Parasitology

cambridge.org/par

Accuracy of real-time polymerase chain reaction to detect *Schistosoma mansoni* – infected individuals from an endemic area with low parasite loads

Fernanda do Carmo Magalhães[1], Samira Diniz Resende[1], Carolina Senra[2], Carlos Graeff-Teixeira[3], Martin Johannes Enk[4], Paulo Marcos Zech Coelho[5], Edward Oliveira[2], Deborah Aparecida Negrão-Corrêa[1], Stefan Michael Geiger[1] and Mariângela Carneiro[1]

[1]Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, MG, Brazil; [2]Pesquisa Clínica e Políticas Públicas em Doenças Infecciosas e Parasitárias, Instituto René Rachou, Fundação Oswaldo Cruz (FIOCRUZ), 30190-002, Belo Horizonte, MG, Brazil; [3]Núcleo de Doenças Infecciosas, Centro de Ciências da Saúde, Universidade Federal do Espírito Santo, Vitória, ES, Brazil; [4]Secretaria de Vigilância em Saúde, Instituto Evandro Chagas, Ministério da Saúde, Belém, Brazil and [5]Diagnóstico e Terapia de Doenças Infecciosas e Oncológicas, Instituto René Rachou, Fundação Oswaldo Cruz (FIOCRUZ), 30190-002, Belo Horizonte, MG, Brazil

Research Article

Cite this article: Magalhães FdoC *et al* (2020). Accuracy of real-time polymerase chain reaction to detect *Schistosoma mansoni* – infected individuals from an endemic area with low parasite loads. *Parasitology* **147**, 1140–1148. https://doi.org/10.1017/S003118202000089X

Received: 15 January 2020
Revised: 24 May 2020
Accepted: 26 May 2020
First published online: 2 June 2020

Key words:
Diagnosis; low parasite load; RT-PCR; *Schistosomiasis mansoni*

Author for correspondence:
Fernanda do Carmo Magalhães,
E-mail: nandademagalhaes@yahoo.com.br

Abstract

Due to the efforts to control schistosomiasis transmission in tropical countries, a large proportion of individuals from endemic areas present low parasite loads, which hinders diagnosis of intestinal schistosomiasis by the Kato-Katz (KK) method. Therefore, the development of more sensitive diagnostic methods is essential for efficient control measures. The aim was to evaluate the accuracy of a real-time polymerase chain reaction (RT-PCR) to detect *Schistosoma mansoni* DNA in fecal samples of individuals with low parasite loads. A cross-sectional population-based study was conducted in a rural community ($n = 257$) in Brazil. POC-CCA® was performed in urine and feces were used for RT-PCR. In addition, fecal exams were completed by 18 KK slides, saline gradient and Helmintex techniques. The combined results of the three parasitological tests detected schistosome eggs in 118 participants (45.9%) and composed the consolidated reference standard (CRS). By RT-PCR, 117 out of 215 tested samples were positive, showing 91.4% sensitivity, 80.2% specificity and good concordance with the CRS (kappa = 0.71). RT-PCR identified 86.9% of the individuals eliminating less than 12 eggs/g of feces, demonstrating much better performance than POC-CCA® (50.8%). Our results showed that RT-PCR is a valuable alternative for the diagnosis of intestinal schistosomiasis in individuals with very low parasite loads.

Introduction

Schistosomiasis is a serious public health problem affecting more than 240 million people worldwide, with another 700–800 million living in areas at risk of infection (Gryseels *et al*., 2006; Steinmann *et al*., 2006; Colley *et al*., 2014). Given the chronic and debilitating character of the disease, the Global Burden of Disease Study estimated that schistosomiasis contributed to 3.51 million disability-adjusted life years (DALYs) in 2015 and led to 10.1 million deaths in 2016 (WHO, 2016; GBD, 2017). In Brazil, the most recent national survey on the prevalence of intestinal schistosomiasis and geohelminths (INPEG 2010–2015) showed that positivity rates for schistosomiasis ranged from 0.02% to 3.5%, with the highest positivity rates found in the southeast (3.5%) and the northeast (1.27%) of the Brazilian territory (Katz, 2018).

Decisions about individual treatment, morbidity studies, identification of communities at risk of infection, cure control and follow-up of control programs are actions that depend primarily on the accuracy of the diagnostic tests (Utzinger *et al*., 2000; Amaral *et al*., 2006). Therefore, the development of simple, low-cost, and sensitive diagnostic methods that can be used in population studies is of fundamental interest for control (Utzinger *et al*., 2001; Enk *et al*., 2008). The Kato-Katz (KK) technique is the parasitological method recommended by the World Health Organization to confirm intestinal schistosomiasis infection and is a quantitative, low-cost and easy to perform test (WHO, 1993; WHO, 2002). This method is very efficient to diagnose moderate and intense infections. However, its low sensitivity for the diagnosis of individuals with low parasite loads was considered to be an important drawback (Engels *et al*., 1996; Kongs *et al*., 2001; Berhe *et al*., 2004; Bergquist *et al*., 2009). Therefore, this method underestimated the prevalence of *Schistosoma mansoni* infection in areas of low endemicity (prevalence less than 10%) or in treatment-reinfection studies (Utzinger *et al*., 2001; Ferrari *et al*., 2003; Enk *et al*., 2008; Coelho *et al*., 2009; Siqueira *et al*., 2015; Oliveira *et al*., 2018). As an alternative, immunological tests, such as the point-of-care rapid urine test for the detection of *S. mansoni* circulating cathodic antigen

(POC-CCA®) are commercially available. The POC-CCA® test is fast, easy to perform, and showed better sensitivity than the KK technique in different regions of Africa and Asia (Stothard *et al.*, 2009; Standley *et al.*, 2010; Colley *et al.*, 2013). However, results obtained with the POC-CCA® in low endemicity areas disagreed to a significant degree with other more sensitive parasitological tests (Siqueira *et al.*, 2016; Oliveira *et al.*, 2018).

