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Comparison of parasitological methods for the identification of soil-transmitted helminths, including *Strongyloides stercoralis*, in a regional reference laboratory in northwestern Argentina: An observational study

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ABSTRACT

Soil-transmitted helminths (STH) are a significant public health problem in impoverished communities of tropical and subtropical areas. Improved diagnostic methods are crucial for Neglected Tropical Diseases programs, particularly for *S. stercoralis*, as traditional methods are inadequate. Thus, it is important to identify the most accurate and efficient methods for the diagnosis of STH. We performed a retrospective study analyzing laboratory data at the Instituto de Investigaciones de Enfermedades Tropicales from 2010 to 2019. The study included data from outpatients referred for stool analysis and public health interventions from urban and rural communities in northern Salta province, Argentina. Samples were included in this analysis if processed through sedimentation/concentration, Baermann, Harada-Mori and McMaster's, with a subgroup that also included Agar plate culture method (APC). Sensitivity was calculated against a composite reference standard. Of the 5625 samples collected, 944 qualified for this analysis, with a prevalence of 11.14% for *A. lumbricoides*, 8.16% for hookworm, 1.38% for *T. trichiura*, and 6.36% for *S. stercoralis*. The sedimentation/concentration method was the most sensitive for *A. lumbricoides* (96%), compared to the McMaster method, with a sensitivity of 62%. Similarly, for hookworms, sedimentation/concentration was more sensitive than McMaster's, Harada-Mori, and Baermann with sensitivities of 87%, 70%, 43%, and 13%, respectively. Most of these infections were of light

Abbreviations: STH, Soil transmitted helminths; WHO, World health organization; DALYs, Disability adjusted life years; NTD, Neglected tropical diseases; NAAT, Nucleic-acid amplification tests; IIET, Instituto de Investigaciones de Enfermedades Tropicales; APC, Agar plate culture; EPG, Egg per gram; PPV, Positive predictive values; NPV, Negative predictive values; IQR, Interquartile range;; qPCR, Quantitative polymerase chain reaction..

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intensity. For *S. stercoralis*, Baermann and sedimentation/concentration methods were the most sensitive, with 70% and 62% respectively, while Harada-Mori was the least sensitive. In a subset of 389 samples also analyzed by the APC, Baermann was more sensitive than APC for detecting *S. stercoralis*, and both methods were superior to Harada-Mori. Parasitological methods, mostly when used combined, offer adequate opportunities for the diagnosis of STH in clinical and public health laboratories. The incorporation of *S. stercoralis* into the control strategies of the World Health Organization requires rethinking the current diagnostic approach used for surveys. With sedimentation/concentration and Baermann appearing as the most sensitive methods for this species. Further studies, including implementation assessments, should help in identifying the most adequate and feasible all-STH diagnostic approach.

1. Introduction

Soil-transmitted helminths (STH) include a group of nematodes, such as *Ascaris lumbricoides*, *Trichuris trichiura*, the hookworms (*Ancylostoma duodenale* and *Necator americanus*) and *Strongyloides stercoralis* whose control and elimination as a public health problem, has been targeted by the World Health Organization (WHO) (Becker et al., 2018; WHO, Word Health Organization, 2020a). STH generate a greater burden of disease in impoverished communities of tropical and subtropical areas that suffer from inadequate water and sanitation services and are responsible for 3.3 million disability-adjusted life years (DALYs) (Alemu et al., 2022; Bartlett et al., 2022). Currently, most STH control programs include only *A. lumbricoides*, *T. trichiura* and hookworms as the target species, while *S. stercoralis*, has been recently added to WHO's strategy with the aim of establishing an efficient control program in school-age children by 2030 (WHO, Word Health Organization, 2020a, 2020b).

