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ORIGINAL ARTICLE

Prevalence of *Strongyloides stercoralis* infection and other soil-transmitted helminths by cross-sectional survey in a rural community in Gisagara District, Southern Province, Rwanda

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Background: *Strongyloides stercoralis* is one of the most neglected tropical diseases. Sparse, dated central African and Rwandan data on seroprevalence are available to guide public health efforts and clinical care.

Methods: In February 2016 we conducted a community-based cross-sectional study among 539 asymptomatic participants in a rural area in the Gisagara District, Southern Province, Rwanda. Direct faecal smear (DFS) and modified Koga agar plate culture (APC) were used to detect *S. stercoralis* infection in a single stool sample. Data on other soil-transmitted helminths diagnosed by DFS were also recorded.

Results: Four intestinal helminth infections were diagnosed, with *S. stercoralis* (17.4%) and hookworms (8.2%) seen most often. APC, compared with DFS, increased the diagnosis rate for *S. stercoralis* from 1.9% to 17.4% ($p<0.01$). The prevalence was higher in farmers and those with lower socio-economic status. Females were less often infected than males (odds ratio 0.6 [95% confidence interval 0.3 to 0.9], $p=0.02$).

Conclusions: *S. stercoralis* is highly prevalent among the general population in a rural area of Gisagara District, Southern Province, Rwanda. Access to effective diagnosis and treatment is needed for this neglected disease.

Keywords: Epidemiology, Helminths, Neglected diseases, Parasitology, Rwanda, Strongyloides

Introduction

Strongyloides stercoralis is a soil-transmitted helminth (STH) and one of the most neglected tropical diseases. Based on estimates using newer diagnostic tools, about 370 million people are infected with *S. stercoralis* worldwide, with prevalence rates as high as 60% in endemic areas, especially Southeast Asia, sub-Saharan Africa, the West Indies and Latin America.[1–3] While many of those infected may remain asymptomatic, chronic, progressive and fatal hyperinfection can occur, especially in immunocompromised hosts, due to the organism's ability to autoinfect the host.

Scant data are available on its prevalence in Central Africa, with the only published studies more than 20 years old and relying on direct faecal smears (DFSs).[4–6] More sensitive diagnostic tools, such as the Baermann funnel (BF) method and Koga agar plate culture (APC), were not employed in these early studies, almost certainly leading to underestimation of the true prevalence rate.[7] Very sparse data are thus available to guide public efforts directed against *S. stercoralis* in the region. Large upheavals in communities related to war and genocide, with subsequent significant improvements in living conditions, as well as more recent governmental initiatives encouraging people to wear shoes, have occurred since these early surveys, all factors likely to have significant effects on prevalence, although in differing directions. Annual mass deworming programs with albendazole

or mebendazole have been used as the control programme for STHs in Rwanda since 2007, with the main objective being to reduce the prevalence and morbidity associated with STH infections among school-aged children. It is should be noted however that standard mass deworming medication regimens (typically single-dose albendazole or 3 d of mebendazole) do not provide effective treatment for *S. stercoralis*.[8]

We thus sought to investigate the prevalence of current *S. stercoralis* infection among a rural-dwelling, asymptomatic adult population in Rwanda using the modified Koga APC technique and DFS.

Materials and methods

Study location and timing

The study was conducted among the general population residing in two sectors, Muganza and Gakoma, in the Gisagara District, Southern Province (see Appendix 1 for a map of the area studied and involved study site locations). These sectors include areas of marshes located near the Akanyaru River, where rice and maize agriculture constitute the main economic activity. Most of the population of these sectors are subsistence farmers and live under basic or inadequate hygiene conditions. Our study was carried out during the dry season (2–20 February 2016).

Study design, participant selection and sampling

This was a cross-sectional, community-based, descriptive, epidemiological study. The study participants included a sample of household members >21 y of age from all 101 villages in the Muganza and Gakoma sectors. Seventy households were selected randomly from the list of all households residing within all villages within the Muganza and Gakoma sectors and were invited by community-based health care personnel to attend on the study days. All household members presenting on the study days and not subject to exclusion criteria were consecutively enrolled until the desired sample size was reached. Participants <21 y of age, those who refused to sign a consent form, visitors from other districts and those who were unable to provide a stool sample were excluded.