Molecular approaches and the use of polymerase chain reaction (PCR) were also used for the detection of human schistosomiasis in fecal (Pontes *et al.*, 2002; Allam *et al.*, 2009; Gomes *et al.*, 2010; Oliveira *et al.*, 2010; Carvalho *et al.*, 2012; Carneiro *et al.*, 2013; Espirito-Santo *et al.*, 2014; Siqueira *et al.*, 2015; Senra *et al.*, 2018), plasma (Wichmann *et al.*, 2009), serum (Pontes *et al.*, 2002; Espirito-Santo *et al.*, 2014), or urine samples (Enk *et al.*, 2012). In these PCR-based assays, different targets were used to detect *Schistosoma* DNA; among them, a tandem repeat sequence of 121 base pairs (bp) successfully used in a PCR system to detect the parasite in snails and for the monitoring of cercariae in water bodies (Hamburger *et al.*, 1991, 1998*a*, *b*). Different studies, which used this sequence for detection of intestinal schistosomiasis, showed encouraging results, with high levels of sensitivity and specificity to identify *S. mansoni* infection (Pontes *et al.*, 2003; Gomes *et al.*, 2010; Carneiro *et al.*, 2013; Siqueira *et al.*, 2015). Therefore, especially in low endemicity settings and in individuals with a low parasite burden real-time PCR (RT-PCR) might be a valuable tool to be considered and applied. However, accuracy evaluation of this technique is still required in an epidemiological setting and needs to be compared with a robust reference standard, e.g. in comparison with different and extensive parasitological tests to detect active infection. Therefore, the aim of this study was to evaluate and compare the efficacy of RT-PCR for the detection of active schistosomiasis infection in an endemic setting and with a special focus on individuals with low parasite loads.

Material and methods

Ethical considerations

This study was approved by the Ethics Committees of the René Rachou Institute (FIOCRUZ) and the Federal University of Minas Gerais and all project details have been registered on the Brazilian Platform for Research with Human Subjects (Plataforma Brasil) under the following number: CAAE#21824513.9.0000.5091. As such, the information presented in this paper is part of an interinstitutional research project, performed in three different Brazilian states (Minas Gerais, Maranhão, and Pará), with the scope to provide data on the performance of parasitological, immunological and serological tests for the diagnosis of intestinal schistosomiasis and geo-helminth infections under different eco-epidemiological situations and up to 1 year after chemotherapy.

An informed consent form was read and signed by the participants and by the parents or legal guardians of minors before enrollment. Literate children were also asked to read and sign an adapted informed consent form. Pregnant and breastfeeding women were excluded from the study. Infected individuals were treated for schistosomiasis with Praziquantel (40 mg kg^{-1} for adults and 60 mg kg^{-1} for children), Albendazole (400 mg single dose) for intestinal helminths, and Metronidazole (250 mg/2x/5 days) for control of parasitic protozoa.

Study design

A cross-sectional population-based study was carried out in the rural area of Brejo do Amparo (Fig. 1), in the municipality of Januária, in the northern area of the state of Minas Gerais, Brazil (15° 29′ 16″ S 44° 21′ 43″ O). This rural community is located along the margins of the Tocantins brook, where populations of *Biomphalaria glabrata* snails were found during malacological surveys. According to the Brazilian Schistosomiasis Control Program and local health authorities, the estimated prevalence of schistosomiasis in this area was 20% in 2010, and no control interventions have been put into place in the locality in the two years prior to the present study. The 257 individuals studied herein were previously evaluated in a study that investigated parasitological and immunological diagnostic techniques for the detection of schistosomiasis in an endemic area with low parasite loads (Oliveira *et al.*, 2018).

As detailed by Oliveira *et al.* (2018), three fecal samples were collected on consecutive days. The samples were received at the field laboratory in Januária, homogenized and processed for evaluation. At least 50 grams of feces were collected in the first fecal sample using a 500 mL plastic container. From this first fecal sample, subsamples were retrieved to perform the KK technique (14 slides approximately 41.7 mg), Saline gradient (500 mg) and a modified Helmintex technique® (30 g). Also, an additional 500 mg sample was sieved through the mesh and stored at −20°C until molecular testing was performed. The second and third fecal samples were collected in the following days in 80 mL plastic cups and were used to perform additional KK slides (2 slides each). A first-morning urine sample was also collected from the participants and used to detect circulating antigen of *S. mansoni* (POC-CCA® urine test), as previously described (Oliveira *et al.*, 2018). The diagnostic methods applied to detect intestinal schistosomiasis are described in the flow diagram in Fig. 1. The intensity of infection was estimated by multiplying the mean number of *S. mansoni* eggs found in the two slides of KK prepared with the first stool sample by 24 to determine the number of eggs per gram of feces (EPG). According to the World Health Organization (WHO, 2002), the intensity of *S. mansoni* infection can be categorized as light (1–99 EPG), moderate (100–399 EPG), or heavy (⩾400 EPG). Individuals who showed a positive result for *S. mansoni* infection in either the Helmintex® technique or the Saline gradient, but were negative in the two KK slides, were classified as infected with a parasite burden of less than 12 EPG.

For a more accurate diagnosis of schistosomiasis a combination of different parasitological methods was defined (KK, Saline gradient, Helmintex®). The results of this combination (Consolidated Reference Standard) showed that among the 257 individuals, 118 (45.9%) had active *S. mansoni* infection. The prevalence of schistosomiasis estimated by each parasitological method was as follows: the modified Helmintex technique showed 40.4% of prevalence (88/218); Saline gradient was 21.3% (42/197) and KK method with 18 slides of tree stool samples was 34.5% (81/235). However, when we evaluated KK technique using the two first slides of one fecal sample (based on the WHO recommendation), the prevalence was 20.4% (48/235) (Oliveira *et al.*, 2018).

