Accuracy, acceptability, and feasibility of diagnostic tests for the screening of *Strongyloides stercoralis* in the field (ESTRELLA): a cross-sectional study in Ecuador

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Summary

Background **WHO recommends the implementation of control programmes for strongyloidiasis, a neglected tropical disease caused by *Strongyloides stercoralis*. Specific recommendations on the diagnostic test or tests to be used for such programmes have yet to be defined. The primary objective of this study was to estimate the accuracy of five tests for strongyloidiasis. Secondary objectives were to evaluate acceptability and feasibility of use in an endemic area.**

Methods **The ESTRELLA study was a cross-sectional study for which we enrolled school-age children living in remote villages of Ecuador. Recruitment took place in two periods (Sept 9–19, 2021, and April 18–June 11, 2022). Children supplied one fresh stool sample and underwent blood collection via finger prick. Faecal tests were a modified Baermann method and an in-house real-time PCR test. Antibody assays were a recombinant antigen rapid diagnostic test; a crude antigen-based ELISA (Bordier ELISA); and an ELISA based on two recombinant antigens (Strongy Detect ELISA). A Bayesian latent class model was used to analyse the data.**

Findings **778 children were enrolled in the study and provided the required samples. Strongy Detect ELISA had the highest sensitivity at 83·5% (95% credible interval 73·8–91·8), while Bordier ELISA had the highest specificity (100%, 99·8–100). Bordier ELISA plus either PCR or Baermann had the best performance in terms of positive and negative predictive values. The procedures were well accepted by the target population. However, study staff found the Baermann method cumbersome and time-consuming and were concerned about the amount of plastic waste produced.**

Interpretation **The combination of Bordier ELISA with either faecal test performed best in this study. Practical aspects (including costs, logistics, and local expertise) should, however, also be taken into consideration when selecting tests in different contexts. Acceptability might differ in other settings.**

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For the Spanish translation of the abstract see Online for appendix 1

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Introduction

Strongyloidiasis is a neglected tropical disease caused by the soil-transmitted helminth *Strongyloides stercoralis*. The infection particularly impacts people in marginalised and low-income communities.[1,2] The most recent global estimates show that around 600 million people might be infected, particularly in tropical and subtropical areas.[3] *S stercoralis* infection causes a chronic disease characterised by intermittent to unrelenting respiratory symptoms, abdominal pain, diarrhoea, dermatological features, and other clinical manifestations.[1,4] In patients who become immunosuppressed, a rapidly fatal hyperinfection and systemic disease can occur. This is characterised by aberrant systemic migration of autoinfective larvae, often leading to septicaemia, with or without meningitis.[1]

WHO has recently committed to promoting the control of strongyloidiasis within the 2030 targets for soil-transmitted helminth control,[5] and specific recommendations are upcoming. Currently, the aim is to establish an efficient strongyloidiasis control programme in school-age children, envisaging preventive chemotherapy with ivermectin in areas of high endemicity. The implementation of such ambitious activity requires appropriate diagnostic tools, but fundamental gaps exist in this field.[6] At the moment there is no consensus method for the diagnosis of *S stercoralis* infection in either an epidemiological or clinical context.[2] Methods commonly used for the diagnosis of other soil-transmitted helminths, such as Kato-Katz or other classical stool concentration techniques, are not appropriate for the diagnosis of strongyloidiasis.[2,7] Several parasitological (eg, Baermann method, culture techniques), serological, and molecular diagnostic methods have been described for the diagnosis of *S stercoralis* infection.[8] These techniques have been mainly developed for clinical use, but could be adapted for use in control programmes. However, assessing the sensitivity and specificity of these tests in the field is

See Online for appendix 2

Research in context

Evidence before this study

We searched PubMed, Embase, LILACS, and the Cochrane Library using the key words reported in appendix 2 (pp 2–3). We retrieved 3446 records, after removing duplicates. These records were evaluated for their relevance on the topic of this study by two authors. Studies carried out in the field usually compared a limited number of techniques, none of them including a rapid test and multiple serology assays. Accuracy was generally evaluated using parasitological tests as reference standard; in a few cases a composite reference standard was used. A thorough evaluation of feasibility of the tests was also missing.