The minimum required sample size was calculated to be 514 participants, based on an estimated mean prevalence of 14% from Ugandan and Tanzanian studies (six and eight surveys with prevalences of 19.3% and 7.9% respectively[2]), with the aim of measuring prevalence to within a 3% margin of error at a 95% confidence level. The sample size was further increased by 3% to account for potential data loss due to non-response, consent withdrawal or recording errors. The targeted sample size was therefore 529 participants.

Field and laboratory procedures

A specially designed questionnaire, translated into the Kinyarwanda language and checked for intelligibility and meaning (Appendix 2), was verbally administered to each participant to obtain sociodemographic data. Oral information provided by study participants was recorded on paper copies of the questionnaire by bilingual research assistants.

Study participants provided stool samples for DFS and APC and blood for complete blood count and serology. The samples were collected in the laboratory of the nearest health centre.

Blood was collected under aseptic conditions in pre-labelled Vacutainer ethylenediaminetetraacetic acid (EDTA) and dry tubes. A stool container was given to each participant to provide a stool sample. Collection containers for stool and blood shared the same study code for each participant. DFS was performed immediately on a light microscope for *S. stercoralis* larvae and helminth eggs, then another portion of the same stool sample was placed in the middle of a horse blood agar plate, which was sealed and transported to the laboratory of the University Teaching Hospital of Butare, Huye, Rwanda, where it was incubated at room temperature (24°C) and visually examined by experienced laboratory technicians for the development of larval tracks within 5 d.

The blood collected in the EDTA tube was examined for eosinophils (absolute number and percentage) using an XS-500i (Sysmex, Kobe, Japan). The serum collected in the dry tube was tested for human immunodeficiency virus (HIV) serology (colloidal and determine reagents); part of this serum sample was frozen for *S. stercoralis* antigen enzyme-linked immunosorbent assay to be performed at a later time. Stool or helminth samples were not stored.

Statistical analysis

The key outcome of interest was the prevalence of *S. stercoralis* by APC in a single stool sample, with *S. stercoralis* and other STH prevalence by DFS, HIV serological status and peripheral blood eosinophilia being secondary outcomes of interest and sociodemographic variables (age, gender, sector of residence, occupation, economic status) as well as access to safe water and the use of footwear at work being exposures of interest and potential predictors of infection status.

Questionnaire and laboratory data collected for each participant were entered in EpiData version 3.1 (EpiData Association, Odense, Denmark) then exported to SPSS version 23 (IBM, Armonk, MY, USA) for statistical analysis. Only participants with complete records (stool and blood samples examined with all proposed diagnostic methods and completed questionnaires) were retained for statistical analysis.

The prevalence of *S. stercoralis* infection was defined as the proportion of individuals with positive stool samples on either DFS or APC. The presence of other helminths was determined based on an experienced laboratory technician's reading of the DFS. Quantitative variables (age, peripheral blood eosinophil count) were recoded categorically prior to analysis.

The χ^2 test was used for qualitative variables, with application of Fisher's exact test as required for low expected cell counts, in order to assess the association between variables. The association between sociodemographic factors and infection was expressed in terms of the odds ratio (OR) with calculation of the 95% confidence interval (CI) around the OR. For all statistical tests of association, a p-value <0.05 was considered significant. A logistic regression model was constructed including sociodemographic factors found to be significantly associated with infection status on univariate analysis and *S. stercoralis* infection status by APC.

Ethics statement

All study participants were adults >21 y of age who consented to participate in the study after explanation about the type and role of the study and its risks and benefits. They gave informed consent prior to any study procedures being carried out and were free to withdraw at any time. The study was reviewed and written approval was given by the Research Committee of the College of Medicine and Health Sciences Institutional Review Board (CMHS/IRB) in September 2015. All relevant authorities, including the mayor of Gisagara District and leaders of the health sector, were informed about the aim and procedures of the study and gave their verbal consent for the study to proceed.

All people diagnosed as having infections with worms (*Ascaris lumbricoides*, hookworms, *Trichuris trichiura*) were treated with mebendazole 100 mg every 12 h for 3 d. Those with infections with *S. stercoralis* were registered and informed of their status and will be treated with ivermectin when it becomes available in Rwanda.