DNA extraction from the fecal samples and RT-PCR

Total DNA was extracted from 500 mg of stool from the first fecal sample of each participant using the commercial QIAamp® DNA Stool Mini Kit, following the manufacturer's instructions (Qiagen GmbH, Hilden, Germany). To improve the accuracy of the molecular test, DNA from fecal samples were amplified using a set of primers and probes complementary to a 90-bp DNA fragment contained within the 121-bp tandem repeat sequence of *S. mansoni* strain Sm 1–7 (GenBank accession number M61098) described by Hamburger *et al.* (1991). The primers forward 5′-CCG ACC AAC CGT TCT ATG A-3′ and reverse 5′-CAC GCT CTC GCA AAT AAT CTA AA-3′ and the probe 5′-6[FAM]/TCG TTG TAT CTC CGA AAC CAC TGG ACG/[(3BHQ1)] were synthesized by

https://doi.org/10.1017/S003118202000089X Published online by Cambridge University Press

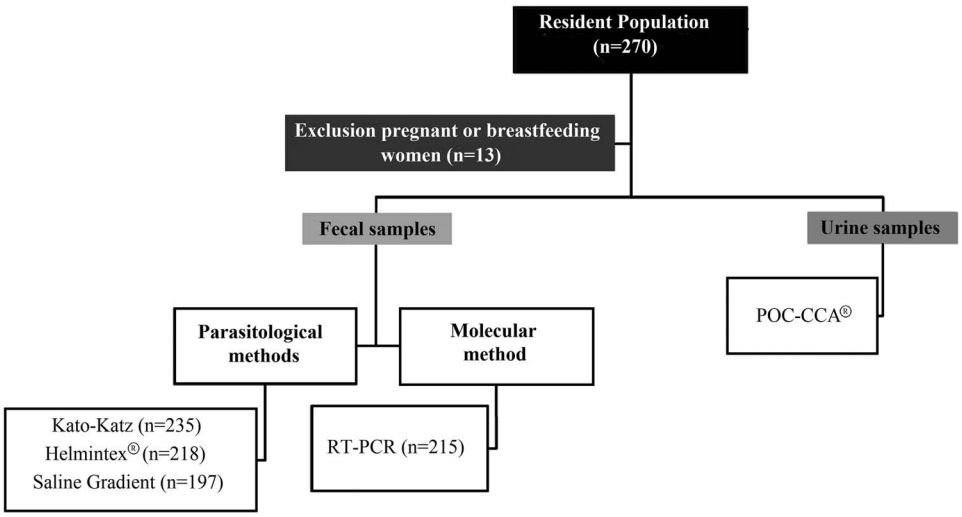

Fig. 1. Flow diagram describing the endemic population enrolled in the study and the diagnostic methods applied to detect intestinal schistosomiasis. The Kato-Katz (KK) technique, saline gradient, modified Helmintex technique®, real-time polymerase chain reaction (RT-PCR) and rapid urine test (POC-CCA®) were also applied.

Sigma Life Sciences (Woodlands, Texas, USA). The amplification reaction was performed in a final volume of 25 μL containing: 12.5 μL of TaqMan® Universal PCR Master Mix (Life Technologies, Thermo Fisher Scientific Inc., USA), 0.1 μM of each *S. mansoni* primer, 0.25 μM of probe, BSA 0.1 μg/μL; 2 M $MgCl_2$; 4 μL of 5-fold diluted DNA. For each run, positive (DNA extracted from adult worms) and negative (no DNA template) controls were performed. The amplification reaction was conducted on the StepOnePlus™ RT-PCR System (Thermo Fisher Scientific Inc., USA) using the universal cycling protocol with 45 cycles and annealing temperature of 60°C. As an internal quality control of DNA isolation and of the absence of PCR reaction inhibitors, all clinical samples were RT-PCR-amplified for the human β-actin gene (*ACTB*). For that, the forward 5′-CCA TCT ACG AGG GGT ATG-3′ and reverse 3′GGT GAG GAT CTT CAT GAG GTA-5′ primers and the 56-JOE/CCT GCG TCT GGA CCT GGC TG/[(3BHQ1)] probe were used. The cut-off cycle threshold (C_t) for positive and negative samples was defined based on a standard curve established with serial dilutions of *S. mansoni* DNA extracted from adult worms. Samples presenting internal control JOE (β-actin probe) amplification and FAM (Schisto probe) target with C_t undetermined and $C_t > 42$ were classified as negative, while samples presenting internal control JOE (β-actin probe) amplification and FAM (Schisto probe) with $C_t \leqslant 42$ were classified as positive. Samples that did not show internal control JOE amplification were classified as invalid and excluded from further analysis.

Data entry and statistical analysis

Data were processed with Open Epi, version 3.01 (Dean *et al.*, 2013) and GraphPad Prism, version 5.0 (La Jolla, CA, USA). In order to evaluate the performance of the different diagnostic tests, a consolidated reference standard (CRS) was established, which included a combination of methods: the KK technique with 18 slides from three stool samples, saline gradient (500 mg) and the modified Helmintex technique® (up to 30 g of feces).

Data normality was verified by the Shapiro-Wilk test. The Kruskal–Wallis test was used to compare the means of continuous variables, with P values $\leqslant 0.05$ considered significant. The overall prevalence of *S. mansoni* infection in the endemic area was calculated by dividing the number of egg-positive individuals found by CRS by the total number of participants.

The intensity of infection was determined according to the C_t value observed by RT-PCR (which reflects fecal parasite-specific DNA load), or by microscopy, as determined by the EPG counts observed in two KK slides from the first stool sample. Spearman's correlation coefficient was used to calculate the concordance between the intensity of infection values established by microscopy and RT-PCR. Statistically significant differences were considered at $P \leqslant 0.05$.

