the parallel combined use of the Kato-Katz and agar plate method to increase sensitivity in the diagnosis of hookworms in epidemiological studies.

1. Introduction

Health care and public health programs for the control of soil-transmitted helminthiasis demand highly accurate tests to evaluate strategies for the control and elimination of parasitic infections, to secure effective surveillance, and to properly prioritize interventions. In addition, these tests should be widely available and should be able to detect low-intensity infections (Knopp et al., 2009; McCarthy et al., 2012; Tchuem Tchuenté, 2011). Currently, there is no agreement about what test or combination of tests should be used as a Gold Standard for the diagnostic of hookworm infection (Knopp et al., 2009; Utzinger et al., 2008).

The WHO has recommended the use of the Kato-Katz technique due to its easy implementation and low cost (Montresor et al., 1998; World Health Organization, 1991). However, the literature reports sensitivity values between 14% and 77.2% for detection of hookworms in a single stool sample analyzed with the Kato-Katz technique (Anantaphruti et al., 2000; Glinz et al., 2010; Habtamu et al., 2011; Knopp et al., 2009; Komiya and Kobayashi, 1966; Nikolay et al., 2014; Nuñez-Fernandez et al., 1991; Speich et al., 2014; Tarafder et al., 2010), although one study showed 100% sensitivity (Machicado et al., 2012). The modified Ritchie-Frick test (Beck et al., 1965), which is a concentration-sedimentation method, has been frequently used in Colombia for the detection of hookworm infection in epidemiological studies; however, a

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recent study (Lopez et al., 2013) found that the sensitivity of this method was only 12%. In clinical settings, direct microscopic examination is routinely used with diagnostic purposes, though it also has low sensitivity for hookworm infection (6.8% − 42.8%) (Jongwutiwes et al., 1999; Lopez et al., 2013; Machicado et al., 2012; Nikolay et al., 2014). The low sensitivity and wide inter-study variation of these three tests may be related to low egg counts, daily variability in excretion (Booth et al., 2003; Knopp et al., 2009; Tarafder et al., 2010), and the time between sample collection and reading of slides, which is crucial to accurately detect hookworm eggs (Dacombe et al., 2007).

Although the Harada-Mori technique, which requires incubation and hatching of fecal samples on filter paper (Departments of the Air Force and the Army, 1974), may have higher sensitivity (75.3%–91.6) (Anantaphruti et al., 2000; Hasegawa et al., 1992; Komiya and Kobayashi, 1966), it is not routinely implemented in most basic parasitology and field laboratories for the diagnosis of hookworm infection. The consequent use of low sensitivity, imperfect tests could lead to an underestimation of the true prevalence of the parasite, making it difficult to monitor the activities of infection control. The use of suboptimal tests could also hide the persistence of a traditionally neglected public health problem (WHO, 2012).

Parallel to this, a growing body of evidence show that other tests such as the agar plate culture technique, with reported sensitivity for hookworm infection between 45.2% and 91.3 (Glinz et al., 2010; Inês et al., 2011; Jongwutiwes et al., 1999), or the FLOTAC method, may have higher sensitivities than Kato-Katz for the diagnostic of soil-transmitted helminthiasis in low prevalence populations (Glinz et al., 2010).

To address the limitations of individual techniques, some authors suggested to use more than one test to detect different parasitic forms (Mendes et al., 2005). However, there are few studies directly comparing the diagnostic performance of the various tests available for detection of hookworms in the same population (Gonçalves et al., 2014).

One additional complexity of estimating the diagnostic accuracy of these tests is the lack of agreement about a gold standard for hookworm detection. To deal with this situation, several studies resort to the use of a parallel combination of tests as a gold standard (Glinz et al., 2010; Habtamu et al., 2011; Knopp et al., 2011, 2009; Speich et al., 2014), but in consequence, these studies must assume a 100% specificity of all involved tests. Although specificity of parasite identification is expected to be high (Nikolay et al., 2014; Tarafder et al., 2010), a 100% may be unrealistic, and may distort the estimations of other measures of test performance as well as the prevalence. Bayesian approaches using latent class models (Nikolay et al., 2014; Tarafder et al., 2010) may be a more appropriate response to the problem of estimating test sensitivity while dealing with an unobserved true disease status.