Added value of this study

We evaluated five diagnostic assays for *Strongyloides stercoralis* (two of them were prospectively evaluated for the first time in this study) in terms of accuracy, as well as acceptability and feasibility of use in an endemic area. We also provide negative and positive predictive values for single tests and test combinations at different prevalence scenarios, so that we can comment on their performance in areas of low and high infection prevalence. The application of Bayesian latent class analysis further contributed to obtain robust estimates of accuracy of all tests under evaluation, overcoming limitations due to the lack of a diagnostic gold standard for strongyloidiasis.

Implications of all the available evidence

Our results aim at informing the upcoming WHO recommendations for the implementation of control programmes for strongyloidiasis in endemic areas. The combination of a serological assay plus either Baermann method or PCR might be recommended for the assessment of baseline prevalence of strongyloidiasis.

difficult due to the absence of a gold standard test (ie, perfect test) for strongyloidiasis. Accurate assessment therefore requires the use of multiple different tests on the same individuals, combined with the use of one of the several available types of latent class model to analyse the data.[9–13] Currently, a target product profile for diagnostic assays for use in preventive chemotherapy programmes for strongyloidiasis has not been established by WHO. Furthermore, other characteristics should be considered in addition to accuracy when choosing tests to apply in these programmes, such as feasibility of field use (including costs, ease of sample transport and storage, required laboratory equipment, and required personnel training and skills), and acceptability by communities.

This study aimed to provide fundamental information on the performance and applicability of diagnostic methods for *S stercoralis* infection to inform the forthcoming strongyloidiasis control programmes envisaged by the WHO 2030 disease control targets. The methods evaluated in the study were selected on the basis of their theoretical suitability for use in the context of control programmes, either in the field or in a centralised laboratory. The primary objective was to estimate the sensitivity and specificity of selected diagnostic methods for the diagnosis of *S stercoralis* and their predictive values in different population prevalence scenarios. Secondary objectives were to appraise the acceptability of sample collection methods for each test by the target population and feasibility of each assay for deployment as field-based diagnostics in an endemic area.

Methods

Study design and participants

This was a cross-sectional study using a school-based approach. The study was conducted in rural communities of San Lorenzo canton, located in the north of the Esmeraldas province of Ecuador, 18 km from the border with Colombia. The study took place in primary schools of 16 villages in eight of the 12 municipalities of San Lorenzo, mainly inhabited by people of African heritage. The remaining four municipalities were excluded either because they were involved in mass distribution of ivermectin previously (due to the onchocerciasis elimination campaign) or because of security issues. Recruitment was carried out during two periods: Sept 9 to Sept 19, 2021, and April 18 to June 11, 2022. All school-age children 5–12 years of age living in the target communities were considered eligible for participation.

Ethical clearance was granted from the Comité de Ética de Investigación en Seres Humanos de la Universidad Central del Ecuador, CEISH-UCE, number 0001-FCM-UCE-BIO-2021. The study followed the principles of the current edition of the Declaration of Helsinki. The investigators informed the children and their parents or guardians about the aims and procedures of the study. Parental or legal guardian permission was demonstrated by signing or fingerprinting the study consent form, together with the child assent. Written consent was also sought for taking and sharing photographs of individuals.

STARD-BLCM guidelines were followed to report the results of the study (appendix 2 p 4).[13]

Procedures

Informative and educational meetings about strongyloidiasis, the objectives of the study, and the methods for collecting samples were held by CECOMET (Centro de Epidemiologia Comunitaria y Medicina Tropical, Esmeraldas, Ecuador) staff in each community using educational leaflets (appendix 2 pp 35–42) and audio-visual tools adapted to the local culture.

Dates of sample collection were planned in agreement with the communities, the local authorities, and the school managers. Shortly before the date agreed for

sample collection, CECOMET staff distributed empty plastic containers and instructed school staff and parents to collect an adequate volume of stool passed on the morning of the study field visit, to be kept unrefrigerated. On the day of sample collection, each participant was invited to hand in the collected faeces and undergo a finger prick for testing the assays based on blood samples.

Deidentified participant data were collected using a case report form (CRF) and then transferred into a REDCap electronic CRF (eCRF), where diagnostic assay results were also collected. In the field, individual staff members were dedicated to the performance and reading of a single assay type, and were unaware of the results of the other field-based assay type performed by their colleagues. A different staff member entered the results of field-performed assays into the eCRF while in the field, and results were not accessible to the staff performing and reading the lab-based assays. One staff member performed, read, and entered into the eCRF all laboratory-based tests at Universidad Central del Ecuador (UCE), Quito.