Sensitivity, specificity, positive likelihood ratio, positive (PPV) and negative predictive values (NPV), and concordance (kappa statistics) were calculated for comparison of the performance and accuracy of the tests. The degree of concordance between the different tests was established according to the following categorization: (1) $\kappa < 0.01$, no agreement; (2) $0.01 \leqslant \kappa \leqslant 0.20$, bad agreement; (3) $0.21 \leqslant \kappa \leqslant 0.40$, weak agreement; (4) $0.41 \leqslant \kappa \leqslant 0.60$, moderate agreement; (5) $0.61 \leqslant \kappa \leqslant 0.80$, good agreement; and (6) $\kappa > 0.81$, excellent agreement (Landis and Koch, 1977).

Results

Characterization of the study population

The 257 individuals analysed in the present study were between 2 and 88 years of age, with a median age of 32 years old (interquartile range 15–51 years). No statistically significant difference was observed in the distribution between males (47.5%) and females (52.5%).

Among these 118 individuals, 86.4% ($n = 102$) presented less than 100 EPG, 10.2% ($n = 12$) had a moderate infection (100–399 EPG), and 3.4% ($n = 4$) were heavily infected (⩾400 EPG). Seventy-four (62.7%) infected individuals presented only *S. mansoni* infection, while 44 (37.3%) were co-infected with other parasites. The parasitological techniques also identified 23 (8.9%) individuals eliminating eggs of hookworms; eight (3.4%) individuals with *Enterobius vermicularis*, one (0.4%) infected with *Trichuris trichiura*, and one (0.4%) infected with *Strongyloides stercoralis*. Of all the 118 individuals infected with *S. mansoni*, ten (8.5%) were coinfected with hookworms and five (4.2%) with *Enterobius vermicularis*.

Molecular test results

Among the 215 individuals for which the RT-PCR assay was performed, 117 showed positive results, representing a prevalence of 54.4% (95% CI 47.7–60.9). In comparison to the CRS, the present study demonstrated RT-PCR with 91.4% of sensitivity, 80.2% of specificity, and a positive likelihood ratio of 4.6 (95% CI 4.2–5.0). The concordance between the RT-PCR and the CRS, evaluated by kappa statistics, was 0.71 (95% CI 0.58–0.85) and diagnostic accuracy 85.6% (95%CI 80.3 −89.7), with 95 (44.2%)

https://doi.org/10.1017/S003118202000089X Published online by Cambridge University Press

individuals showing positive results and 89 (41.4%) individuals showing negative results in all the methods. The PPV was 81.2% (95% CI 73.2–87.2).

Only 9 individuals that were recorded with eggs in any of the parasitological exams showed no reactivity in the RT-PCR assay. On the other hand, RT-PCR showed a DNA sequence compatible with *S. mansoni* in 22 individuals that were considered egg negative in all the parasitological techniques (Table 1).

Given the low parasite load observed in the population (<100 EPG), we described the performance of the RT-PCR in relation to the parasite load (high, moderate and low). As shown in Table 2, RT-PCR readily detected all infected individuals with moderate or heavy intensity infections, i.e. patients eliminating more than 100 EPG. Among infected individuals with low parasite load, the RT-PCR also detected 100% of the individuals with a parasite load between 50 and 99 EPG (4/4) and between 12 and 49 EPG (25/25) (Table 2). Moreover, the RT-PCR assay detected the *S. mansoni* infection in 87% ($n = 53$) of the individuals eliminating less than 12 EPG. Noteworthy, six of the nine individuals that had parasite eggs in at least one parasitological test, but showed a negative result by RT-PCR, were identified as egg positive only by the Helmintex® technique. The other three individuals were diagnosed egg positive only by the KK technique with an increased number of slides (12 slides in the first stool sample), or when three consecutive fecal samples were analysed with six different slides.

In addition, when we evaluated the possibility of cross-reactivity with other intestinal helminths, only four (18.2%) of the 22 individuals that were positive only by RT-PCR had a hookworm infection.

Fig. 2, demonstrated that RT-PCR intensity (C_t amplification cycles) is correlated with parasite load of individuals with high/medium/low parasite load. The samples from individuals infected with high (>400 EPG) or moderate (100–400 EPG) parasitic burden required a significantly lower number of PCR-amplification cycles ($C_t = 24.6 \pm 1.7$ and 28.2 ± 3.5, respectively) for detection of *Schistosoma* DNA (Fig. 2A). In contrast, samples from individuals infected with very low parasite load (<12 EPG) needed a higher number of PCR-amplification cycles ($C_t = 33.8 \pm 3.7$). As a result, a highly significant inverse correlation was observed between individual egg counts and the C_t values ($r = -0.66$, $P < 0.001$) (Fig. 2B).

As for the RT-PCR results for the different age groups, the positivity rate was higher among younger children and in individuals over 60 years of age when compared with the CRS (Fig. 3). Thus, the prevalence per age group increased from 31.7% to 38.9% found through the parasitological techniques (CRS) to 50.0% and 53.3% by RT-PCR, respectively (Fig. 3).

Among the 215 individuals who underwent the RT-PCR test, all individuals provided samples to perform the two-slide KK (SPL1 K1-K2), 172 for the KK six slides of three fecal samples (SPL1-3 K1- K2) and 194 for the Helmintex®. The performance of these parasitological methods was compared with RT-PCR (Table 3) The two KK slides, which corresponded to 83.4 mg of feces, revealed low sensitivity (36.0%; 95% CI 27.8–45.1); accuracy (64.6%; 95% CI 57.8–70.8) and kappa statistics (0.33; 95% CI 0.23–0.44). The KK six slides of three fecal samples, which corresponded to 500 mg of feces (41.7 mg × 6 = 250 mg), demonstrated increased sensitivity and concordance (kappa statistics). The modified Helmintex® technique (30 g of feces) demonstrated sensitivity of 68.3% (95% CI 58.8–76.4), concordance of 0.60 (95% CI 0.47–0.74) and diagnostic accuracy 79.9% (95%CI 73.7- 84.9).

