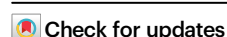


Integration of diagnostics for *Strongyloides stercoralis* into the soil-transmitted helminths control programme in Rwanda

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The WHO recently issued guidelines for public health control of *Strongyloides stercoralis* in endemic areas. We aimed to evaluate the feasibility of implementing the WHO recommendations in Rwanda, and our secondary objective was to estimate *S. stercoralis* prevalence. We conducted a community-based cross-sectional study in two Rwandan districts (Gisagara and Rubavu) including a training session focused on diagnostics for *S. stercoralis*: parasitological assays (Baermann and agar plate culture, APC) and a novel rapid diagnostic test (RDT). Technicians' perceptions of each assay were evaluated via a questionnaire; 1,415 individuals were screened. A critical aspect of the parasitological assays was the length of training, but there was no issues with RDT implementation. Based on the combination of Baermann and APC diagnostics, prevalence was 1.1% (95% CI 0.5–2.3) in Gisagara, and 3.9% (95% CI 2.6–5.7) in Rubavu, which was similar for the RDT. Overall, we found the implementation of *S. stercoralis*-specific tests was feasible, though intense training was crucial.

Strongyloides stercoralis is one of the soil-transmitted helminths (STH), infecting an estimated 300–600 million people globally^{1,2}.

Strongyloidiasis, caused by *S. stercoralis* is a neglected tropical disease (NTD) transmitted through fecal contamination in areas with inadequate sanitation, leading to environmental contamination by human faeces³. Strongyloidiasis is sometimes clinically inapparent, while a proportion of individuals present unspecific symptoms (e.g., urticaria, abdominal pain, wheezing, etc) of diverse intensity^{4,5}. However, in immunocompromised hosts, infection predisposes the individuals to an accelerated auto-infective cycle resulting in a dramatic increase in larval load (hyperinfection) and, in severe cases, systemic parasite dissemination. This can result in severe complications

including sepsis and meningitis due to enteric bacteria carried by the migrating larvae. As such managing hyperinfection is challenging with a high risk of mortality^{5,6}.

Strongyloidiasis is unfortunately still largely underdiagnosed, due to the low sensitivity of the routine parasitological techniques (i.e., direct stool examination, classical sedimentation/flotation concentration techniques, and Kato-Katz (KK) technique) to detect *S. stercoralis*⁷. This issue has impacted both individual diagnosis and the assessment of infection prevalence, which has led to a longstanding underestimation of the burden of strongyloidiasis¹. In August 2024, the WHO issued the first guideline for public health control of strongyloidiasis^{8,9}. The guideline recommends to use either the Baermann sedimentation

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technique or stool agar plate culture (APC) to estimate local prevalence, and to implement mass drug administration (MDA) with single-dose ivermectin (200 µg/kg orally) in all age groups from five years, in case of infection prevalence 5% or higher observed in school-age children (SAC) or 10% or higher observed in community-based surveys. Compared to KK, the techniques recommended for *S. stercoralis* diagnosis are more time-consuming and require specialised parasitological training to accurately differentiate the larvae of *S. stercoralis* from those of other parasites, particularly hookworm^{10,11}. The WHO guideline does not exclude the use of antibody-based assays, which can be advantageous in areas where blood sampling is already conducted for other NTDs⁸. This is in line with the WHO policy of integrating different control programmes to share logistics, infrastructure, and biological samples, reducing costs¹².

Since the guideline for the strongyloidiasis control was recently introduced, there is a need for operational studies aimed at evaluating the optimal integration of *S. stercoralis*-specific diagnostics into the existing control programmes for other NTDs.

The “*Strongyloides stercoralis* control in Rwanda” (“SCAN”) study, was conducted as a pilot study to evaluate the feasibility of the integration of *S. stercoralis* diagnostic techniques into the national STH and schistosomiasis control programme. Secondary objectives were (i) to estimate the prevalence of *S. stercoralis* infection in selected districts of Rwanda that can guide appropriate intervention, and (ii) to evaluate the performance of a novel immunochromatographic rapid diagnostic test (RDT) for the screening of strongyloidiasis at the community level.

In this study, we demonstrate that integrating *S. stercoralis* diagnostics into the STH control programme in Rwanda is feasible, with high participant compliance, affordable costs, and successful implementation of all diagnostic methods. However, the study highlights the need for intensive training of technicians in copro-parasitological techniques to ensure high competency, particularly in distinguishing *S. stercoralis* larvae from other nematodes. While the RDT was easier to use, its diagnostic performance requires further analysis. In addition, there are currently no commercially available RDT devices, currently limiting the immediate deployment of such tests in prevalence surveys of strongyloidiasis.