Diagnostic tools with adequate accuracy, reproducibility and affordability are a requirement for the development and monitoring of Neglected Tropical Diseases (NTD) control programs (Souza et al., 2021). In the case of STH, it needs to be redefined in view of the incorporation of *S. stercoralis*, which requires parasitological methods different from those used for *A. lumbricoides*, *T. trichiura* and hookworms (Krolewiecki and Nutman, 2019). While classical parasitological methods are often adequate for baseline surveys, mapping disease distribution and monitoring the progress of interventions, the need for improved diagnostics becomes a priority as infection prevalence declines and programmatic decisions become dependent on the performance and operational characteristics of the diagnostic methods applied in field laboratories (Bergquist et al., 2009; Souza et al., 2021).

Besides their use in public health, parasitological methods are still the first line methods in multiple clinical laboratories around the world that serve individualized care of patients in low prevalence settings, migrants and travelers (Shane et al., 2017). Even in reference centers that have incorporated molecular-based methods, there is growing concern on the lack of standardized protocols, trained microscopists and overall availability of these “vintage” methods that are still necessary in particular clinical situations and also as reference methods for validating nucleic-acid amplification tests (NAAT) and serological diagnostic approaches (van Lieshout and Roestenberg, 2015).

While NAAT appear as more sensitive than parasitological methods, particularly in low burden infections and serology assays are only available for *S. stercoralis* (Bisoffi et al., 2014; Cimino et al., 2015), there is a need to identify the most suitable diagnostic approach for the particular requirements of each laboratory setting. Therefore, rather than the pure accuracy of each diagnostic method, the objective of this study is to present an analysis of over 900 samples, from a regional stool microscopy-based reference lab in northwestern Argentina (WHO, Word Health Organization, 2020c), to identify the most accurate and efficient array of parasitological diagnostic methods with special attention to *S. stercoralis*.

2. Material and methods

2.1. Study design

A retrospective analysis of the records of the parasitology laboratory at “Instituto de Investigaciones de Enfermedades Tropicales” (IIET) between 2010 and 2019. The IIET is located in San Ramon de la Nueva Oran, Salta, Argentina, 46 km south of the border with Bolivia, an area endemic for STH. The database comprises information from outpatients and from different public health interventions and surveys carried out in the area. Information includes the results of diagnostic methods applied to stool sample as well as sex and age.

2.2. Parasitological methods

All single stool samples from individual cases were collected without preservatives and analyzed within 24 h through four different methods: sedimentation/concentration, McMaster, Harada-Mori and Baermann; a separate analysis with samples also evaluated with the Agar Plate Culture method (APC) is included.

Stool samples were processed according to the standard laboratory operating procedure; upon arrival at the laboratory, samples were coded, weighted and kept at room temperature through processing, with the different methods briefly described as follows.

2.2.1. Sedimentation/concentration

In the same cup in which the sample was collected, equal quantities of fresh feces (between 3 and 50 g) and tap water were mixed. The mixture was then homogenized until obtaining a semi-liquid consistency. Approximately 5 mL of the mix was filtered using four layers of gauze, placed in a funnel, and deposited in a 15 mL conical tube. Then, the mixture was then centrifuged at 2500 r.p.m. for 5 min, discarding the supernatant. Finally, the tube was washed with tap water by vigorously agitating the sediment, resuspending the sediment, and then a drop of sediment was extracted and deposited on a microscope slide with a drop of Amido Schwartz dye, and examined microscopically at 100× and 400× (Garcia, 2001).

2.2.2. McMaster

2 g of feces were filtered and homogenized with 30 mL of saturated saline. Two flotation chambers (0.5 mL each) were filled for each sample and examined after 3 min. Eggs were counted and multiplied by 50 to calculate the eggs per gram (EPG) of feces for each identified species (Barda et al., 2014).

2.2.3. Harada-Mori

1 g of feces was spread in the center of a narrow strip of filter paper and inserted into a 50 mL conical tube containing 5 mL of tap water, with the lower end of the paper touching the water. The tube was sealed with paraffin paper pierced with a needle for aeration and then maintained in an upright position at 28 °C for 7 days and then examined in a microscope at 100× and 400× (Garcia, 2001).