We also compared the performance of POC-CCA and RT-PCR. Among the 215 individuals evaluated by RT-PCR, 196 provided samples for the rapid urine test. Of these, 70 (35.7%) were positive by RT-PCR and reactive by POC-CCA® and 64

Table 1. Performance of the real-time polymerase chain reaction (RT-PCR) in comparison with a consolidated reference standard (CRS)

		Consolidated Standard Reference							
		Positive *N* (%)	Negative *N* (%)	Total *N* (%)	Sens. % (95% CI)	Specif.% (95%CI)	PPV (95%CI)	LR + (95%CI)	Accuracy (95%CI)	Kappa (95%CI)
RT-PCR	Positive	95 (44.2)	22 (10.2)	117 (54.4)	91.4 (84.4–95.4)	80.2 (71.8-86.5)	81.2 (73.2–87.2)	4.61 (4.21–5.05)	85.6 (80.3–89.7)	0.71 (0.58–0.85)
	Negative	9 (4.2)	89 (41.4)	98 (45.6)						
	Total	104 (48.4)	111 (51.6)	215 (100.0)						

Data shows the Sens. %: Sensitivity; Esp. %: Specificity; PPV: positive predictive value; LR+: Likelihood ratio for positive results, accuracy and kappa statistic. Consolidated standard reference (CSR) was used as a reference test.

https://doi.org/10.1017/S003118202000089X Published online by Cambridge University Press

Table 2. Sensitivity (%) of the real-time PCR (RT-PCR) and the point-of-care rapid urine test (POC-CCA®) for the detection of schistosomiasis in relation to the individual parasitic load, as defined by quantitative egg counts from two slides Kato-Katz (KK)

Classification according to parasite load (EPG value) Sensitivity (%) of each diagnostic method					
Diagnostic tests	High (EPG >400) % Sensitivity	Moderate (EPG: 100-399) % Sensitivity	(EPG: 99-50) % Sensitivity	Low* (EPG: 49-12) % Sensitivity	(EPG <12) % Sensitivity
RT-PCR	100 (04/04)	100 (08/08)	100 (04/04)	100 (25/25)	86.9 (53/61)
POC-CCA®	100 (04/04)	100 (10/10)	100 (04/04)	77.8 (21/27)	50.8 (34/67)

Data show the sensitivity (%) of each diagnostic method and the number of individuals detected positive for intestinal schistosomiasis *vs* the total number of examined individuals (in brackets), according to parasite load classification. *Individuals with a light infection were arbitrarily divided into three subgroups with egg counts of 99–50 eggs per gram of feces (EPG), 49–12 EPG and less than 12 EPG.

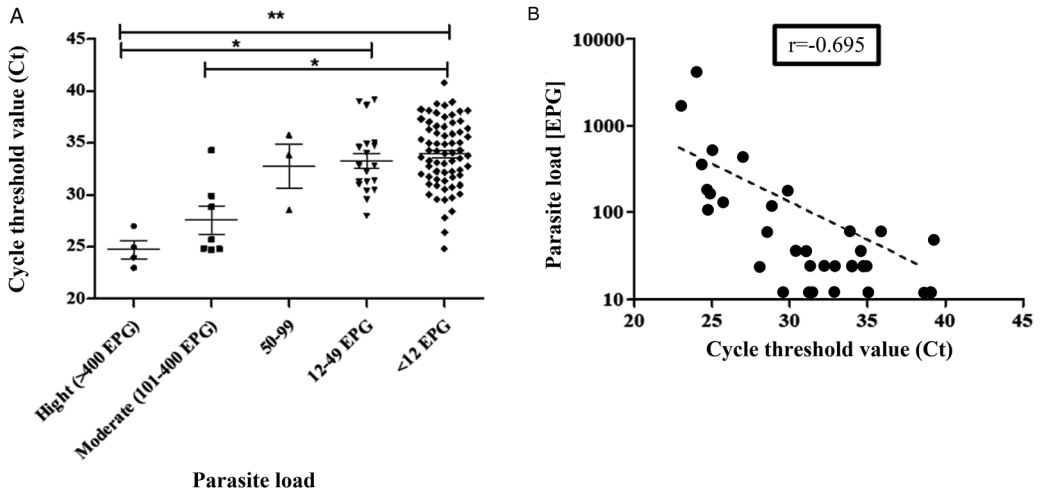

Fig. 2. A. Infection intensities, as determined by parasite load (EPG values) in relation to the number of cycle threshold (C_t) values for the detection of *S. mansoni* DNA. The solid line indicates the median and interquartile range of C_t values. B. Correlation between individual parasite load (EPG values) and C_t values for amplification of *S. mansoni* DNA in fecal samples ($P < 0.0001$).

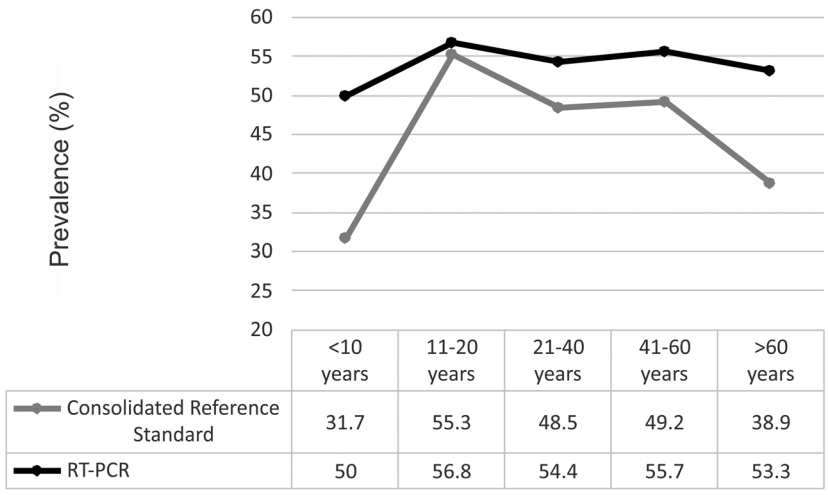

Fig. 3. Prevalence profile of intestinal schistosomiasis in an endemic population divided by age, according to a parasitological consolidated reference standard and to real-time PCR analysis of one fecal samples (500 mg).