Attending the above mentioned reasons, this study was aimed to assess the diagnostic accuracy of five tests: direct microscopic examination, Kato-Katz, Harada-Mori, culture in agar plate, and modified Ritchie-Frick techniques, as well as the combination thereof, for the diagnosis of hookworm infection (i.e. *Necator americanus* and *Ancylostoma duodenale*) in a sample of school-aged children from a rural Colombian community. In addition, we estimated the prevalence of hookworm infection in this population.

2. Material and methods

2.1. Study population

All children aged 5–15 years old, living in the town of La Virgen, Quipile municipality, state of Cundinamarca, Colombia, in September 2011, were invited to participate in the study. The prevalence of soil-transmitted helminthiasis in this population has been documented through multiple cross-sectional surveys since 1995 (Fernández-Niño et al., 2007), as part of a health education and treatment of soil-transmitted helminthiasis control program, including mass chemotherapy starting in early 2011 (Fernández-Niño et al., 2015).

2.2. Inclusion criteria

Participants had to be between 5 and 15 years, attending school at the local community, had to voluntarily provide informed assent, had written informed consent from the parent or legal guardian, provide their identification data, age, place of residence, school and class, and provide the sufficient amount of stool sample to perform five diagnostic tests.

2.3. Outcomes

Direct microscopic examination and the modified Ritchie-Frick technique were used to search for all parasitic forms; the Kato-Katz technique was used to search for eggs; the Harada-Mori and agar culture methods were used to search for larvae. The results of the Kato-Katz and modified Ritchie-Frick tests presenting quantitative results were recoded dichotomously, so that any sample with at least one observed egg was considered positive. Since these two tests use multiplication factors to estimate the number of eggs per gram (epg), a positive test would correspond to 24 epg for the Kato Katz (Montresor et al., 1998; World Health Organization, 1991) and 160 epg for the Ritchie-Frick technique (Beck et al., 1965). All of these tests rely on morphology for species identification, but eggs of *Necator americanus* and *Ancylostoma duodenale* cannot be morphologically differentiated, thus we will use the term hookworm to refer to either species along this paper.

2.4. Field and laboratory procedures

Fecal samples were collected in the early morning in plastic containers, then labeled, and kept on ice until processing. The samples were transported to the Parasitology Laboratory located at the Universidad Nacional de Colombia, Bogotá within 4 h of collection. The samples were processed by laboratory personnel blinded to the identity of the children who provided the sample.

The laboratory procedures for the direct examination, Kato-Katz (Katz et al., 1972; World Health Organization, 1991), Harada-Mori (Departments of the Air Force and the Army, 1974) and modified Ritchie-Frick (Beck et al., 1965) techniques have been described in detail before (Lopez et al., 2013; Machicado et al., 2012). For the agar plate technique we used Koga culture medium, placed 2 g of feces in the center of the plate and incubated it at 37 °C for 48 h. After this time, larvae were recovered in 10% formalin, centrifuged at 500*g* for 5 min, observing the sediment for specific parasite identification. The test was considered macroscopically positive for the presence of larvae when bacterial growth was observed on agar furrows resulting from larvae migration at 24, 48 and 72 h post-inoculation. Microscopic differentiation of the larvae at the species level was based on morphological characteristics (Beck et al., 1965; Jongwutiwes et al., 1999; Katz et al., 1972; Koga et al., 1991).

2.5. Statistical analysis

Initially, we compared basic socio-demographic characteristics of the study population with the distribution of the same variables in the source population to assess representativeness. Given that the proportion of voluntary participation was higher among children attending elementary school than among those in high school, comparisons were performed stratifying by educational cycle (elementary vs. high school).