Children with at least one test positive for *S stercoralis* were treated with ivermectin (3 mg tablets) at a dose of 200 µg/kg, single intake. The results of the study were discussed within communities, and with the municipalities and health district staff.

Test methods

The assay procedures are detailed in appendix 2 (pp 5–7).

Blood drops from a finger prick (figure 1A) were immediately tested using a cassette immunochromatographic rapid diagnostic test (RDT), developed by the Institute for Research in Molecular Medicine (INFORMM) of the Universiti Sains Malaysia[14] (figure 1B). For the first time in this study, this assay was evaluated prospectively and using whole blood from a finger prick. Staff performing the RDT received brief training using a video tutorial provided by the test producer. The results of the RDT were interpreted qualitatively (positive or negative).

Blood samples from the finger prick were also collected on Whatman filter paper, air-dried (figure 1C), and transported as dried blood spots to UCE, where two ELISA assays were run: the InBios Strongy Detect IgG ELISA (InBios International, Seattle, WA, USA; based on two recombinant antigens, NIE and SsIR[15,16]), evaluated for the first time prospectively in this study, and the *Strongyloides ratti* IgG ELISA (Bordier Affinity Products, Crissier, Switzerland; based on crude antigen).[17] We henceforth refer to these as the Strongy Detect ELISA and Bordier ELISA, respectively. The cutoff for positivity of the Strongy Detect ELISA is not provided by the manufacturer and was calculated as detailed in appendix 2 (pp 6, 11). The cutoff for positivity of the Bordier ELISA was calculated as normalised optical density, as per the manufacturer's instructions (appendix 2 p 6). The Bordier ELISA was previously evaluated for use from dried blood spots;[18] for the Strongy Detect ELISA, we received instructions for use from the manufacturer.

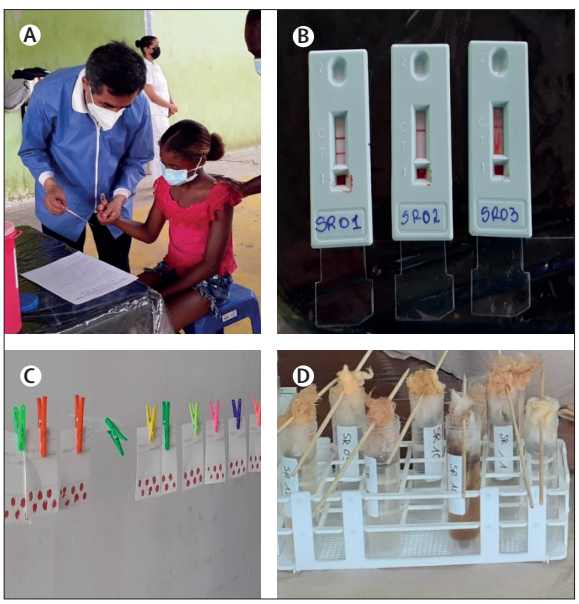

Figure 1: **Data collection and field-based testing**
(A) Staff member collecting demographic and clinical data, and obtaining blood drops from a finger prick. (B) From right to left: rapid diagnostic test on execution, negative result, and positive result. (C) Blood spots drying on filter papers. (D) Baermann method.

The faecal tests performed were a modified Baermann method (figure 1D)[19] and an in-house real-time PCR test based on Verweij's protocol.[20] Both were carried out from the same stool sample provided by the participant. One aliquot of unfixed faeces was used to perform the Baermann procedure in the field; a second aliquot was preserved in 70% ethanol and transported at room temperature to UCE, where PCR was carried out. A senior clinical microbiologist from the IRCCS Sacro Cuore Don Calabria hospital (Negrar, Verona, Italy) performed the Baermann procedure and the microscopic identification of larvae during the first of the two fieldwork periods, and trained the UCE staff in performing the same diagnostic method in the second period.

Statistical analysis

Sample size calculation was performed using PASS 2019 software (NCSS, Kaysville, UT, USA). Sensitivity and specificity of the diagnostic tests were based on literature data as follows: Baermann—sensitivity 50%, specificity 98%;[21] Bordier ELISA—sensitivity 90%, specificity 93%;[17] Strongy Detect ELISA—sensitivity 70%, specificity 91%;[15] real-time PCR—sensitivity 60%, specificity 95%;[20] RDT—sensitivity 90%, specificity 74%.[22] We calculated that 640 samples were required to estimate the sensitivity and specificity of any test with a 95% confidence interval (CI) with a width of at least 10%, and for an assumed