Results

A total of 539 participants from two sectors (Muganza and Gakoma) in the Gisagara District, Southern Province, Rwanda were enrolled in the study and completed all required data records (see Table 1). All 539 subjects approached for study participation consented and provided a single stool sample and completed the questionnaire. Participants were between 21 and 84 y old (median age 42 y); the great majority (523 [97%]) were farmers working barefoot. The majority of participants were female (81.4%) and from Gakoma (440 [81.6%]). Most were from low economic status households (Ubudehe level 1 and 2; 81.8%).

The *S. stercoralis* detection rates for DFS and APC were 10/539 (1.9%) and 94/539 (17.4%) respectively, yielding an overall prevalence of 17.4% (Table 2). All specimens positive by DFS were also positive by APC, but APC had a higher diagnostic yield for *S. stercoralis* when compared with DFS. Being male, coming from Gakoma and being from a lower economic status household were all associated with *S. stercoralis* infection on univariate analysis, with these differences only being significant with the greater sensitivity achieved with the APC, although similar trends were apparent for DFS. There was a trend towards increased *S. stercoralis* infections with older age, although this did not reach statistical significance. On construction of a linear regression model, male gender (F=4.3, p=0.038) and coming from Gakoma (F=8.7, p=0.003) were significantly associated with *S. stercoralis* status as measured by APC, with economic status falling just short of statistical significance (F=2.9, p=0.054).

The overall prevalence of other soil-transmitted helminths was 12.8% (*Ascaris lumbricoides*, 4.3%; hookworms, 8.2%; *Trichuris trichiura*, 0.4%) and the rates of coinfection with *S. stercoralis* are detailed in Table 3. Among 69 participants diagnosed with other STHs (12.8%), 16 had *S. stercoralis* infection (17%). No other helminth infection was statistically associated with *S. stercoralis* positivity in the study population.

In the study, only 7 of 539 participants were diagnosed with HIV, yielding an HIV prevalence of 1.3%, and none of the HIV-positive participants had *S. stercoralis* infection. This number was insufficient to statistically determine any association between *S. stercoralis* and HIV infection.

Table 1. Sociodemographic characteristics of the study participants

Characteristics	n	%
Gender		
Male	100	18.6
Female	439	81.4
Age (y)		
21–40	242	44.9
41–60	222	41.2
61–80	71	13.2
>80	4	0.7
Sector		
Gakoma	440	81.6
Muganza	99	18.4
Economic status (Ubudehe)		
Level 1	149	27.6
Level 2	292	54.2
Level 3	98	18.2
Access to safe water		
Potable	478	88.7
Non potable	61	11.3
Protection at work		
Barefoot	523	97
Wearing shoes	16	3
Occupation		
Farmer	524	97
Public sector worker	16	3
Private sector worker	1	0.2
HIV status (as tested)		
Positive	7	1.3
Negative	532	98.7

Ubudehe refers to a Rwandan social system for classifying household economic status. The levels range from 1 to 5 and lower numbers refer to lower economic status.

Moderate (1.21–4%) or severe (>4%) eosinophilia was present in the majority of participants, with rates of 157/539 (29.1%) and 331/539 (61.4%), respectively. Among the participants infected with *S. stercoralis*, 84 of 94 (88.4%) had severe eosinophilia and the remaining 10 had moderate (7/94) or mild (3/94) eosinophilia.

Discussion

Our study showed the prevalence of *S. stercoralis* infection to be 17.4% as detected by APC and DFS on a single stool test in a rural area of the Gisagara District, Southern Province, Rwanda, where people largely work in riverside rice fields and without shoes. The modified Koga APC technique increased the diagnostic

Table 2. Prevalence of *S. stercoralis* infection based on the DFS and APC methods in the general population in the Gisagara District, Southern Province, Rwanda, univariate analysis