(32.7%) were negative in both tests. Interestingly, 24 (12.2%) individuals negative by RT-PCR were reactive in the rapid urine test and were classified as with either trace results or with weak reactivity. In addition, 38 (19.4%) individuals were not reactive by the POC-CCA® test but showed *S. mansoni*-specific DNA amplification in RT-PCR from fecal samples (Table 3). As such, the concordance between the POC-CCA® test and the RT-PCR was considered weak ($\kappa = 0.37$). Further evaluation of the POC-CCA® test indicated low sensitivity (64.8%) when compared to the RT-PCR. The specificity was 72.7%, the likelihood ratio for a positive result was 2.38, and the PPV was 74.5% (Table 3). As shown in Table 2, the lack of concordance between RT-PCR and POC-CCA® was mainly due to the low agreement of the latter diagnostic test in identifying infected individuals with less than 50 EPG. From the 112 infected individuals who were identified by parasitological tests, the POC-CCA® detected 77.8% of the

https://doi.org/10.1017/S003118202000089X Published online by Cambridge University Press

Table 3. Performance of different parasitological and immunochromatographic methods for the detection of intestinal schistosomiasis in comparison with real-time PCR (RT-PCR)

RT- PCR										
	TP	TN	FP	FN	Total	Sens. % (95%CI)	Specif.% (95%CI)	PPV (95%CI)	LR + (95%CI)	Accuracy (95%CI)	Kappa (95%CI)
SPL1 K1-K2	41	92	0	73	206	36.0 (27.8–45.1)	100 (96.0–100)	100 (91.4–100)	–	64.6 (57.8–70.8)	0.33 (0.23–0.44)
SPL1-3 K1-K2	61	66	3	42	172	59.2 (49.6–68.2)	95.7 (88.0–98.5)	55.8 (48.1–63.1)	13.6 (6.9–26.8)	73.8 (66.8–79.8)	0.50 (0.37–0.64)
Helmintex®	71	84	6	33	194	68.3 (58.8–76.4)	93.3(82.2–96.9)	92.2 (84.0–96.4)	10.2 (7.3–14.4)	79.9 (73.3–84.9)	0.60 (0.47–0.74)
POC-CCA®	70	64	24	38	196	64.8 (62.6–73.2)	72.7 (62.6–80.9)	74.5 (64.8–82.2)	2.4 (2.16–2.62)	68.4 (61.6–74.5)	0.37 (0.23–0.51)

TP, true positive; FP, false positive; FN, false negative; TN, true negative.
Data show the Sens. %, Sensitivity; Esp. %, Specificity; PPV, positive predictive value; LR+, Likelihood ratio for positive results, accuracy and kappa statistic of concordance for the Kato-Katz technique obtained with the analysis of one fecal sample using two slides (SPL1 K1-K2) or obtained from two slides prepared from each of three fecal samples (SPL1-3 K1-K2), or Helmintex®, or with POC-CCA® methods. RT-PCR (real-time polymerase chain reaction) was used as reference test.

individuals eliminating 12–49 EPG and only 50.8% of those eliminating less than 12 EPG.

Discussion

In the current study, we demonstrated that RT-PCR of DNA isolated from fecal samples using primer pairs targeted to a 90-base pair sequence inside of the highly repeated 121-base pair sequence of *S. mansoni* showed high sensitivity (91.4%) to identify infection and good concordance ($k = 0.71$) with an accurate parasitological reference standard. In addition, the RT-PCR showed better performance in identifying cases with low (<100 EPG) and very low (<12 EPG) parasite loads than when compared with POC-CCA® (100% *vs* 77.8% and 86.9%; *vs* 50.8%).

Molecular tests have been used as alternative diagnostic methods for the detection of *Schistosoma* species (Pontes *et al.*, 2003; Lier *et al.*, 2006; Allam *et al.*, 2009; Gomes *et al.*, 2009; Lier *et al.*, 2009). In these PCR-based assays, different targets have been used to detect *Schistosoma* DNA, including the ribosomal subunits 18 s rDNA, 28 s rDNA and SSU-rRNA (Gomes *et al.*, 2006; Sandoval *et al.*, 2006), mitochondrial genes (nicotinamide adenine dinucleotide hydrogen – *NADH-I*, *NADH-3* and nternal transcriber-spacer-2 sequence – *ITS2*), and the cytochrome c oxidase -*COX I* (Ten Hove *et al.*, 2008; Lier *et al.*, 2009). Among them, Gordon *et al.* (2015) applied RT-PCR assay, utilizing primers which amplify a fragment of the NADH dehydrogenase I (nad1) mitochondrial gene, to identify *S. japonicum* DNA in feces of 560 individuals living in six small villages in the municipality of Palapag, Philippines. The authors showed that the RT-PCR assay was positive in 90.2% of the study population, while only 22.9% of them showed parasite egg in feces examined by KK method, suggesting that prevalence of *S. japonicum* was much higher in the area. Similarly, Meurs and collaborators (2015), using ITS2-based multiplex RT-PCR, detected 13–15% more positive individuals than with conventional KK stool exams in fecal samples of individuals of endemic areas from Senegal and Kenya. These data indicated that the molecular approach would increase the sensibility of the schistosmiasis diagnosis in areas where the intensity of infection is low and it could currently control efforts to eliminate the disease. In addition, RT-PCR has been suggested as a useful and more sensitive tool for detecting animal hosts and for controlling transmission of zoonotic schistosomiasis (Van Dorssen *et al.*, 2017; He *et al.*, 2018).