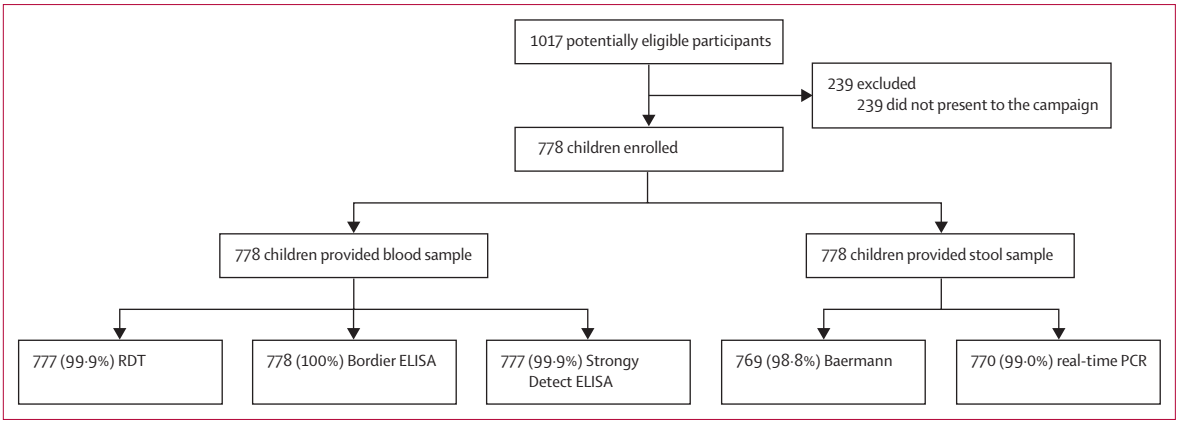

Figure 2: **Study flow chart**
RDT=rapid diagnostic test.

prevalence of 15%,[23] which was deemed to be feasible in terms of recruitment in the area.

Participants' data were summarised using descriptive statistics, measures of variability, and measures of precision. Cohen's kappa coefficient was used to test the agreement between PCR and Baermann.[24] Accuracy of single tests and test combinations was assessed with Bayesian latent class analysis (BLCA). This allows the observed data to be analysed in the absence of a gold standard test and therefore with imperfect knowledge regarding the true status of individuals.[25,26] We used a fixed-effects type latent class model that is commonly used within veterinary epidemiology,[13] rather than the random-effects type described by Dendukuri and Joseph,[10] due to the availability of existing code for optimising test cutoff values using this approach.[26] Key features of this model are: (1) test properties (sensitivity and specificity) differ between tests but remain constant between individuals and villages for the same test; (2) prevalence differs between villages; and (3) observed test results are independent conditional on the true status of individuals and the correlation structure imposed on the model. We based the correlation structure specification on biological knowledge of the similarity in antibody test targets between tests—ie, we assume independence between direct and indirect methods, but allow for correlation between the direct methods (Baermann, PCR) and pairwise correlation between each of the three indirect methods (Bordier ELISA, Strongy Detect ELISA, RDT). Given the fact that the diagnostic assays used test for different targets of the infection process, including the parasite and its DNA (direct assays) and the antibody response (indirect assays), we believe that this binary latent state reflects something very close to true *S stercoralis* infection positive status and true negative status.

Positive (PPV) and negative (NPV) predictive values were also calculated using BLCA. Uncertainty was quantified as 95% Bayesian credible intervals. Fixed prevalences of 15% and 30%, as well as the lowest, highest, and mean prevalence estimates observed within villages, were considered for the computations. BLCA was also used to define a cutoff for positivity of the Strongy Detect ELISA. Sensitivity analyses were performed considering different priors for the tests and excluding one subpopulation (ie, village) at a time from the model to ensure that estimated test characteristics were robust. Primary results were obtained using informative priors for the sensitivity and specificity of the RDT and minimally informative priors for the sensitivity and specificity of all other tests. The prior information for the sensitivity and specificity of the RDT test was obtained from the results of previously published data on the same test.[22] Full details of the model are provided in appendix 2 (pp 8–12).

The acceptability of the sampling methods was assessed as the proportion of people consenting to participate out of the whole target population to whom participation to the study was offered, and the actual provision of the faecal or blood samples by each participant. The feasibility of each technique was evaluated as the proportion of samples for which a result could be obtained out of all samples that were collected for the performance of the technique. A qualitative evaluation of the practical implementation of each technique was also carried out by means of group discussion of the six operators involved. The discussion was guided by the use of a checklist which included, for each assay: the ease of collecting a sample, performing the assay, and reading the result; the time required to perform the assay; and concerns regarding the disposal of the material.