Results

Thirty-six lab technicians participated in the study. Of those, 15 (41.7%) were trained in all steps of all three techniques; 22 (61.1%) received training in all technical steps (i.e., sample processing) of the two faecal techniques; 23 (63.9%) were trained in microscopy; and 21 (58.3%) participated in any of the RDT steps.

None of the 35 technicians who performed the Baermann technique had prior experience with the technical steps of the method; Fig. 1 illustrates the technicians’ opinions regarding the ease of implementing the various steps of the method. The reasons for designating each step as “difficult” or “very difficult” at any one evaluation time are outlined in Table 1.

Of the 30 technicians who were involved in training with any step of the APC, only two declared having previous experience with the technique. Figure 1 shows the technicians’ opinions about the ease of implementation of the different steps of the test. Reasons for considering each step “difficult” or “very difficult” at any one evaluation time are reported in Table 2.

The RDT was performed using whole blood from finger pricks in Gisagara and plasma from venipuncture in Rubavu. All technicians involved were already experienced with RDT sero-assay techniques. Figure 1 illustrates the technicians’ perceptions of the ease of implementation of the different steps. No difficulties or safety concerns were reported for sample collection, analysis, or interpretation, regardless of the sampling method (finger-prick or venipuncture).

Costs

For *S. stercoralis*-specific assays, the estimated cost for materials and equipment for the collection, processing and analysis of one sample was 1.83 euro for Baermann, 1.77 euro for APC, 1.53 euro for the RDT using blood plasma from venipuncture, and 1.24 euro for the RDT using finger prick blood. The estimated cost per sample for Kato Katz (2 slides per sample) was 1.95 euros. When considering the cost of personnel in addition to that of material for the processing of 72 samples (average number of participants/day in this study) from sample collection to result, the estimate for Kato Katz alone was 459.88 euros; for Kato Katz plus Baermann 756.79 euros (64.6% increase); for Kato Katz plus APC 782.19 euros (70.0% increase); for Kato Katz plus RDT from finger prick 653.41 euros (42.1% increase); and for Kato Katz plus RDT from venipuncture 674.12 euros (46.6% increase). The cost of material accounted for 28.8% to 35.9% of these costs.

Prevalence of infection

A total of 1415 individuals consented to participate in the study: 719 in Gisagara and 696 in Rubavu Districts. Female participants constituted 744/1415 (52.6%), the median age was 16 (IQR 10–39); children were 322/719 (44.8%) of the participants in Gisagara and 319/696 (45.8%) in Rubavu. All participants provided both stool and blood samples.

Overall, 35 participants tested positive for *S. stercoralis* larvae on microscopy. Considering the combination of Baermann and APC as true positive results, the prevalence of *S. stercoralis* infection was 1.1% (95% CI: 0.5–2.3%) in Gisagara, and 3.9% (95% CI: 2.6–5.7%) in Rubavu. Comparable figures (1.4% and 3.6%, respectively) were obtained with the RDT. Table 3 details the results by age group and district.

The combination of faecal tests identified more cases in adults than in SAC, though the difference was not statistically significant ($p = 0.1264$). On the other hand, the RDT showed a significantly higher number of positives in adults than in SAC ($p = 0.0005$). The diagnostic performance of the RDT will be discussed in another publication, pending the results of real-time PCR, which is being done in the context of a capacity-building study.

Discussion

In this study, we evaluated the implementation of diagnostic methods for *S. stercoralis* within the ongoing STH and schistosomiasis control programme in Rwanda, initiated in 2007. Participants’ compliance was excellent, with all individuals providing the required biological samples in sufficient volumes. All samples were processed using the planned techniques, from sample collection to testing, without any logistical or technical disruptions. The current study employed experienced technicians who are involved in STH and Schistosomiasis mapping studies as part of the NTD control programme in Rwanda. At the beginning of the study, technicians reported some difficulties implementing the Baermann and APC methods; these challenges were solved with practice.

Initially, the majority of technicians reported “insufficient previous training” as the main reason for difficulties encountered in the implementation of both methods. By the third evaluation timepoint, none of the steps were reported as “difficult” or “very difficult”, suggesting that a structured training programme (incorporating hands-on practice with real samples) significantly enhanced technicians’ competency over time. Other common reasons noted for technical difficulties included “technical problems” and “larvae identification”. Although cases of misidentification (i.e., the technician presenting a possible *S. stercoralis* larva, which was subsequently identified as something else by the supervisors) were not formally quantified in this study, supervisors reported an initial poor performance that improved gradually. Hence, we recommend that NTD national programmes should not underestimate the amount of time dedicated to tailored