The sensitivity and specificity of the five tests were estimated using two approaches: in the first one, frequentist, we assumed the parallel combination of all five tests to be the Gold Standard, so that every individual with at least one positive test was considered truly infected,

and individuals were considered free of infection only if they tested negative for hookworms in the five tests. The sensitivity, specificity and prevalence for each test were estimated using the classification produced by the so defined Gold Standard. In addition, we estimated the kappa coefficient to assess the agreement in excess of chance between the tests with the highest sensitivities. The second approach, Bayesian, used a latent class model where the true infection status of each of the participating individuals was considered as a latent class variable dependent on the prevalence (Gonçalves et al., 2014; Joseph et al., 1995). The probability of a positive outcome for an individual in any of the tests was assumed as a random Bernoulli variable, whose parameter for infected individuals is the sensitivity, and for the uninfected is 1-specificity. The sensitivity, specificity and prevalence were modeled as binomially distributed variables with parameters n and p, where n is the number of participants in the observed sample, and p is sampled from the beta prior distributions.

The latent class model was implemented in WinBUGS 1.4.3, which uses a Gibbs sampling algorithm to estimate the multivariate posterior distributions by iteratively sampling and updating the prior conditional distributions by means of Markov-chain Monte Carlo simulations (Dendukuri and Joseph, 2001; Joseph et al., 1995). The Sensitivity, specificity and prevalence were estimated from 20,000 such simulations originated in two parallel-processed Markov chains with randomly initialized parameters. After assessing convergence of the two Markov chains with the Gelman-Rubin diagnostics, we discarded the first 1000 simulations as a burn-in and used the next 10,000 simulations from each chain to estimate the sensitivity, specificity, and prevalence. We implemented 3 models with different assumptions about the specification of the prior distribution: Model 1 assumed that specificity came from a distribution common to all tests, while sensitivities were considered inherently different for each test, and were derived from a summary of the available literature. Thus, mean sensitivity for Kato-Katz was estimated to be about 50% (Anantaphruti et al., 2000; Glinz et al., 2010; Habtamu et al., 2011; Knopp et al., 2009; Komiya and Kobayashi, 1966; Machicado et al., 2012; Nikolay et al., 2014; Nuñez-Fernandez et al., 1991; Speich et al., 2014; Tarafder et al., 2010), for the agar Plate 70 % (Glinz et al., 2010; Inês et al., 2011; Jongwutiwes et al., 1999), for Harada Mori 80% (Anantaphruti et al., 2000; Hasegawa et al., 1992; Komiya and Kobayashi, 1966), for Ritchie-Frick 15% (Lopez et al., 2013), and for direct exam 25% (Jongwutiwes et al., 1999; Lopez et al., 2013; Machicado et al., 2012; Nikolay et al., 2014). Model 2 kept the same assumptions about specificity, but replaced the prior distributions of the sensitivities with distributions based on the observed data (empirical Bayes). Model 3 is similar to model 2, but it allows for the tests to be correlated by means of a random effect defined at the individual level, which is analogous to infection intensity and induces a correlation structure between tests (Dendukuri and Joseph, 2001; Limmathurotsakul et al., 2010; Pan-ngum et al., 2013). The expected values of the priors used in our analysis along with their credible intervals are summarized in Table 1.

2.6. Ethical considerations

This research followed the ethical guidelines delineated in the Declaration of Helsinki (WMA General Assembly, 2013), the Resolution No. 008430 of 1993 of the Ministry of Health of Colombia (Colombia Ministry of Health, 1993), and Colombia's Children Protection Act (Congreso de la República de Colombia, 1989). The research protocol was approved by the IRB of the National University of Colombia Medical School (Act No. 15 of 2009).