2.2.4. Baermann

5 to 10 g of fresh feces were mixed with activated charcoal. The stool sample was transferred to a petri dish with a double layer of tissue paper at the bottom and then covered by single layer of tissue paper at the top to form a small pouch. Incubation was maintained for 24 h at 26 °C. After incubation, stool samples were suspended for 1 h in lukewarm water at room temperature (which is in our case between 25 and 37°C) and filtered using a conventional Baermann apparatus (a strainer on top of a funnel connected to a clamped rubber hose) supported by a funnel stand, the single layer tissue paper side of the pouch was facing the strainer. Then, the lower 10 mL from the water contained in the hose was drained off, centrifuged at 2000 r.p.m. for 5 min, and sediment was examined microscopically at 100× and 400× for the identification of larvae (Garcia, 2001).

2.2.5. Agar plate culture (APC)

Agar was prepared at the concentration indicated by the manufacturer, (laboratory Britania S.A. CABA-Argentina), autoclaved, and distributed in Petri dishes at a thickness of 0.5 cm without any additives or antibiotics. Then, one gram of fresh feces was placed in the center of the Petri dish and incubated at 37 °C for 48 h. Finally, it was observed in a microscope at 400× (Garcia, 2001).

2.3. Data analysis

An ad-hoc database was created for the purpose of this analysis in Microsoft Excel, where data was included in a de-identified manner. Infection intensities for *A. lumbricoides* and hookworms were classified into light, moderate or high intensity according to WHO (Ephrem et al., 2017). Although this classification was intended for Kato-Katz method and not for McMaster method, which represents a limitation, it was known that both methods correlated well (Levecke et al., 2011; Barda et al., 2014). The performance of each parasitological method was evaluated by calculating the sensitivity, positive and negative predictive values (PPV & NPV) for each species of STH. A composite reference standard was used as a reference diagnostic test, where a sample that tested positive for at least one method was considered positive. For the cases of *A. lumbricoides*, the composite reference standard was the combination of the sedimentation/concentration and McMaster. For hookworms, the combination of five or four methods (without APC) was used as a composite reference standard, while for *S. stercoralis* the combination of four methods was used as a composite reference standard (sedimentation/concentration, Harada-Mori, APC and Baermann, or three methods (without APC)). McNemar test was used to compare the sensitivity and relative predictive values to compare PPV and NPV-and a non-parametric Kruskal Wallis test was performed to evaluate the relationship between sample volume and positivity. *P*-values <0.05 were considered statistically significant. All the statistical analyses were performed using R Software (R Foundation for Statistical Computing. Vienna, Austria), "DTComPair" and "PropCIs" packages in RStudio version 4.3.3.

2.4. Ethical considerations

The entire database was de-identified. The project was evaluated and approved by the Institutional Review Board at Hospital San Vicente de Paul, Oran, Salta Argentina, thorough an approval letter dated on September 4th, 2019.

3. Results

3.1. Characteristics of the study data

A total of 5625 samples were included in the database. Out of the total, 944 had complete information on four diagnostic methods (sedimentation/concentration, Harada-Mori, McMaster and Baermann), and 389 had complete information on five diagnostic methods (sedimentation/concentration, Harada-Mori, McMaster, Baermann, and APC).

The mean age of the study population was 10 years old (IQR: 6–21), 55.9% were female and 44.1% male. Prevalence of STH species were 11.44, 8.16, 6.36, and 1.38% for *A. lumbricoides*, hookworm, *S. stercoralis*, and *T. trichiura*, respectively (Table 1), consistent with the prevalence informed in the area (Echazú et al., 2015). Most infections were of light and moderate intensity (Table 2). Due to the low prevalence of *T. trichiura* it was excluded from subsequent analyses, and it was only considered in the calculations of prevalence and infection intensity.

Table 1. A includes information on the overall findings in the study population for any STH and for each species. Hookworm species, were identified through Harada-Mori and Baermann methods in 38 cases. Out of these, 10 cases (26%) were due to *A. duodenale*, 27 (71%) due to *N. americanus*, and 1 case (3%) presented both species.