	DFS, n/N (%)	OR (95% CI)	APC, n /N (%)	OR (95% CI)
Total	10/539 (1.9)		94/539 (17.4)	
Age (y)				
21–40	6/242 (2.5)	REF	34/242 (14)	REF
41–60	4/222 (1.8)	0.7 (0.2 to 2.6)	44/222 (19.8)	1.5 (0.9 to 2.4)
61–80	0/71 (0)	—	14/71 (19.7)	1.5 (0.7 to 2.9)
>80	0/4 (0.0)	—	2/4 (50)	6 (0.8 to 44)
Gender				
Male	4/100 (4)	REF	25/100 (25)	REF
Female	6/439 (1.4)	0.3 (0.09 to 1.2)	69/439 (15.7)	0.6 (0.3 to 0.9)*
Sector				
Gakoma	9/440 (2)	REF	89/440 (20.2)	REF
Muganza	1/99 (1)	0.5 (0.06 to 3.9)	5/99 (5.1)	0.2 (0.08 to 0.5)*
Economic status (Ubudehe)				
Level 1	3/149 (2)	1.9 (0.2 to 19)	35/149 (23.5)	3.4 (1.5 to 7.8)*
Level 2	6/292 (2.1)	2 (0.2 to 17)	51/292 (17.5)	2.3 (1.0 to 5.0)*
Level 3	1/98 (1)	REF	8/98 (8.2)	REF
Occupation				
Farmer	9/524 (1.7)	0.2 (0.02 to 1.9)	90/524 (17.2)	0.5 (0.2 to 1.7)
Public sector worker	1/14 (7.1)	REF	4/14 (28.6)	REF
Private sector worker	0/1 (0)	—	0/1 (0.0)	—
HIV status				
Positive	0/7 (0)	—	0/7 (0.0)	—
Negative	10/532 (1.9)	REF	94/532 (17.7)	REF

*Statistically significant ($p<0.05$).
REF: referent for univariate statistical comparison; CI: confidence interval; OR: odds ratio; —: OR not calculable because one of the cells contains zero.

Table 3. Prevalence of other STHs in the study population cross-tabulated by *S. stercoralis* status

	S. stercoralis status		p-Value
	Positive, n/N (%)	Negative, n/N (%)	
Ascaris lumbricoides	5/94 (5.3)	18/445 (4)	ns
Hookworms	10/94 (10.6)	34/445 (7.6)	ns
Trichuris trichiura	1/94 (1.1)	1/445 (0.3)	ns

hookworms: *Ancylostoma duodenale* or *Necator americanus*; ns: not statistically significant.

yield 9-fold (1.9% to 17.4%) compared with DFS on a single stool sample. As DFS is currently the only diagnostic test usually used in Rwandan laboratories, this points to a high rate of missed diagnosis of *S. stercoralis* in routine clinical practice in Rwanda. The prevalence of other STHs was 12.8% and there was no significant correlation between any other helminth and *S. stercoralis*. Among seven participants (1.3%) diagnosed with HIV infection, none had *S. stercoralis*, but the infrequency of HIV in the study population made drawing any conclusions difficult.

This study on the prevalence of *S. stercoralis* infection using multiple diagnostic methods is the first of its type conducted in Rwanda. It was a prospective, cross-sectional study conducted in a well-characterized community population considered to be at an increased risk. In addition, APC can be considered among other gold standard methods to detect active *S. stercoralis* infection and showed a much higher prevalence than would have been detected with DFS alone, as has been previously reported elsewhere.[7,9] Since APC is a simple and cheap technique that requires only materials that are readily available in laboratories in resource-poor settings, the study also demonstrates the possibility that pragmatic use of the APC could increase the diagnostic yield for *Strongyloides* in Rwanda and other similar resource-poor settings.

The prevalence observed in our study population is high and concordant with other cross-sectional studies performed in sub-Saharan Africa,[7,10] although even higher rates have been reported from several studies in at-risk populations from Southeast Asia.[11,12] Variations in reported prevalence are likely to be due to the intersection of multiple factors, including

different sampling methods (at-risk vs. cross-sectional surveying) and the number of stool samples and simultaneous diagnostic methods used (one to three samples and two to three methods), as well as true differences in population prevalence. Even within our study, the sector of origin was a potent predictor of *S. stercoralis* infection, pointing to probable true prevalence variability even within relatively small geographic areas; more research is clearly required to define this.

The correlation between *S. stercoralis* and other STHs was statistically significant in several of the other studies mentioned above;[7,10] in particular, hookworms were more frequently associated with *S. stercoralis*. The positive studies used the Kato–Katz method for detection of hookworm parasites, which is more sensitive than DFS alone, as was used in our study.[13] This may have led to a systemic underestimation of hookworm prevalence in our study and limited the power of the study to detect associations between *S. stercoralis* and hookworm infections.