All individuals diagnosed as infected with pathogen intestinal parasites by any of the five tests received treatment. Those with helminth infections received albendazole, those infected by *Giardia duodenalis* received metronidazole, while those with *E. histolytica/E. dispar/E. moshkovskii* were treated with teclozan. Teclozan is a luminal antiprotozoal agent active against the trophozoite stage of the *E. histolytica/E. dispar/E. moshkovskii* complex (Barar, 2015; Botero et al., 1990). While teclozan is not commonly used in other regions, it remains a cornerstone of luminal antiprotozoal therapy in several countries of South America due to the unavailability of alternative pharmaceutical agents (Edouard et al., 2004; Romero Cabello, 2002). For instance, diloxanide and paromomycin are not registered for clinical use in Colombia and are thus unavailable (Ministry of Health of the Republic of Colombia, 2015).

Table 1
Parameter values and credible intervals for the prior distributions of sensitivity, specificity and prevalence of three Bayesian models of diagnostic tests for hookworm detection.

Parameter	Expected value (credibility interval 95%)		
	Model 1	Model 2	Model 3
Kato-Katz Sensitivity	50% (35%–65%)	Elementary school: 80% (60%–100%) High School: 70% (50%–90%)	
Agar plate Sensitivity	70% (40%–100%)	Elementary school: 90% (80%–100%) High School: 80% (60%– 100%)	
Harada-Mori Sensitivity	80% (65%–95%)	Elementary school: 25% (5%–45%) High School: 5% (0%–10%)	
Ritchie-Frick Sensitivity	15% (0%–30%)	Elementary school: 5% (0%–10%) High School: 20 (0%–40%)	
Direct examination Sensitivity	25% (0%–50%)	Elementary school: 5% (0%–10%) High School: 5% (0%–10%)	
Specificity (All tests)	95% (90%–100%)	95% (90%–100%)	95% (90%–100%)
Prevalence	15% (5%–25%)	15% (5%– 25%)	15% (5%–25%)
Correlation between tests allowed in the model	No	No	Yes

3. Results

According to the school census, 277 children between 5 and 15 years were attending school at La Virgen in 2011. Of these, 175 (63%) voluntarily participated in our study, had the consent of their parents, and provided a stool sample sufficient to perform five diagnostic tests. The final study population included 77 girls (44%) and 98 boys (56%). Elementary students were more likely to participate than high-school students (85% vs. 35%). Within each school level, participants and non-participants were similar in terms of age and gender.

The frequentist analysis showed a higher detection rate of hookworms among elementary school students as compared to high school, except for the modified Ritchie-Frick technique. The prevalence of infection estimated using the set of 5 parallel diagnostic tests was 15.79% for elementary school and 14.29% for high school. Prevalence estimates for individual tests are shown in Table 2. In the 21 samples that tested positive according to the Kato-Katz technique, egg counts ranged between 24 and 1560 epg, with a mean of 313 and median of 168 epg.

In our data, and using the frequentist perspective, the tests could be separated in two groups according to the concordance of their results:

Table 2
Estimated prevalence and 95% confidence intervals of Hookworm Infection in La Virgen, Colombia, 2011, according to 5 diagnostic tests.

Diagnostic test	Elementary school (n = 133)	High School (n = 42)
	% (95% CI)	% (95% CI)
Harada-Mori	4.1 (0.5–7.7)	0 (n.a.)
Direct Exam	0.8 (0–2.2)	0 (n.a.)
Ritchie-Frick	0 (n.a.)	2.4 (0–7.1)
Kato-Katz	12.8 (7.1–18.5)	9.5 (0.5–18.6)
Agar plate	13.6 (7.7–19.6)	11.9 (1.9–21.9)
All tests combined	15.8 (9.5–22.1)	14.3 (3.5–25.1)

Table 3
Comparison of the estimated prevalence of hookworm infection, sensitivity and specificity of five tests for hookworm detection, using frequentist and Bayesian approaches in a sample of school-age children in La Virgen, Colombia, 2011.