3.2. Performance of parasitological methods

In each case, the diagnostic specificity and PPV were considered 100%. From both methods evaluated for *A. lumbricoides*, sedimentation/concentration showed higher sensitivity and NPV than McMaster ($p < 0.01$). Sedimentation/concentration was also found to be significantly more sensitive for hookworms, followed by McMaster, Harada-Mori and Baermann ($p < 0.01$ in each comparison). For *S. stercoralis*, sedimentation/concentration and Baermann methods were the most sensitive, without significant differences between them ($p = 0.38$). The sedimentation/concentration method was the most effective method of detection, as it not only detected the majority of the samples positive by other methods, also was the only method detecting 38% of *A. lumbricoides* and 17% of hookworm samples; while for *S. stercoralis*, Baermann and sedimentation/concentration were the most effective methods, being the only methods positive for this species in 32% and 38% of the positive samples, respectively (Table 3).

Regarding the dataset of 389 samples that in addition to the 4 methods mentioned above were also analyzed by the APC method, the results showed that for *S. stercoralis*, Baermann was found to be more sensitive than the APC method ($p = 0.038$). Baermann, sedimentation/concentration, and APC methods were all more sensitive than the Harada-Mori method ($p < 0.05$). For hookworms, Baermann and APC methods had lower sensitivity than sedimentation/concentration, without any significant differences between them ($p = 0.15$). Among this subgroup of 389 samples which included 39 samples with *S. stercoralis*, 5 (12,82%) were identified exclusively through APC; and for the 50 cases with hookworms, there were no positive cases exclusively by APC method.

For the sedimentation/concentration method, the median volume of stools used was 27 g (IQR 20–39). No significant differences were found in the volume of feces between negative and positive samples (Kruskal Wallis test. *A. lumbricoides* $p = 0.31$, hookworms $p = 0.36$ and *S. stercoralis* $p = 0.85$).

4. Discussion

This retrospective study aimed at identifying the most accurate and operationally feasible set of parasitological methods for an adequate assessment in endemic areas where interventions to control the prevalence (deworming) and decrease exposure (water and sanitation) are underway, therefore finding infection intensities that are moderate to low in most cases (Elgendy et al., 2016; Engels et al., 1996; Werkman et al., 2018).

Specifically, we evaluated the performance and characteristics of a group of parasitological methods for the identification and quantification of STH, considering the new targets for STH control outlined by WHO to be achieved by 2030 (Stuyver and Levecke, 2021). We describe the diagnostic utility of different methods for clinical laboratories in endemic areas in resource limited settings where new molecular based methods are not available or when available, not integrated to quality control assessment certifications (Cools et al., 2020). Current recommendations for parasitological methods in STH surveys require adaptations in order to contribute to programmatic decision-making and allow achieving the new targets. Among these requirements, the capacity to detect *S. stercoralis* is the most significant. Our results show the feasibility of an array of diagnostic methods to estimate the prevalence of *S. stercoralis* without compromising the ability of identifying and quantifying eggs from the other relevant species of STH.

The Kato-Katz method is widely used for STH surveys; however, relying on the results from a single stool sample leads to underestimation of the prevalence of infection, especially with low-intensity infections. Additionally, this diagnostic method is not suitable for detecting hookworm and *S. stercoralis* infections, which represents an important disadvantage (Elgendy et al., 2016; Engels et al., 1996).

The use of a combination of parasitological methods would help overcome diagnostic challenges posed by low STH burdens.

Table 1
A Frequency of STH Infections based on a composite reference standard including sedimentation/concentration, McMaster, Harada-Mori and Baermann methods ($n = 944$).

STH Infection	Positive total	%	95% CI
Any STH	216	22.88	20.14–25.61
A. lumbricoides	108	11.44	9.35–13.52
Hookworms	77	8.16	6.35–9.95
S. stercoralis	60	6.36	4.74–7.96
T. trichiura	13	1.38	0.58–2.17

The methods for detecting *A. lumbricoides* and *T. trichiura* species included sedimentation/concentration and McMaster methods. For hookworm detection, sedimentation/concentration, McMaster, Harada Mori, and Baermann methods were utilized. Meanwhile, for *S. stercoralis*, sedimentation/concentration, Harada Mori, and Baermann methods were applied.