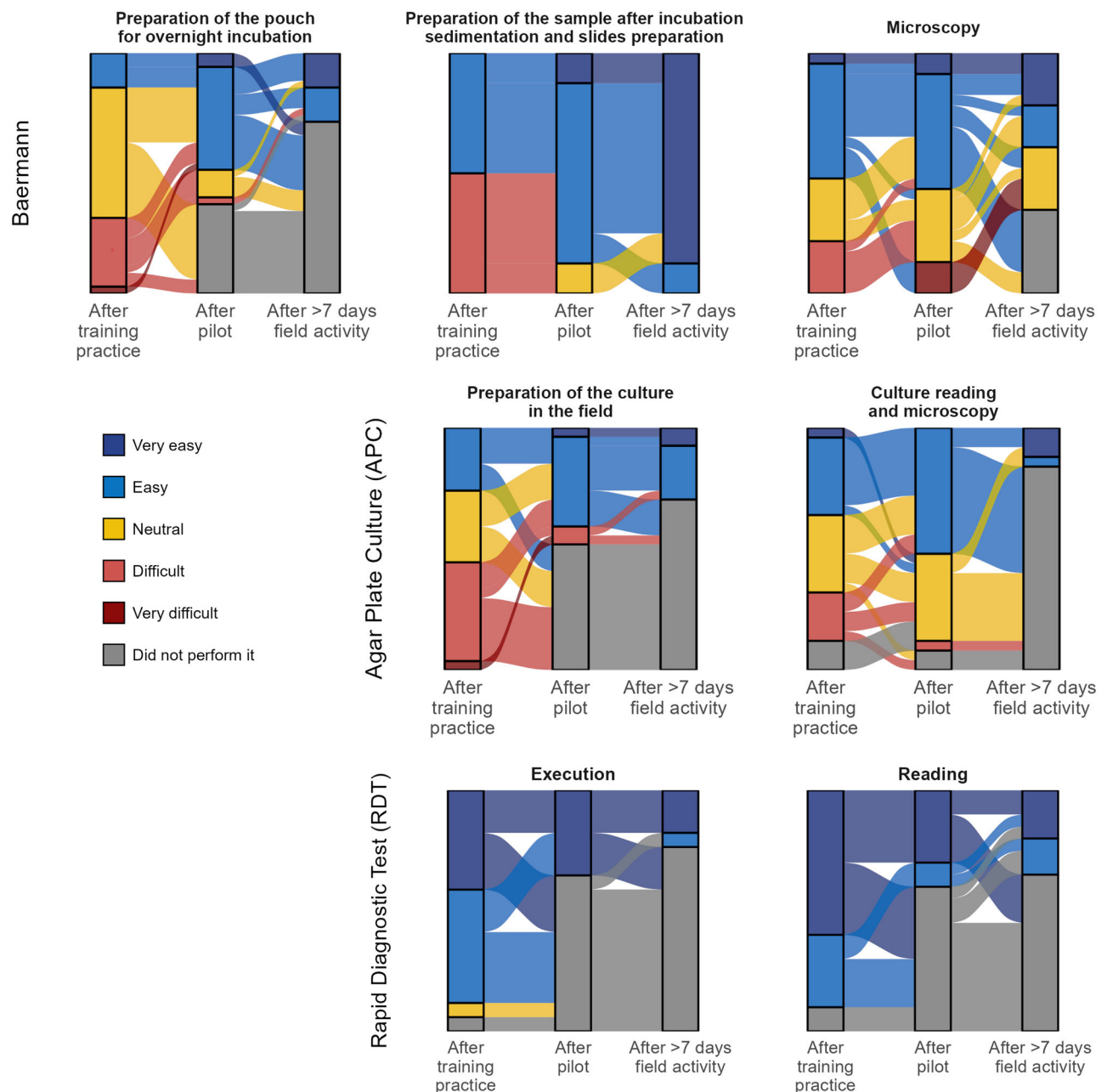


Fig. 1 | Technicians' evaluation. Technicians' opinions about the ease of implementation of the different steps of the tests.