	Frecuentist method	Bayesian Model 1	Bayesian Model 2	Bayesian Model 3
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Sample of elementary school children				
Kato-Katz sensitivity	81% (58.1–94.6)	60.9% (47.9–72.9)	86.6% (71.6–96.8)	86.0% (69.8–96.6)
Agar plate sensitivity	90% (68.3–98.8)	87.2% (69.7–97.6)	92.0% (82.5–98.0)	91.9% (82.5–97.9)
Harada-Mori sensitivity	26.3% (9.2–51.2)	64.6% (48.7–79.0)	29.2% (15.1–45.7)	26.8% (13.0–44.1)
Ritchie-Frick sensitivity	0% (0–16.8)	8.7% (2.0–19.7)	4.1% (1.1–9.0)	3.7% (0.9–8.2)
Direct exam sensitivity	4.8% (0.1–23.8)	14.0% (3.8–29.4)	5.2% (1.7–10.6)	4.7% (1.5–9.7)
Kato-Katz specificity	100% (96.8–100)	96.7% (93.6–98.9)	97.1% (94.2 - 99.1)	97.1% (94.2–99.1)
Agar plate specificity	100% (96.8–100)	96.4% (93.1–98.8)	96.5% (93.3–98.8)	96.7% (93.5–98.9)
Harada-Mori specificity	100% (96.4–100)	97.9% (95.5–99.5)	97.9% (95.4–99.5)	97.9% (95.5–99.5)
Ritchie-Frick specificity	100% (96.6–100)	98.0% (95.6–99.5)	98.0% (95.5–99.5)	98.0% (95.6–99.5)
Direct exam specificity	100% (96.8–100)	98.0% (95.7–99.5)	98.0% (95.7–99.5)	98.0% (95.7–99.5)
Prevalence	15.8% (9.5–22.1)	12.9% (8.1–18.4)	13.1% (8.4–18.6)	13.6% (8.7–19.2)
Sample of high-school children				
Kato-Katz sensitivity	66.7% (22.3–95.7)	52.3% (37.7–66.5)	71.0% (51.0–87.5)	70.7% (50.7–87.3)
Agar plate sensitivity	83.3% (35.9–99.6)	76.2% (47.6–95.5)	81.8% (61.3–95.6)	81.4% (60.2–95.6)
Harada-Mori sensitivity	0% (0–52.2)	74.5% (56.8–89.0)	4.8% (1.2–10.4)	4.8% (1.2–10.5)
Ritchie-Frick sensitivity	16.7% (0.4–64.1)	17.1% (5.4–33.9)	20.8% (6.4–41.6)	19.8% (5.6–40.8)
Direct exam sensitivity	0% (0–45.9)	19.8% (4.2–43.8)	4.7% (1.2–10.3)	4.6% (1.2–10.2)
Kato-Katz specificity	100% (90.3–100)	95.5% (90.7–98.6)	96.0% (91.6–98.8)	96.0% (91.6–98.8)
Agar plate specificity	100% (90.3–100)	94.9% (90.0–98.3)	95.6% (90.9–98.7)	95.5% (91.0–98.7)
Harada-Mori specificity	100% (90–100)	96.7% (92.7–99.1)	96.6% (92.7–99.1)	96.6% (92.6–99.1)
Ritchie-Frick specificity	100% (90.3–100)	96.7% (92.7–99.1)	96.7% (92.7–99.1)	96.7% (92.7–99.1)
Direct exam specificity	100% (90.3–100)	96.7% (92.8–99.1)	96.7% (92.5–99.1)	96.7% (92.7–99.1)
Prevalence	14.3% (3.5–25.1)	11.5% (5.6–19.0)	12.8% (6.5–20.7)	13.2% (6.7–21.4)

the Kato-Katz method and the agar plate culture grouped together, with an overall agreement of 94.8% and kappa coefficient of 0.76. These two methods provided similar prevalence estimates and had overlapping confidence intervals. In addition, these intervals contained the estimated prevalence values derived from the combination of all tests. The Harada-Mori, Ritchie-Frick and direct examination techniques instead, provided prevalence estimates inconsistent across tests, and which were out of the confidence interval for the prevalence estimated by the combination of the five tests. Under the frequentist approach, the prevalence estimated from the culture agar plate was higher than that estimated with the Kato-Katz test, and both were higher than the estimates derived from the Harada Mori, Ritchie-Frick and direct examination results.

