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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

Rafael E. Barreto, Javier Narváez*,[1], Natalia A. Sepúlveda, Fabián C. Velásquez, Sandra C. Díaz, Myriam Consuelo López, Patricia Reyes, Ligia I. Moncada

Departamento de Salud Pública, Facultad de Medicina, Universidad Nacional de Colombia, Carrera 30 # 45-03, Bogotá, Colombia

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

* Corresponding author. at: Carrera 30 # 45–03 Universidad Nacional de Colombia Facultad de Medicina, Edificio 471, Departamento de Salud Pública, Oficina 150 Bogotá, Colombia.
E-mail addresses: rebarretor@unal.edu.co (R.E. Barreto), janarvaezg@unal.edu.co, jn2471@cumc.columbia.edu (J. Narváez), nasepulvedah@unal.edu.co (N.A. Sepúlveda), fcvelasquezc@unal.edu.co (F.C. Velásquez), scdiazd@unal.edu.co (S.C. Díaz), mclopezp@unal.edu.co (M.C. López), preyesh@unal.edu.co (P. Reyes), limoncadaa@unal.edu.co (L.I. Moncada).
[1] Current address: 100 Haven Ave., Apt. 4G New York, NY 10032, U.S.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,