Table 1 | Reasons for considering the different steps of the Baermann technique difficult or very difficult and safety concerns

Reason for difficulty and safety concerns	n/N (% of technicians providing the motivation)		
	Preparation of the pouch for overnight incubation	Sedimentation and slide preparation	Microscopy
Insufficient previous training	9/35 (25.7%)	12/25 (48.0%)	7/23 (30.4%)
Technical difficulty in performing the step	1/35 (2.9%)	3/25 (12.0%)	3/23 (13.0%)
Insufficient assistance in case of need/doubts	0/35 (0%)	0/25 (0%)	0/23 (0%)
Insufficient space	0/35 (0%)	0/25 (0%)	0/23 (0%)
Insufficient time for the workload	3/35 (8.6%)	2/25 (8.0%)	0/23 (0%)
Time-consuming	1/35 (2.9%)	2/25 (8.0%)	0/23 (0%)
Labour-intensive	0/35 (0%)	1/25 (4.0%)	0/23 (0%)
Difficulty in parasite identification	-	-	3/23 (13%)
Perceived unsafety in relation to personal protection	3/35 (8.6%)	1/25 (4.0%)	1/23 (4.3%)
Environmental pollution	4/35 (11.4%)	3/25 (12.0%)	0/23 (0%)

Table 2 | Reasons for considering the different steps of the APC technique difficult or very difficult and safety concerns

Reason for difficulty and safety concerns	n/N (% of technicians) providing the motivation	
	Preparation of the culture in the field	Culture reading and microscopy
Insufficient previous training	10/27 (37.0%)	6/25 (24.0%)
Technical difficulty	3/27 (11.1%)	0/25 (0%)
Insufficient assistance in case of need/doubts	0/27 (0%)	0/25 (0%)
Insufficient space	0/27 (0%)	0/25 (0%)
Insufficient time for the workload	2/27 (7.4%)	1/25 (4.0%)
Time-consuming	0/27 (0%)	0/25 (0%)
Labour-intensive	0/27 (0%)	0/25 (0%)
Difficulty in parasite identification	-	2/25 (8.0%)
Perceived unsafety in relation to personal protection	2/27 (7.4%)	1/25 (4.0%)
Environmental pollution	3/27 (11.1%)	1/25 (4.0%)

training for the correct application of *S. stercoralis*-specific coproparasitological techniques, particularly in areas where *S. stercoralis* is co-endemic with hookworm.

The prevalence of *S. stercoralis* reported in this study was comparable to the 1.9% observed in a previous study carried out in 2016 in Gisagara by faecal direct smear, which is a parasitological technique known for its exceedingly low sensitivity compared to Baermann and APC methods¹³. In the same study, a 17.4% prevalence was reported using horse blood agar culture, a method which is, however, not recommended for *S. stercoralis* detection⁷. Although in nine years the epidemiology of strongyloidiasis may have changed, the figures from 2016 and from this study differ too much to be attributable to individual treatment with ivermectin (no MDA with ivermectin has ever been done in Rwanda). Of note, ivermectin has never been used for clinical case management due to its unavailability in Rwanda until COVID-19 period, despite being recommended by the national treatment guidelines for strongyloidiasis, trichiuriasis and scabies cases. Furthermore, the 2016 study relied on visual detection of “larval tracks”, a characteristic exhibited also by other migrating larvae, suggesting potential misdiagnosis¹⁴.

As opposed to coproparasitological techniques, the use of the RDT was straightforward at all steps and with either blood or plasma samples. Most staff members were already familiar with similar tests such as those for malaria. An RDT would be particularly useful for fieldwork, and would not require long training for its deployment. No concerns were raised about waste disposal of the tests, contrary to a previous study where this aspect was flagged as an issue¹⁵. It is important to note that the RDT used in this study was a dipstick format, whereas the previous study used a plastic cassette.

The point-of-care format, the short time needed for training (one day or less), the possible use by staff with no specific laboratory expertise (i.e., possible use by community health workers), the short time to obtain the results (less than 1 h), the high throughput (at least ten tests per hour per tester) are among the characteristics of the RDT that comply with the recently issued target product profile (TPP) for *S. stercoralis* diagnostics¹⁶.

Overall, the prevalence data obtained by the RDT were in line with those from the faecal tests. However, the discrepancies between RDT and the faecal test results in SAC may be due to adults having more long-lasting infections, permitting enhanced antibody production compared to children. Further data are needed to explore these

aspects, especially since SAC are commonly the target population for other STH prevalence surveys¹⁷. An evaluation of the RDT's diagnostic accuracy, incorporating stool PCR as a reference standard, is currently underway.

Recently, a cost-effectiveness analysis was conducted to support the development of the TPP for diagnostics in *S. stercoralis* control programmes¹⁸. The analysis showed that an RDT could be more cost-efficient than Baermann, provided it demonstrated good specificity (at least 72% for adequate decision-making and 84% for ideal decision-making) and cost around one US dollar per test. The materials and equipment costs for the Baermann technique estimated in our study were comparable to those reported in Ecuador¹⁵, which informed the cost-effectiveness analysis. However, the RDT used in Ecuador was nearly twice as expensive as the one deployed in this study, highlighting variability in assay costs. Of note, neither RDT is available on the market yet, so their cost may change once commercially available. The APC was not included among the assays considered in that cost analysis; in this study, its cost was comparable to that of Baermann; both, in any case, costed less than two euros per test in terms of material and equipment needed per sample. When considering personnel costs in the particular setting of Rwanda for the processing of samples collected in one day of field work from reception of the sample to result (Supplementary File), the cost increase of integrating one *S. stercoralis*-specific assay to Kato Katz for STH and *Schistosoma* was less than 50% for RDT, while reached 65% and 70% for Baermann and APC, respectively. About one third of the total cost was due to equipment and materials, while two thirds were due to personnel, highlighting the importance of a thorough evaluation of total survey costs and cost-effectiveness analysis of the actual field logistic deployed, when planning an integrated control programme, even if using the same sample matrix.