The results of the Bayesian simulation approach under three different model specifications are summarized in Table 3. The same table allows to compare these results with the point estimators and confidence intervals calculated using the frequentist approach. Models based on the Bayesian approach resulted in lower prevalence estimates than those based on the frequentist approach. In the model with literature-driven prior distributions, the agar plate and Harada-Mori techniques exhibited the highest sensitivity. However, in models with data-driven priors, the agar plate and Kato-Katz methods consistently showed the highest sensitivities among all tests.

4. Discussion

Accurate and sensitive diagnostic methods are necessary for the clinical and public health evaluation of helminth infections, as well as for monitoring treatment and control interventions (Speich et al., 2014).

In this study population, the intensity of hookworm infection in all cases was mild; the highest count of epg by the Kato-Katz test was 1560, that occurred in a 12 years old child. All others were less than 800 epg.

This study showed that the Kato-Katz and agar plate tests had a significantly better diagnostic performance compared with the Ritchie-Frick, and direct examination tests in the identification of hookworm infection. This is mainly due to the higher sensitivity of the first two tests: in this study, the sensitivity of the agar plate for hookworm infection was estimated to be between 76% and 92%, depending on the estimation method and subpopulation; in the same manner, the sensitivity of the Kato-Katz test was estimated to be between 52 and 87%. The specificity of all tests was high, estimated by different models to be between 94 and 98%. However, the assumption of specificity equal to 100% seems to be excessive, since the posterior credibility intervals of Bayesian analysis did not include in any case the scenario of perfect specificity. Prevalence estimates obtained with the frequentist approach and the Bayesian models are consistent between them, and their confidence or credibility intervals extensively overlap. Estimates from Bayesian models resulted in more precise intervals than the frequentist estimates. Models 2 and 3, which differed only by allowing correlation between the tests, provided similar results, and were more plausible under the observed data than our literature-driven model 1.

This work suggests that, the simultaneous use of Kato-Katz and agar plate culture methods in parallel may allow to increase the net sensitivity to a value between 85% and 98%, depending on which of the different estimation models is deemed as the most appropriate (frequentist or Bayesian models), and what is the intended target population. Net specificity under the same conditions, would be between 89 and 92%. These estimates correspond to samples with mild infection intensities.

In our sample, the Harada Mori test, the Ritchie-Frick concentration technique, and the direct examination test did not provide additional positive cases besides those detected with the other two methods. One of the causes described in the literature (Anamnart et al., 2010) to explain the low sensitivity of the Ritchie-Frick method is the effect of formaldehyde on hookworm eggs, in addition to the effect of the gauze pore size in the recovery of larvae and eggs.

Similar results about the superiority of Kato-Katz versus sedimentation-concentration methods for the diagnosis of hookworms have been reported before (Lopez et al., 2013; Nuñez-Fernandez et al., 1991). However, the Kato-Katz method is not capable of detecting *Strongyloides stercoralis* infections, which may be a disadvantage in regions where strongyloidiasis is still endemic (Becker et al., 2015). In contrast, other authors reported that formaldehyde-ether is more

sensitive than Kato-Katz when using a single stool sample (Speich et al., 2014), and that spontaneous sedimentation performed better than Kato-Katz in terms of egg counts per gram of feces (Machicado et al., 2012).