Statistical analyses were performed in R (version 4.2.1) using runjags[27] and irr packages. Bayesian latent class models were run using a Markov chain Monte Carlo method implemented using JAGS software[28] (appendix 2 p 8).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

The communities comprised 1017 school-age children. The study flow is displayed in figure 2. Due to the high participation, we enrolled more children (n=778) than the target sample size (n=640), demonstrating good acceptance of the activity. Of the 778 children enrolled, 410 (52·7%) were male and 368 (47·3%) female. The median age of the children was 9·59 years (IQR 7·42–11·22).

Both faecal and blood samples were supplied by all enrolled children (hence, 100% acceptance of sampling methods), and the quantity of biological samples provided was enough to perform all assays in the near totality (≥98·8%) of cases. The calculated cutoff of Strongy Detect ELISA was an optical density of 0·18, similar to what was obtained (optical density of 0·179) from the BLCA for cutoff optimisation.

641 (82·4%) of 778 samples showed perfect agreement between all tests. Cross tabulation of the tests' results is reported in appendix 2 (p 13). Figure 3 shows the number of positive results on each test. Agreement between PCR and Baermann was moderate (Cohen's kappa coefficient 0·57; 95% CI 0·42–0·72); 737 of 778 samples (17 positive and 720 negative) had the same results on both Baermann and PCR.

The test sensitivity and specificity estimates of each assay expressed as median and 95% credible intervals are given in the table. True prevalence estimates for each village are presented in appendix 2 (p 14). The PPV and NPV of each test and of test combinations at the average prevalence estimated for the study area (8·5%) and at the other prevalence levels are shown in figure 4.

Results of sensitivity analysis are showed in appendix 2 (pp 15–34). The model fit was found to be adequate based on examination of the predicted frequencies of test result combinations with the observed frequencies. The posterior distribution of specificity for the RDT conflicted with the prior obtained from the literature, indicating that the test performed differently in our study compared with previous estimates, but the overall results were robust to different prior specifications including a less informative prior for RDT specificity. The sensitivity analysis showed that results were qualitatively unaffected by different choices for priors and exclusion of individual villages.

From the qualitative evaluation of the practical implementation of the assays, the main concerns related to the implementation of the Baermann method and waste disposal. The Baermann method was judged to be extremely time-consuming and material-consuming, and cumbersome for the microscopist, who had to read multiple coverslips from each sample and dedicate further time for the differentiation of *S stercoralis* larvae from those of hookworm. 50 samples per operator per day was perceived as the maximum processable even by an experienced operator. Concern was expressed regarding the amount of plastic materials used and to be disposed of on site, especially for the collection and processing of stool for the Baermann procedure, but also for the performance of the RDT, the cassette of which is made of plastic.

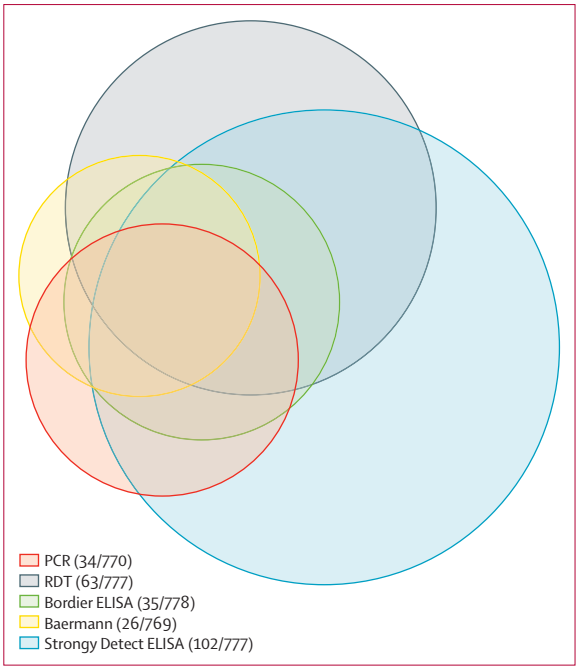

Figure 3: **Euler diagram showing the proportion of samples that were positive on the different tests**
RDT=rapid diagnostic test.