This study has several limitations. Mass treatment with ivermectin was not recommended in either of the investigated Rwandan districts, as the prevalence of *S. stercoralis* infection was below the 10% threshold established for community-based surveys in the WHO guidelines^{8,9}. Consequently, one limitation of this study is our inability to provide information on potential challenges associated with implementing ivermectin distribution. The small number of *S. stercoralis* cases might also have impacted the training and its evaluation. Indeed, the low prevalence could have resulted in an increase of the time required for slide reading (more time is needed to read the whole sediment when no larvae are detected, compared to cases where larvae are found and the slide is therefore discarded after the first *S. stercoralis* larva identification), but also in a reduced attention from technicians, who might become gradually accustomed to examine and therefore expect mainly negative samples. Furthermore, technicians had only limited chance to examine *S. stercoralis* larvae from the study samples. A further limitation is that we did not quantify the proportion of larvae or larvae-like objects that were misidentified as *S. stercoralis* under microscopy, as this was technically challenging. Furthermore, we did not incorporate a feasibility assessment of PCR performance in a central laboratory or collect blood samples (e.g., dried blood spots) for laboratory-based seroassays, as the Rwanda NTD programme did not foresee the potential to implement these screening strategies. Moreover, a comprehensive cost assessment of an entire strongyloidiasis screening initiative integrated into a control programme for STH, or of extensive training for coproparasitological diagnosis of *S. stercoralis*, were beyond the scope of this study.

The strengths of this study include a formal assessment of the feasibility of each technique at multiple stages, allowing for a thorough evaluation of critical steps that may require retraining. Additionally, the participant sample size was sufficiently large to provide a representative estimate of *S. stercoralis* prevalence in each district, in accordance with the WHO recommendations.

Table 3 | Results of the tests per age group and district

Test	Adults, N = 774			SAC, N = 641		
	Overall N = 774 ¹	Gisagara N = 397 ¹	Rubavu N = 377 ¹	Overall N = 641 ¹	Gisagara N = 322 ¹	Rubavu N = 319 ¹
APC	4/774, 0.5%	4/397, 1.0%	N.A.	1/641, 0.2%	1/322, 0.3%	N.A.
Baermann	20/774, 2.6%	3/397, 0.8%	17/377, 4.5%	10/641, 1.6%	0/322, 0.0%	10/319, 3.1%
RDT	33/774, 4.3%	10/397, 2.5%	23/377, 6.1%	2/641, 0.3%	0/322, 0.0%	2/319, 0.6%
Baermann + APC	24/774, 3.1%	7/397, 1.8%	17/377, 4.5%	11/641, 1.7%	1/322, 0.3%	10/319, 3.1%

¹n/N, %

APC agar plate culture, RD rapid diagnostic test, N.A. not applicable.