The higher sensitivity of the agar plate culture versus other methods for the diagnosis of hookworm is in agreement with previous reports (Inês et al., 2011; Jongwutiwes et al., 1999), which also showed that the sensitivity of the agar plate increases with time post-inoculation. Besides, Koga agar culture is well suited for detection of both, hookworm and *Strongyloides stercoralis*, although distinction of these species based on the morphology of larvae alone may be inaccurate (Becker et al., 2015). While the use of molecular methods including multiplexing assays (WHO, 2012) and PCR (Becker et al., 2015) may contribute to improve diagnosis performance and species identification, and to better monitor programs for the control and elimination of neglected diseases, these are not readily available in most endemic countries, outside scientific research settings.

On the other hand, direct exam and sedimentation-concentration methods have the advantage that they are able to concurrently detect helminths and protozoa in the same sample, which is a major asset in clinical settings.

Several authors have pointed out that a single sample Kato-Katz has low sensitivity for the diagnosis of hookworm eggs, which worsens with the time elapsed between sample collection and observation. Dacombe et al. (Dacombe et al., 2007) and Gonçalves et al. (Gonçalves et al., 2014) suggested that the decrease in sensitivity along time may be due to larvae emerging from the eggs and/or to degeneration of the eggs as time elapses between the emission and microscopic observation. However, in this study we only observed larvae in the agar plates after 48 h post-inoculation. This suggests that average hatching time may be greater than previously considered by the aforementioned authors.

As described above, there were no large differences in major demographic variables between the participant and non-participant population, with the exception of the proportion of children living in a rural vs. urban area among elementary school students. The strengths of this study include the participation of highly qualified laboratory personnel performing the detection of parasitic forms, the use of multiple diagnostic methods and a latent class Bayesian model to address the lack of consensus about an appropriate gold standard for the diagnosis of hookworm infection.

5. Conclusions

In the absence of consensus about a gold standard for hookworm detection, some studies have considered the possibility of combining two or more tests (de Carvalho et al., 2012; Knopp et al., 2008). Based on our results, we suggest using a parallel combination of the Kato-Katz method and agar plate culture in order to increase sensitivity in the diagnosis of hookworm infection in epidemiological studies. This recommendation is based on the combined ability of the two tests to detect most of the cases present in our study population, while the Harada Mori, Ritchie-Frick and direct examination tests did not identify additional cases. The parallel combination of the Kato-Katz test and agar plate may be an advantageous candidate for a Gold Standard, since these two tests do not require sophisticated instrumentation and can be performed in a low complexity laboratory setting.

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Appendix. Combination of five diagnostic tests to estimate the prevalence of hookworm infection among school-aged children from a rural area of Colombia

Models used in the Bayesian analysis

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#Model 1 (elementary and high school)
    #Specificity is common to all tests
    #Sensitivities are inherent to each test
    #Priors are derived from literature for both elementary and high school students
    #Tests are assumed to be independent, conditional on true disease status
```

```
Model{

    #Likelihood
    for(i in 1:n){
        d[i]~dbern(pr)
        for(k in 1:n.test){
            test[i,k] ~ dbern(p[i,k])
            p[i,k] <- se[k]*d[i]+(1-sp[k])*(1-d[i])
                                                    }
                                        }
    #Prior
    for(k in 1:n.test){
        sp[k] ~ dbeta(71.25, 3.75)
                                                }
se[1] ~ dbeta(21.7222, 21.7222)
se[2] ~ dbeta(5.8333, 2.5)
se[3] ~ dbeta(21.9556, 5.4889)
se[4] ~ dbeta(3.25, 18.4167)
se[5] ~ dbeta(2.75, 8.25)
pr ~ dbeta(7.5, 42.5)
            }
```

#Model 2 (elementary school)

#Specificity is common to all tests
#Sensitivities are inherent to each test
#Priors are derived from observed data for elementary school students
#Tests are assumed to be independent, conditional on true disease status