Aside from the DNA sequences discussed above, a tandem repeat sequence of 121 base pairs (bp) has also been successfully used in PCR-based approaches for the detection of the parasite in snails and for the monitoring of cercariae in water bodies (Hamburger *et al.*, 1991, 1998*a*, *b*). The amplification of this sequence has been adapted for the diagnosis of *Schistosoma* in human feces and some published data showed high sensitivity and specificity of these molecular approach to identify *S. mansoni* infection (Pontes *et al.*, 2002, 2003; Gomes *et al.*, 2010; Carneiro *et al.*, 2013; Siqueira *et al.*, 2015). The authors argued that given the high number of copies of this repeat region, the technique enables the detection of fractions of a single *S. mansoni*-derived cell, in contrast to the microscope detection, which requires the presence of entire eggs, and regardless of the differences in the number of eggs (EPG) in fecal samples (Pontes *et al.*, 2002).

In agreement with the results using the others DNA-target, studies also showed that -molecular diagnosis using as target the 121-bp tandem repeat DNA sequence of *Schistosoma,* similar target that we used in current study, were more sensible compared to the KK parasitological technique to identify *S. mansoni* infection (Rabello *et al.*, 2002; Pontes *et al.*, 2003; Gomes *et al.*, 2009; Gomes *et al.*, 2010). As an example, Carvalho *et al.* (2012)

https://doi.org/10.1017/S003118202000089X Published online by Cambridge University Press

reported that a conventional PCR system detected 4.8 times more infected individuals than with two KK-slides among individuals from low endemic areas. In addition, other studies which used a RT-PCR to amplify the same DNA-target showed a positivity rate 12 times higher than the conventional KK or spontaneous sedimentation techniques (Espirito-Santo *et al.*, 2014). However, most of the studies testing PCR-based assays as a diagnostic tool for schistosomiasis in individuals with low parasite load compare their data with conventional KK-method. Given the known low sensibility of the conventional KK-method to identify individuals with low-intensity infections, this method should not be used as a reference test to assess the accuracy of a new diagnostic strategy for *Schistosoma* infection. In the current study, we had, for the first time, used an extensive combination of parasitological tests to allow robust evaluation of RT-PCR performance to diagnose *S.mansoni* infection in individuals with low-intensity infection. In addition, we also optimized the RT-PCR reaction by selecting as target a smaller fragment of DNA (90 bp) inside the repeated 121-bp sequence. Our data clearly showed that this RT-PCR technique allowed to identify 100% of infected individuals with more than 12 EPG, that is the lowest parasite load that could be identified by 2 slides of KK, and about 87% of infected individuals eliminating less than 12 EPG, who were identified only by more extensive parasitological analysis obtained through the Consolidated Reference Standard. Therefore, the RT-PCR assay employed in current study showed high sensibility (91.4%) and good accuracy (85.6%) to diagnose active *S. mansoni* infection in low-intensity infected individuals of endemic area. A good performance of PCR-assay using the 121 bp tandem repeat DNA sequence to diagnose *S. mansoni* infection was also reported by Senra and collaborators (2018) who evaluated the performance of a PCR-ELISA system. The authors reported that the PCR-estimated prevalence of schistosomiasis (25.2%) was only slightly higher than that observed by examining 12 KK slides (18.4%), with sensitivity and specificity of 97.4% and 91.1%, respectively.

In the analysis of concordance, nine individuals were identified as positive by the consolidated reference standard but were undetected by the RT-PCR. The disagreement between false-negative individuals by RT-PCR may be associated with the very low parasite load, which may result in the lack of eggs and sufficient genetic material in the small amount of feces (500 mg) analysed by the molecular test. This is in agreement with the parasitological exams since the majority of the infected individuals were identified by tests that used a higher amount of feces (30 grams Helmintex technique®) or a higher number of fecal samples or slides (three fecal samples collected in consecutive days for KK). Former studies of RT-PCR using as target the 121-bp tandem repeat estimated that the assay was able to amplify minimum amounts of egg DNA in fecal samples and estimated that the assay would detect as low as 0.2 (Gomes *et al.*, 2010) or 2.4 EPG (Pontes *et al.*, 2002); however this sensibility is lower than the limitation of Helminthex (1 egg in 30 g feces). Also, a specific RT-PCR developed to target the 28S ribosomal RNA gene was validated for the detection of different *Schistosoma* species in international travelers and migrants and demonstrated high analytical sensitivity and a detection limit of 0.2 EPG (Cnops *et al.*, 2012). Pontes *et al.* (2003) and Gomes *et al.* (2009) obtained false-negative results using conventional PCR and attributed those to the following: (1) the presence of amplification inhibitors in fecal samples; (2) variations in distribution and release of *S. mansoni* eggs in the feces (Engels *et al.*, 1996); and (3) DNA degradation during sample transport from the endemic area to the laboratory (Gomes *et al.*, 2009). However, in our study, we were able to exclude the latter possibility, since our internal control (the human β-actin gene) was amplified.