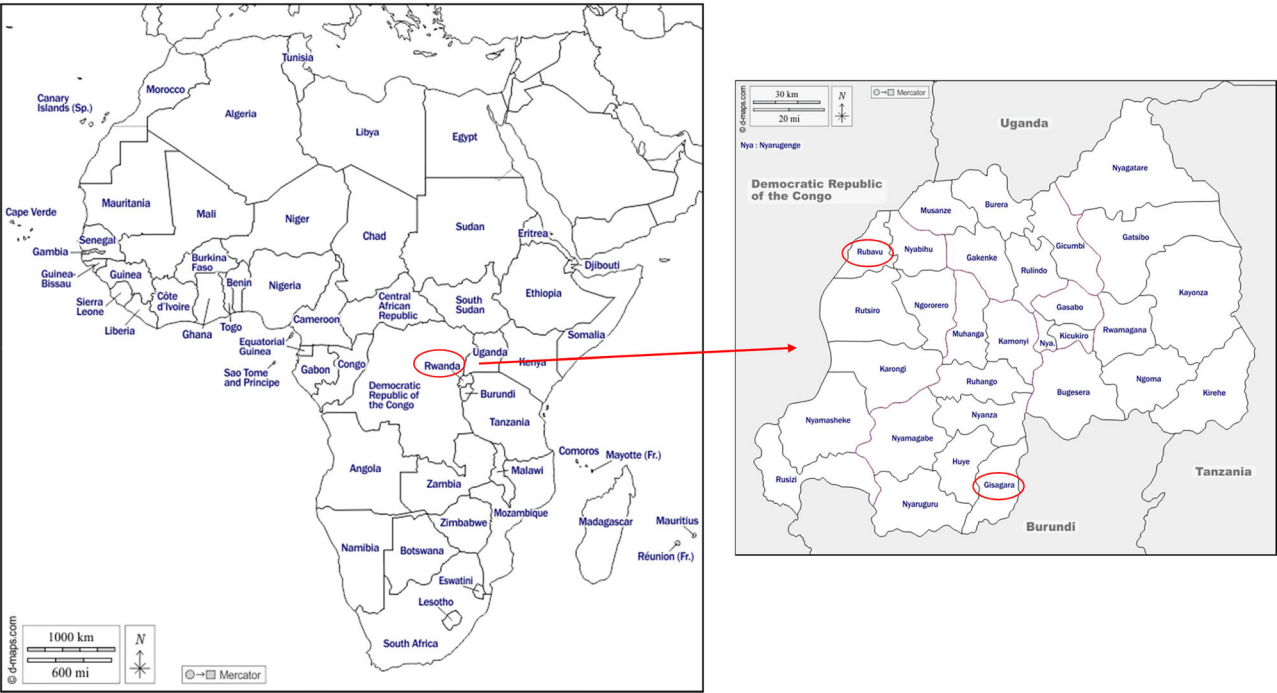


Fig. 2 | Study sites. Maps showing the study sites (created with d-maps.com: <https://d-maps.com/index.php?lang=it>).

Methods

This was a community-based cross-sectional study, approved by the Rwanda National Ethics Committee (No477/RNEC/2024).

Participants were enrolled in two districts of Rwanda, Gisagara and Rubavu (Fig. 2). The diagnosis of strongyloidiasis was conducted on adults aged from 16 years and above and SAC (from five to 15 years of age) residing in the selected districts. Participants were selected from the lists of households (HH) updated from the previous census conducted for the 2020 countrywide mapping survey for STH and schistosomiasis to facilitate contact and logistical arrangements. Thirty-six HH (see sample size calculation) were randomly selected from each of ten villages per district, with one participant invited per HH for each target age group. If the selected HH lacked sufficient members in both age groups, the following HH on the list was considered to provide participants to the missing age groups. The inclusion criterion was the obtainment of written informed consent (for children’s participation, consent was sought from parents/guardians). The exclusion criterion was the inability to supply an adequate stool sample.

Field operators comprising laboratory technicians and data entry clerks were selected from the Rwanda Biomedical Centre (RBC)’s National Reference Laboratory (NRL), and various district hospitals and health centres, following the procedures routinely

used for the national control programme. Each technician/entry clerk was provided an information sheet about the study purpose, which included ethically required clauses such as voluntary participation, confidentiality of their information. Oral consent was then sought. The recruited personnel underwent a six-day training session in Kigali from 17th to 22nd September 2024, including a pilot survey. All technicians were trained on all diagnostics by nine trainers. Slides and handouts were provided in English; training sessions were carried out both in English and Kinyarwanda. This training focused on laboratory practice of *S. stercoralis*-specific diagnostic techniques: Baermann, APC, and a lateral-flow RDT. It was facilitated by staff from the University of Rwanda (UR), RBC’s Neglected Tropical Disease program and the NRL, and the IRCCS Sacro Cuore Don Calabria Hospital (Verona, Italy). Additionally, senior staff at the NRL provided refresher training on the KK technique. After training, a two-day pilot simulation took place in Kigali on 19th and 20th September 2024, involving 142 volunteers from two different sites in the city outskirts. Field activities were conducted from 23rd September to 11th October 2024, with two field teams formed, each led by two supervisors. The study team was split into sub-teams, and each sub-team was assigned a diagnostic test. Every technician could process 72 samples (i.e., 144 slides) daily.