In our study, HIV infection was observed in only seven participants (1.3%) and none of them had *S. stercoralis* infection nor any other STH. This number was too low to determine a statistical association or difference between *S. stercoralis* infection and HIV in our study. This observation was thus unable to confirm what was found in a prospective study done in Brazil looking at the efficacy of APC to detect *S. stercoralis* in HIV-infected vs. non-HIV-infected individuals. Among 424 participants in that study, HIV-infected participants were more susceptible to *S. stercoralis* infection than those who were HIV negative.[14]

Eosinophilia was extremely frequent in the study population, and unlike in Western populations, thus this was unhelpful in distinguishing those with STHs among the study population from those without. Based on the data obtained, eosinophilia would be a poor screening test for STHs in Rwanda, with very low specificity for the diagnosis.

The limitations of our study include the use of one effective method (APC) on a single stool sample only. Analysing one stool sample with a single test is not sufficient to determine the true prevalence of *S. stercoralis* infection; a satisfactory level of sensitivity requires analysing multiple stool samples with multiple different, sensitive diagnostic methods. APC, the Baermann method and DFS are faecal-based techniques that have been commonly used in *S. stercoralis* infection prevalence studies. Their sensitivities were 89%, 72% and 21%, respectively, with a specificity of 100% for each, according to a review of studies conducted between 1980 and 2013 designed to assess the test performance characteristics of faecal-based methods.[15] The recommended gold standard is a combination of all three methods, but due to limited study resources, only two techniques (DFS and APC) were used in our study.

The DFS and APC we used in our study have sensitivities of 21% and 94%, respectively, with a high reported specificity of 100% for both APC and DFS, as estimated by the prevalence studies that used a combination of three tests (DFS, BF and APC) designed to assess their sensitivity.[16] When APC is used on a single stool, the sensitivity has been estimated at 78.5%.[9] All measured prevalences in our study would likely have been higher if a more rigorous approach (more diagnostic methods, multiple stool samples) had been used, but logistical constraints imposed by funding and follow-up of enrolled participants made this difficult.

A second challenge to interpretation of our results is posed by the possibility of motile hookworms being mistaken for *S. stercoralis* on the modified Koga APC. In other studies, 50–60% of hookworms detected on a single DFS were also detected by an APC method.[7] As we did not examine each plate for differences in the patterns of motile tracks observed between hookworms and *S. stercoralis*, up to 5% (8.2%×0.6) of the positive APC plates ascribed to *S. stercoralis* may have in fact been due to incorrect classification of hookworm species. In contrast, testing of three stool samples would be likely to increase the detected *S. stercoralis* prevalence compared with a single stool specimen, probably by a significant amount, likely between 40% and 70% based on other published studies. As such, the reported prevalence in our study is an estimate subject to biases in both directions and requiring further validation.

Our plan in the future is to extend the study in the same study area as well as in other areas in Rwanda with potential risk factors, using more than one effective method on more than one stool sample. Another study using the same sample is being done looking at risk factors and the clinical effect of *S. stercoralis*. We plan to join the advocacy for the availability of effective treatment for *S. stercoralis*, which is a key public health priority as ivermectin is not readily available at present.[17]

Conclusions

S. stercoralis is highly prevalent among an at-risk population residing in a rural area in the Gisagara District, Southern Province, Rwanda, with an estimated prevalence of 17.7% by APC upon analysing a single stool sample. The examination of multiple stool samples with multiple diagnostic methods would likely lead to an even higher detected prevalence. Upgrading of laboratory detection facilities for STHs and provision of efficacious treatment (in particular, ivermectin) should be public health priorities for Rwanda.

Supplementary data

Supplementary data are available at Transactions online (http://trstmh.oxfordjournals.org/).

Authors' contributions: DCH conceived the study. DCH, AT, AN, CN, CB and TDW designed the study. AT and AN wrote the study protocol. AT, AN, CN and DCH carried out fieldwork. AT, AN and CB performed the laboratory analysis. AT and AN entered and checked the data. AT, DCH and TDW performed the data analysis. AT, DCH, VD and TDW wrote the final manuscript. All authors approved the manuscript prior to submission. DCH, AT and AN are guarantors of the paper.

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Competing interests: None declared.

Ethical approval: Written approval was given for the study by the Research Committee of the College of Medicine and Health Sciences Institutional Review Board (CMHS/IRB) in September 2015. All study procedures were carried out in accordance with the Helsinki Declaration of the World Medical Association.

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