Table 2
A Distribution of infection intensities among McMaster positive samples by species (n = 944).

Species	Light (%)	Moderate (%)	Heavy (%)
A. lumbricoides	53 (79.1)	14 (20.9)	0
T. trichiura	9 (81.8)	1 (9)	1 (9)
Hookworm	41 (75.9)	13 (24.1)	0

World Health Organization. epg = eggs per gram. *A. lumbricoides*: light intensity 1–4999 epg, moderate intensity 5000–49,999 epg, heavy intensity >50,000 epg. *T. trichiura*: light intensity 1–999 epg, moderate intensity 1000–9999 epg, heavy intensity >10,000 epg. Hookworms: light intensity1–1999 epg, moderate intensity 2000–3999 epg, heavy intensity >4000 epg.

Table 3
A Diagnostic performance of different methods for the diagnosis of STHs against the composite reference standard (n = 944). NPV: negative predictive value.

Method	Species	Positive (%)	Sensitivity % (CI 95%)	NPV % (CI 95%)	Positive exclusively by this method (%)
Concentration sedimentation	*A. lumbricoides*	104 (11.0)	96 (93–99)	99 (98–1)	41 (37.96)
	Hookworms	67(7.1)	87 (80–95)	99 (98–1)	13 (16.88)
	S. stercoralis	37(3.9)	62 (49–75)	97 (96–99)	11 (18.33)
McMaster	*A. lumbricoides*	67(7.1)	62 (53–71)	95 (94–98)	4 (3.70)
	Hookworms	54(5.7)	70 (60–80)	97 (96–98)	2 (2.59)
Harada Mori	*S. stercoralis*	19(2.01)	32 (19–44)	96 (94–97)	4 (6.66)
	Hookworms	33(3.5)	43 (32–54)	95 (93–96)	4 (5.19)
Baermann	*S. stercoralis*	42(4.5)	70(57–82)	98 (97–99)	19 (31.66)
	Hookworms	10(1.1)	13(5–20)	92 (91–94)	2 (2.59)

Although identification of moderate and high burden infections is relevant from a public health perspective for morbidity control, when the goal is to interrupt transmission, the negative predictive value of the diagnostic approach requires sensitivities that are not achievable with methods such as Kato-Katz (Werkman et al., 2018), and in contexts without access to quantitative polymerase chain reaction (qPCR), methods with increased sensitivity as sedimentation/concentration and Baermann offer opportunities for improved assessments; and in the clinical evaluation of individual cases that require accurate diagnosis, similar conditions apply (Ngwese et al., 2020).