Diagnosics

Detailed Standard Operating Procedures (SOPs) for the *S. stercoralis*-specific assays, are available as Supplementary Materials (Supplementary File). Briefly, the Baermann technique adapted for field use from Gelaye et al.¹⁹, involved overnight incubation, thus requiring an overall 24 hours from collection to result. The APC performed only in Gisagara, followed a field-adapted protocol from diagnostic parasitology SOP of IRCCS Sacro Cuore Don Calabria Hospital (Verona, Italy), based on the Koga et al. procedure²⁰, with one culture plate per sample and microscopy after three days of incubation. The APC was not implemented in Rubavu due to logistic and economic constraints. Blood (RDT) and stool (KK) samples were processed on-site, in temporary laboratories set up in nearby administrative offices or health centres. The APC and Baermann technique were initiated on-site (incubation step) and then transported to the designated peripheral laboratories for incubation and microscopic evaluation. Positive samples on microscopy were photographed and ambiguous cases were immediately reviewed remotely by the study team to confirm larval species identification. Each team was provided pre-prepared control slides with *Strongyloides* larvae as reference standards for microscopy.

A blood sample was collected from each participant to perform the RDT, a novel immunochromatographic test (Strongy IgG4 Detect™ Rapid Test) produced by InBios (Seattle, USA) for research use only (RUO), which detects IgG4 against two *Strongyloides* recombinant antigens (NIE and SsIR)²¹. In Gisagara, the RDT was performed using whole blood obtained by finger prick, while in Rubavu, venipuncture plasma was used. The results are available 15–20 min after RDT initiation. The percentage of samples collected from all participants and the proportion of samples examined for each diagnostic technique was documented to evaluate the acceptance and adequacy of the sampling methods.

Evaluation of user appraisal of diagnostic assays

Technicians' previous experience with each *S. stercoralis*-specific assay, along with their perceptions regarding the ease of each of the procedural steps and associated safety concerns were assessed using a structured questionnaire (available as Supplementary material) administered at three-time points: after-training (T1), after the pilot simulation (T2), and after seven days into the study fieldwork (T3).

Each procedural step was rated as “Very easy”, “Easy”, “Neutral”, “Difficult” and “Very difficult”. For the steps marked “difficult” or “very difficult”, respondents could specify the reasons for the difficulty from a predefined non-mutually-exclusive list of options. Safety concerns for each technique's step – such as issues relating to personal protection devices, waste disposal, and environmental contamination – were likewise evaluated through a non-mutually-exclusive list of options. The respondents had the option to elaborate on the difficulties or safety issues encountered. Finally, the per-test costs were calculated from the procurement records of each *S. stercoralis*-specific assay. All the data were recorded electronically using REDCap electronic data capture software hosted at RBC [version REDCap 15.0.14].

Sample size and statistical analysis

The sample size was calculated to estimate the prevalence of *S. stercoralis*, a secondary objective of the study, as no formal power calculation could be performed for the primary qualitative objective of determining the feasibility of the techniques. Due to a lack of reliable prevalence data of *S. stercoralis* in the study areas, the sample size calculations were based on a published model applying Lot Quality Assurance Sampling (LQAS) methods to STH control programmes²². Instead of constructing a precise estimate of a population parameter, LQAS aims to quantify whether the population parameter is above or below some decision cut-off. The process considers specific characteristics of STH control programmes, which apply to *S. stercoralis* as well, including (1) the use of diagnostic tests with imperfect sensitivity

and specificity; (2) the presence of multiple decision cut-offs; (3) the acceptable probability of undertreatment and overtreatment. According to the model (specifically, to the calculations available as S1 Data in the paper by Levecke et al.)²², for decision cut-offs ranging from 2–50% prevalence, with an acceptable under-treatment probability of <5% and of overtreatment <25%, and considering a test with sensitivity around 60% and specificity of 100% (i.e., Baermann test)¹⁵, a sample size of 353 participants per age group (SAC and adults) per district is required. For study purpose, sex distribution was determined based on self-report.

Descriptive statistics included the number of subjects, means, standard deviations, minimums, medians, and maximums for continuous outcomes, and absolute and relative frequencies for categorical outcomes. Missing data was reported using absolute numbers and percentages.

Two-sided 95% confidence intervals for proportions were calculated using the Wilson score formula with continuity correction. Chi-squared test was used to compare the proportions of positives between adults and SAC. Analyses were performed with R statistical software (version 4.4.1).

Cost of material and equipment per sample was estimated first for each assay (Supplementary File). Then, we estimated the cost to process 72 samples (average number of participants per day in this study) including personnel salary and participants compensation, for Kato Katz alone and when Kato Katz was integrated with each of the *S. stercoralis*-specific assays. Assumptions costs estimates are detailed in the Supplementary File.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The raw data used in this study are available in the figshare repository under accession code: <https://doi.org/10.6084/m9.figshare.29380922>.