	Sensitivity	Specificity
RDT	79·4% (72·4–85·9)	93·6% (91·8–95·3)
Baermann	53·9% (43·0–65·1)	99·8% (99·3–100)
PCR	55·6% (44·8–67·0)	99·4% (98·7–100)
Bordier ELISA	65·9% (53·4–79·0)	100·0% (99·8–100)
Strongy Detect ELISA	83·5% (73·8–91·8)	91·7% (89·6–93·8)
PCR plus Bordier ELISA	84·9% (77·2–92·2)	99·4% (98·6–100)
PCR plus Strongy Detect ELISA	92·8% (88·1–96·7)	91·1% (88·9–93·2)
PCR plus RDT	90·9% (86·8–94·6)	93·0% (91·1–94·8)
Baermann plus Bordier ELISA	84·3% (76·3–91·6)	99·8% (99·2–100)
Baermann plus RDT	90·6% (86·4–94·4)	93·4% (91·5–95·1)
Baermann plus Strongy Detect ELISA	92·5% (87·5–96·5)	91·5% (89·3–93·5)

95% credible intervals are shown in parentheses. Combinations assume parallel performance of the two tests (ie, both tests were performed on all individuals, and an individual was considered positive when a positive result was obtained on at least one test). RDT=rapid diagnostic test.

Table: **Posterior medians and 95% credible intervals for sensitivity and specificity of the five diagnostic tests as well as test combinations calculated by Bayesian latent class analysis**

Discussion

We estimated the accuracy, acceptability, and feasibility of five diagnostic tests for the screening of strongyloidiasis in remote villages of San Lorenzo, Esmeraldas province, in Ecuador. In addition, we provide, for the first time, a prospective evaluation of two seroassays, the Strongy Detect ELISA and the RDT. Moreover, the latter was used for the first time with whole blood from a finger prick.[14]

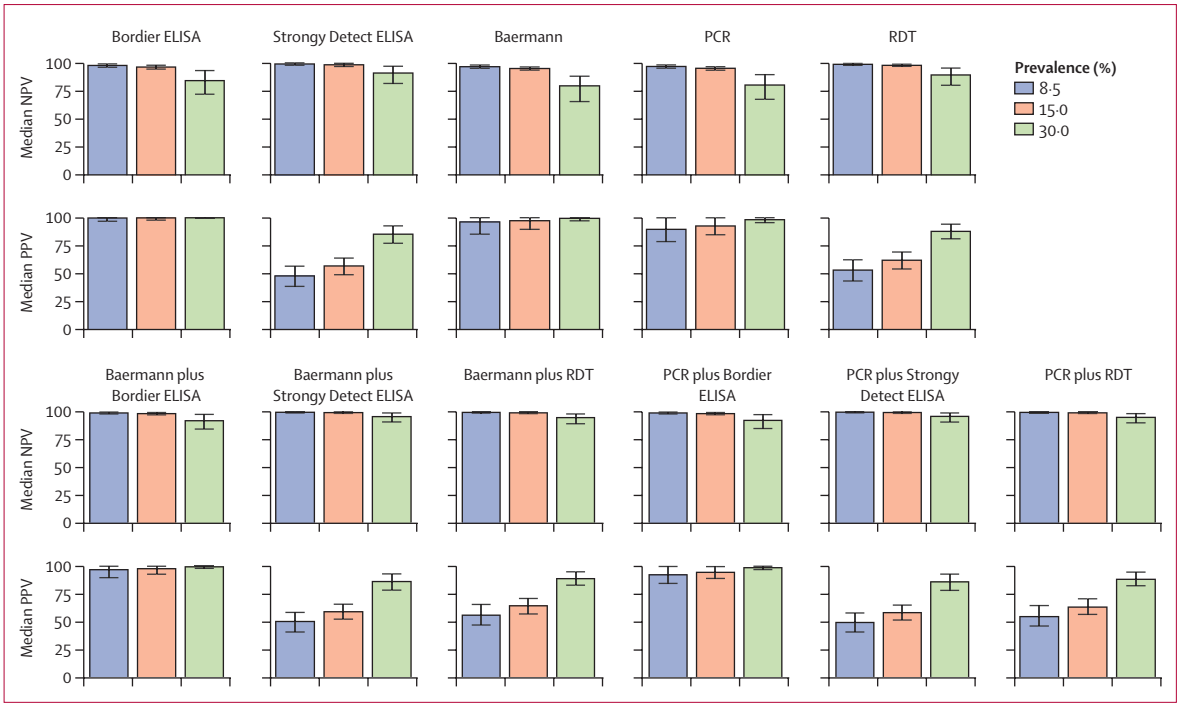

Figure 4: **Estimated medians and 95% credible intervals of PPV and NPV of each test and their combinations for the estimated mean prevalence in the study area (8·5%) and hypothesised 15% and 30% prevalence**
Error bars show 95% credible intervals. PPV=positive predictive value. NPV=negative predictive value. RDT=rapid diagnostic test.