```
Model{

    #Likelihood
    for(i in 1:n){
        d[i]~dbern(pr)
        for(k in 1:n.test){
            test[i,k] ~ dbern(p[i,k])
            p[i,k] <- se[k]*d[i]+(1-sp[k])*(1-d[i])
                                                    }
                                    }
    #Prior
    for(k in 1:n.test){
        sp[k] ~ dbeta(71.25, 3.75)
                                                    }
    se[1] ~ dbeta(12, 3)
    se[2] ~ dbeta(31.5, 3.5)
    se[3] ~ dbeta(4.4375, 13.3125)
    se[4] ~ dbeta(3.75, 71.25)
    se[5] ~ dbeta(3.75, 71.25)
    pr ~ dbeta(7.5, 42.5)
                    }
```

#Model 3 (elementary school)

#Specificity is common to all tests

#Sensitivities are inherent to each test

#Priors are derived from observed data for elementary school students

#Tests are allowed to be correlated by means of a random error parameter defined at the individual level that is interpretable as a continuous disease intensity indicator

```
Model{

    #Likelihood
    for(i in 1:n){
        d[i]~dbern(pr)
        e[i]~dnorm(0,1)
    for(k in 1:n.test){
        test[i,k] ~ dbern(p[i,k])
        logit(p[i,k]) <- a[k]*d[i]+b[k]*(1-d[i])+e[i]*d[i]
                                                    }
                                }

#Prior
for(k in 1:n.test){
        sp[k] ~ dbeta(71.25, 3.75)
        a[k]<-logit(se[k])
        b[k]<--logit(sp[k])
                                                }
se[1] ~ dbeta(12, 3)
se[2] ~ dbeta(31.5, 3.5)
se[3] ~ dbeta(4.4375, 13.3125)
se[4] ~ dbeta(3.75, 71.25)
se[5] ~ dbeta(3.75, 71.25)
pr ~ dbeta(7.5, 42.5)
                }
```

#Model 2 (high school)

#Specificity is common to all tests
#Sensitivities are inherent to each test
#Priors are derived from observed data for high school students
#Tests are assumed to be independent, conditional on true disease status

```
Model{

    #Likelihood
    for(i in 1:n){
        d[i]~dbern(pr)
        for(k in 1:n.test){
            test[i,k] ~ dbern(p[i,k])
            p[i,k] <- se[k]*d[i]+(1-sp[k])*(1-d[i])
                                                    }
                                    }

    #Prior
    for(k in 1:n.test){
        sp[k] ~ dbeta(71.25, 3.75)
                                                }
    se[1] ~ dbeta(14, 6)
    se[2] ~ dbeta(12, 3)
    se[3] ~ dbeta(3.75, 71.25)
    se[4] ~ dbeta(3, 12)
    se[5] ~ dbeta(3.75, 71.25)
    pr ~ dbeta(7.5, 42.5)
            }
```

#Model 3 (high school)

#Specificity is common to all tests

#Sensitivities are inherent to each test

#Priors are derived from observed data for high school students

#Tests are allowed to be correlated by means of a random error parameter defined at the individual level that is interpretable as a continuous disease intensity indicator

```
Model{

    #Likelihood
    for(i in 1:n){
        d[i]~dbern(pr)
        e[i]~dnorm(0,1)
        for(k in 1:n.test){
            test[i,k] ~ dbern(p[i,k])
            logit(p[i,k]) <- a[k]*d[i]+b[k]*(1-d[i])+e[i]*d[i]
                }
            }

    #Prior
    for(k in 1:n.test){
        sp[k] ~ dbeta(71.25, 3.75)
        a[k]<-logit(se[k])
        b[k]<--logit(sp[k])
                }
    se[1] ~ dbeta(14, 6)
    se[2] ~ dbeta(12, 3)
    se[3] ~ dbeta(3.75, 71.25)
    se[4] ~ dbeta(3, 12)
    se[5] ~ dbeta(3.75, 71.25)
    pr ~ dbeta(7.5, 42.5)
            }
```

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