References

- Buonfrate, D. et al. The global prevalence of strongyloides stercoralis infection. *Pathogens* (Basel, Switzerland) **9**, 468 (2020).
- Fleitas, P. E. et al. The Strongyloides stercoralis-hookworms association as a path to the estimation of the global burden of strongyloidiasis: a systematic review. *PLoS Negl. Trop. Dis.* **14**, e0008184 (2020).
- Hall, A., Conway, D. J., Anwar, K. S. & Rahman, M. L. Strongyloides stercoralis in an urban slum community in Bangladesh: factors independently associated with infection. *Trans. R. Soc. Trop. Med. Hyg.* **88**, 527–530 (1994).
- Jember, T. H. et al. Prevalence of Strongyloides stercoralis infection and associated clinical symptoms among schoolchildren living in different altitudes of Amhara National Regional State, northwest Ethiopia. *PLoS Negl. Trop. Dis.* **16**, e0010299 (2022).
- Buonfrate, D., Bradbury, R. S., Watts, M. R. & Bisoffi, Z. Human strongyloidiasis: complexities and pathways forward. *Clin. Microbiol. Rev.* **36**, e0003323 (2023).
- Rojas, O., Montoya, A., Villanueva-Lozano, H. & Carrion-Alvarez, D. Severe strongyloidiasis: a systematic review and meta-analysis of 339 cases. *Trans. R. Soc. Trop. Med. Hyg.* **117**, 682–696 (2023).
- Nieves, E. et al. 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. *Parasite Epidemiol. Control* **26**, e00370 (2024).
- WHO. WHO guideline on preventive chemotherapy for public health control of strongyloidiasis. CC BY-NC-SA 3.0 IGO: Geneva, Switzerland (2024).

9. Lo, N. C. et al. Review of the WHO guideline on preventive chemotherapy for public health control of strongyloidiasis. *Lancet Infect. Dis.* **25**, e146–e152 (2025).
10. Forrer, A. et al. Strongyloides stercoralis and hookworm co-infection: spatial distribution and determinants in Preah Vihear Province, Cambodia. *Parasites Vectors* **11**, 33 (2018).
11. Zainol, D. A. et al. Systematic review of strongyloides stercoralis infection diagnosis in Southeast Asia: insights from parasitological, molecular, and serological approaches. *Am. J. Trop. Med. Hyg.* **111**, 724–735 (2024).
12. WHO. Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva, Switzerland: CC BY-NC-SA 3.0 IGO (2020).
13. Tuyizere, A. et al. Prevalence of Strongyloides stercoralis infection and other soil-transmitted helminths by cross-sectional survey in a rural community in Gisagara District, Southern Province, Rwanda. *Trans. R. Soc. Trop. Med. Hyg.* **112**, 97–102 (2018).
14. Montes, M., Sawhney, C. & Barros, N. Strongyloides stercoralis: there but not seen. *Curr. Opin. Infect. Dis.* **23**, 500–504 (2010).
15. Tamarozzi, F. et al. Accuracy, acceptability, and feasibility of diagnostic tests for the screening of Strongyloides stercoralis in the field (ESTRELLA): a cross-sectional study in Ecuador. *Lancet Glob. health* **11**, e740–e748 (2023).
16. WHO. Call for public consultation – Target Product Profiles (TPP) for Strongyloides stercoralis diagnostics. May 16, 2025.
17. Montresor, A. et al. The global progress of soil-transmitted helminthiasis control in 2020 and World Health Organization targets for 2030. *PLoS Negl. Trop. Dis.* **14**, e0008505 (2020).
18. Kazienga, A. et al. Target product profiles for new diagnostics to inform strongyloidiasis control programs. *PLoS Negl. Trop. Dis.* **19**, e0012774 (2025).
19. Gelaye, W. et al. Performance evaluation of Baermann techniques: the quest for developing a microscopy reference standard for the diagnosis of Strongyloides stercoralis. *PLoS Negl. Trop. Dis.* **15**, e0009076 (2021).
20. Koga, K. et al. A modified agar plate method for detection of Strongyloides stercoralis. *Am. J. Trop. Med. Hyg.* **45**, 518–521 (1991).
21. Scarso, S. et al. Evaluation of novel recombinant antigen-based (NIE/SsIR) immunochromatographic rapid tests for Strongyloides stercoralis: an accuracy study. *Parasites Vectors* **17**, 535 (2024).
22. Levecke, B., Coffeng, L. E., Hanna, C., Pullan, R. L. & Gass, K. M. Assessment of the required performance and the development of corresponding program decision rules for neglected tropical diseases diagnostic tests: monitoring and evaluation of soil-transmitted helminthiasis control programs as a case study. *PLoS Negl. Trop. Dis.* **15**, e0009740 (2021).

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Author contributions

E.S. contributed to data curation, investigation, methodology, writing the original draft; F.T. contributed to conceptualisation, data curation,

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Competing interests

The authors declare no competing interests.

Additional information

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