In our study, the sedimentation/concentration method demonstrated a high sensitivity for all species, including *S. stercoralis*, which together with the Baermann method showed adequate sensitivity for this species. These findings are in line with those of another study from Ethiopia that reported similar sensitivities for the sedimentation/concentration and Baermann methods (Gelaye et al., 2021). Our sedimentation/concentration method has some remarkable characteristics; firstly, samples are collected without preservatives and process them within 24 h, while avoiding the use of formalin and ether to promote the survival and movement of *S. stercoralis* larvae, which in turn facilitates their detection. Secondly, we use a larger sample size (mean 31 g) than other published studies (Hailu et al., 2022; Intapan et al., 2005; Werkman et al., 2018); which may explain the higher performance of this method in our analysis, particularly for *S. stercoralis*. However, we were not able to identify a higher sensitivity correlating with the use of a larger amount of stool in our sample population. In our study, the sedimentation/concentration method showed a sensitivity of 96% and 87% for detecting *A. lumbricoides* and hookworm, respectively, which are similar to those obtained in a study conducted in Brazil using equivalent methods (Periago et al., 2015); sedimentation/concentration was more sensitive than McMaster's, which has a sensitivity of 62% and 70% for *A. lumbricoides* and hookworm, respectively, in agreement with studies conducted in Brazil and Ethiopia (Adugna et al., 2018; Levecke et al., 2011). Although Kato-Katz is the method most frequently used in surveys, it's worth noting, McMaster's method is comparable in performance and included in WHO's recommendations for assessing drug efficacy within programs (Becker et al., 2011; Khurana et al., 2021; Papaiakovou et al., 2019). There were no significant differences in sensitivity between Baermann and sedimentation/concentration for *S. stercoralis*. Several studies conducted in Cambodia, Ethiopia, and Ivory Coast reported similar sensitivities for Baermann at 75%, 73%, and 70%, respectively (Becker et al., 2011; Hailegebriel et al., 2017; Khieu et al., 2013). The Harada-Mori method is particularly useful in identifying the hookworm species involved, which has important epidemiological and morbidity implications given the higher capacity of *A. duodenale* to cause anemia compared to *N. americanus* (Papaiakovou et al., 2019). However, the accuracy and ability of the Harada-Mori method to identify hookworms or S. stercoralis solely through this method is limited, as the sensitivity for hookworm and S. stercoralis diagnosis using Harada-Mori was only 43% and 32%, respectively in our study. In contrast, studies conducted in Thailand and Ethiopia have reported higher sensitivity for this method (Becker et al., 2011; Hailegebriel et al., 2017). This variability among studies might be related to differences in incubation times across studies (Inês et al., 2011).

Among the limitations of our analysis, the retrospective nature is the main one. The lack of a definitive gold standard for the assessment of the true sensitivity of each method is another limitation that is common place for studies on diagnostic methods for STH, which has been partially compensated by the use of a composite reference standard; and although the inclusion of qPCR would have certainly improve the accuracy of the estimations, that methods has shown significant limitations in the diagnosis of *S. stercoralis* and burden estimations for all STH (Buonfrate et al., 2018; Papaiakovou et al., 2019). Despite commonly assumed, a specificity of 100% for parasitological methods in STH is a limitation that could hinder human error and introduce bias; as a regional reference laboratory, this

study was based on the expertise and experience of the microscopists and supervisors in charge of the evaluations.

A warning, as well as a limitation, is that the current study is not an epidemiologic survey but was rather designed to assess the performance of different diagnostic methods, with the epidemiologic data reported elsewhere (Echazú et al., 2015).

Finally, the comparability between studies is limited by subtle and significant differences in the operative procedures across laboratories which places a limitation and calls for harmonization of optimized procedures.

5. Conclusions

Our results indicate that for the incorporation of *S. stercoralis* into the diagnostic targets, limiting the diagnostic approach to sedimentation/concentration and Baermann methods could serve as a sensitive and affordable approach for STH diagnosis, with Harada-Mori being useful only when the identification of hookworm becomes relevant and McMaster (or Kato-Katz) when burden estimations through egg counts are necessary. Still, each method has its limitations and strengths, and choosing the appropriate method depends on the specific parasite being investigated, the objectives of the study and the available resources. Given that these methods are based on microscopy and are suitable for use in laboratories in endemic areas with limited resources, it is important to ensure that quality control assessment certifications are integrated to maintain accuracy and reproducibility.

It is recommended that more research is conducted to develop more sensitive and specific diagnostic methods for STH, especially for species such as *S. stercoralis*. Additionally, it is important to continue to promote public health education and preventive measures to reduce the burden of STH infections in endemic areas.

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CRediT authorship contribution statement

Elvia Nieves: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Pedro Fleitas:** Formal analysis, Visualization, Writing – original draft. **Marisa Juárez:** Investigation, Methodology. **Cristina Almazán:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Gabriela Flores:** Methodology. **Jimena Alani:** Methodology. **Ramón Diaz:** Methodology. **Jorge Martos:** Investigation, Validation. **Pamela Cajal:** Methodology. **Rubén Cimino:** Conceptualization, Formal analysis, Funding acquisition, Investigation. **Alejandro Krolewiecki:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

The database used in this study is included in a separate supplementary document submitted along with this manuscript and can be obtained from the corresponding author upon request.

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