As expected, the faecal methods had low sensitivity and excellent specificity. In the literature, there is a wide range of sensitivity values reported for the Baermann method,[8] which vary, among other factors, depending on technical aspects of the implemented technique, the reference method used for accuracy calculations, and the number of stool samples tested. Campo Polanco and colleagues performed a systematic literature review and estimated a sensitivity of 72% (95% CI 67–76).[29] The discrepancy with our results (53·9%, 95% credible interval 43·0–65·1) might be due mostly to an overestimation of sensitivity in previous studies, which compared the Baermann technique with other poorly sensitive tests based on stool microscopy. A systematic literature review retrieved sensitivity values of PCR (56·5%, 95% CI 39·2–72·4)[30] similar to those found here, when comparison was done against a panel of diagnostic tests including serology. In terms of predictive values, in our study Baermann and PCR had similarly good performance, with high PPV and some decline in NPV in high-prevalence scenarios. The only moderate agreement between the two faecal techniques was also not surprising, in light of the literature.[8]

When considering the seroassays, all evaluated tests missed many cases that were positive on Baermann or PCR, and this can be considered the main flaw of their use as single tests. Bordier ELISA had the lowest sensitivity (66%), but showed excellent specificity (100%). This sensitivity result is in contrast with previous studies that obtained values ranging from 83% to 97·7%.[8] Considering that this assay is based on *S ratti* crude antigen, the higher specificity compared with the other two assays, based on recombinant antigens, was also unexpected. When we evaluated the results in terms of predictive values, we found excellent performance of Bordier ELISA as single test, with some decline in NPV at the highest prevalence tested compared with lower prevalence scenarios, similar to what was shown for the faecal-based tests. However, when used in combination with either PCR or Baermann, the NPV was above 91% in the high-prevalence scenario also.

Strongy Detect ELISA showed the highest sensitivity (84%) and the lowest specificity (92%). This test showed slightly worse sensitivity (78%, 95% CI 72–83) and better specificity (98%, 95% CI 96–100) in a diagnostic study carried out in a clinical setting in a non-endemic country.[15] The different clinical context and methods (dried blood spots *vs* frozen serum samples) might explain the discrepancy. The RDT, based on one of the two recombinant antigens (NIE) present in the Strongy Detect ELISA, had lower sensitivity (79%) and similar specificity (94%) compared with the Strongy Detect ELISA. The figures are very different from those (86% sensitivity and 74% specificity) found in a previous study,[22] although that study was carried out in a different clinical context and used frozen serum samples as opposed to whole blood. The optimisation of the method for using whole blood and the addition of a second

recombinant antigen might also be considered to improve sensitivity. In all prevalence scenarios, both Strongy Detect ELISA and RDT had excellent NPV but low PPV, which improved slightly when the assays were combined with either PCR or Baermann. However, it should be noted that the cutoff value for the Strongy Detect ELISA was determined on the basis of the same dataset from which these sensitivity and specificity estimates were later generated, which will have resulted in a small positive bias in the estimates for these values. Independent verification using an independent sample would therefore be of benefit.

Based on the predictive values, the combination of Bordier ELISA plus a faecal test, either Baermann or PCR, would appear to have the best performance in all prevalence settings. However, the choice of a test or combination of tests to be used for control programmes must be based on a number of parameters, including suitability of performance in the different contexts or phases of the programme and setting-specific conditions. Whereas for individual screening or diagnosis in a clinical context, high sensitivity (hence accepting a low PPV) is of primary importance given the potential harm caused by an untreated strongyloidiasis, for control programmes a high PPV should be privileged, to identify the areas requiring intervention.[5] In these areas, the whole target population will receive preventive chemotherapy; hence, missing some infections would not impact individual access to treatment.

Regarding acceptability, in this setting the procedures were well accepted by the target population. However, context-specific evaluation should be carried out or derived from experiences of other control programmes to allow generalisation. Concerning the feasibility of the different techniques, the availability of laboratory infrastructure as well as the logistics and costs of material supply, sample transportation, and sample processing in a reference laboratory should be weighed against the characteristics and requirements of the techniques implementable directly in the field. In our study, the staff deemed the Baermann method cumbersome and time-consuming; moreover, there were concerns about the amount of plastic waste produced, and to be disposed of safely, in the field. It should be further considered that rigorous training of the staff and regular quality control of the results should be done, in order to obtain reliable morphological identification of *S stercoralis* larvae. On the contrary, the cassette format of the RDT makes it very suitable for use in the field, as it is easy to use and interpret, although plastic cassette disposal should still be considered. Another strength of the RDT is the use of a recombinant antigen, as also true for the Strongy Detect ELISA, which theoretically would be advantageous in terms of reproducibility and mass production, compared with a crude antigen-based seroassay. Currently, however, these recombinant antigen-based tests are not commercially available, contrary to the Bordier ELISA, so it is not known whether they could be affordable and easily supplied. Compared with the ELISA kits, a further advantage of the RDT is that it does not need shipment under controlled temperature. Indeed, maintenance of the cold chain can be an issue in most *Strongyloides*-endemic areas. For the Strongy Detect ELISA, another problem is represented by the current lack of a fixed cutoff for positivity, which is unacceptable in the context of control programmes.

Besides the requirement of laboratory equipment, the supply of some reagents for PCR might also be difficult. Among molecular assays, loop-mediated isothermal amplification (LAMP) might be theoretically preferable for use in low-income and middle-income countries. Some LAMP assays have been implemented for *S stercoralis*,[31,32] and would deserve further evaluation. Finally, standardisation is required for tests to be recommended for control programmes, hence there might be concerns about using in-house techniques, including the real-time PCR used here.[33] Standardisation of the PCR technique should include also pre-analytical procedures.[34]

This study has several limitations. Firstly, the low number of cases positive on the faecal tests might have had an impact on the calculated performance of the antibody tests. However, the use of the BLCA model allowed more robust estimates to be obtained, despite some limitations associated with the use of this method, particularly regarding the core model assumptions.[9] Major potential sources of inaccuracies with the Bayesian framework used are misspecification of prior information, the assumption of constant sensitivity and specificity between populations, and misspecification of the correlation structure between tests. However, our sensitivity analysis indicated that the results presented are qualitatively similar using less informative priors and also unaffected by exclusion of individual villages, and our correlation structure was chosen on the basis of strong biological justifications. We therefore believe that the model inference presented is valid, but we note the availability of alternative specifications including random-effects type models, which might give different results due to different assumptions being made in the analysis.[10] Another limitation of the study is that the sample size was calculated assuming the availability of a gold standard test and might therefore have been underestimated relative to the desired precision estimates specified. Further work is needed to establish a standardised sample size calculation method for use in the absence of a gold standard, as in our study.

Analysing stool samples collected on different days is known to increase the sensitivity of faecal-based tests.[8] However, in our context, reflecting that of control programmes, it would have been difficult to visit communities for several days due to economic and logistical constraints. Secondly, we did not evaluate the performance of the tests after treatment. Although data

are available on the behaviour of some of these tests after treatment,[35,36] the performance of the novel ones should be assessed. Finally, we could not carry out an in-depth qualitative appraisal of the acceptability and feasibility of each sampling modality and diagnostic technique, but we inferred the former from the participation response of the target population and discussed the latter among the staff involved in the study.

In conclusion, the combination of crude antigen-based ELISA and a faecal-based test specific for *Strongyloides* might be the most suitable option for prevalence surveys in *S stercoralis* control programmes. Local expertise and facilities, financial and logistic constraints, and local acceptability can also impact the choice of tests. Further investigation is needed in different geographical areas as well as evaluation of other diagnostic tools that might be feasible to implement.

Contributors

FT, AGG, MA, YV, and DB contributed to study design. FT, AGG, YV, CM, MD, and DB contributed to data curation. FT, CM, MD, and DB contributed to formal analysis. FT, AGG, MA, YV, RP, MM, SV, FRH, and MR contributed to investigation. FT, AGG, MA, YV, CM, and DB contributed to methodology and validation of data. FT, CM, and DB contributed to writing the original draft. AGG, MA, and DB contributed to project administration and supervision. DB contributed to conceptualisation and funding acquisition. All authors reviewed and edited the original draft. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The raw data (with deidentified participant data) are accessible in Zenodo (https://zenodo.org/record/7612076#.ZAg2u3bMLct).

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