On the other hand, for the 22 infected individuals identified by RT-PCR and not by the CRS, two hypotheses should be considered. First, a superior sensitivity of the RT-PCR, which allows the identification of individuals with low parasite loads. As discussed above, the previous study estimated that RT-PCR assays were able to identify as low as 0.2 EPG (Gomes *et al.*, 2010; Cnops *et al.*, 2012), a sensibility higher than most of the parasitological test, but not the Helmintex. As such, a study by Allam *et al.* (2009) showed the high sensitivity of the real-time PCR system, which diagnosed 23% of samples as positive among individuals living in a hypoendemic area that were identified as egg negative for *S. mansoni* infection, using the conventional KK technique. In addition, the possibility of cell-free parasite DNA (cfDNA) detection in the RT-PCR assay should be considered. The cfDNA is released from schistosome stages (schistosomula, adult worms and eggs), and could be the result of dead or decaying parasites within the circulation and tissues, active shedding from the parasite or from disintegrating inactive eggs (Cnops *et al.*, 2012; Weerakoon *et al.*, 2016; Weerakoon *et al.*, 2018). cfDNA can be detected in serum, plasma and bio-fluids such as urine, saliva and cerebrospinal fluid (Pontes *et al.*, 2002; Wichmann *et al.*, 2009; Weerakoon *et al.*, 2016, 2017). Moreover, it was shown that cfDNA can be readily detected in active, low-intensity infections and low prevalence schistosomiasis areas, using Droplet Digital PCR (ddPCR) and RT-PCR (Weerakoon *et al.*, 2017, 2018). The detection of cfDNA released from different stages of *Schistosoma* development by the RT-PCR assay would also allow the diagnose of the infection before the egg elimination.

The second hypothesis is that the false-positive results by RT-PCR may be due to cross-reactions with other helminth parasites. However, the high specificity of this technique was demonstrated by the absence of amplification in individuals infected with other helminths. The primers used in the PCR to diagnose schistosomiasis mansoni are genus-specific and were shown to not cross amplify DNA from *Ascaris lumbricoides*, hookworm, *Taenia solium,* or *Trichuris trichiura* (Pontes *et al.*, 2002; Gomes *et al.*, 2010; Senra *et al.*, 2018).

When the performance of POC-CCA® was compared with that of the RT-PCR, the rapid urine test presented lower sensitivity (64.8%), lower specificity (72.7%) and a low agreement was observed between the methods ($\kappa = 0.37$). Our previous study with the same population resulted in a similar poor performance when POC-CCA® was compared with the consolidated reference standard based on the detection of *S. mansoni* eggs by different parasitological methods (Oliveira *et al.*, 2018). The relatively high percentage of results classified as false positive and false negative in the POC-CCA® might be explained by the discontinuous distribution of eggs in the fecal matter, intermittent egg excretion, a small number of female worms or ageing worms (Engels *et al.*, 1996; Berhe *et al.*, 2004; Gryseels *et al.*, 2006) and cross-reactivity with other intestinal helminths or even other clinical conditions that may lead to a false positive POC-CCA® result (van Dam *et al.*, 1994; Colley *et al.*, 2013; Coelho *et al.*, 2016).

This study presents a few limitations. Stool samples for RT-PCR were collected on a single day (first sample). The performance of the RT-PCR test could likely be improved if fecal sample collection would have been performed on 2 or 3 consecutive days, which would have reduced the variation in egg production in infected individuals. We did not perform this in the present study due to the high costs for the test and the considerable number of examined individuals. The choice of a molecular test for diagnosing *Schistosoma* infection in endemic settings will depend on different factors, including the available infrastructure and the cost-effectiveness (Gomes *et al.*, 2006). Nevertheless, here, the molecular technique was tested in a restricted setting and not

https://doi.org/10.1017/S003118202000089X Published online by Cambridge University Press

on a large epidemiological basis. Therefore, evaluation of its applicability, cost-effectiveness and comparisons with other methods in a larger epidemiological setting and with increased numbers of tested individuals are still in need.

Current strategies for controlling schistosomiasis in Brazil are based on the diagnosis and treatment of positive individuals. Since the goals for schistosomiasis control have shifted from morbidity control to transmission control and eradication (Bergquist *et al.*, 2017), it is of extreme importance to identify individuals with reduced parasite loads, who would continue undiagnosed if only the commonly applied parasitological methods were used and would, therefore, easily contribute to the maintenance of the disease in endemic areas. In conclusion, the RT-PCR methodology presented herein showed high sensitivity to identify active *S. mansoni* infection in individuals with very low parasite loads (<12 EPG) and resulted in a good concordance with a Consolidated Reference Standard, which consisted of different and very extensive parasitological exams.

Acknowledgements. The authors would like to thank the people from the communities of Pé da Serra, Tocantins, and Santana for their collaboration and the warm reception during the field activities. They are also thankful to the municipal government of Januária for the logistic support during the field studies and to the technicians from the Schistosomiasis Control Program. They also thank Dra. Liliane Maria Vidal Siqueira (Fiocruz) for the training and supervision provided during the molecular analyses.

Author Contributions. MC, PMZC, SMG, DN-C, CG-T and MJE conceptualized the study. FM, SR, SMG and CG-T collected the sample. EO, FM, SR and CS performed experiments. SR, FM, EO, MC, FCM, DN-C and MC analysed the data. Resources and project administration were supervised by SMG, DN-C, PMZC and CG-T. Elaboration of the manuscript was done by FCM, DN-C, SMG and MC.

Financial Support. The authors received financial support from the National Brazilian Research Council (CNPq) for research in neglected tropical diseases, DECIT program 2012 #404405/2012-6. DN-C received financial support from Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), within the Edital of PP-SUS and grant #APQ 01637-17. FCM thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brazil), Programa de Pós-Graduação em Parasitologia, for PhD-scholarship. MC would like to thank the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG – Brazil) for the grant within the Pesquisador Mineiro program (PPM-2016) and CNPq for her research fellowship.

Conflict of interest. None to declare

Ethical standards. This study was approved by the Ethics Committees of the René Rachou Institute (FIOCRUZ) and the Federal University of Minas Gerais and is registered at the Brazilian Platform for Research with Human Subjects (Plataforma Brasil) under the following number: CAAE#21824513.9.0000.5091. Informed consent form was read and signed by the participants and by the parents or legal guardians of minors before enrollment. Literate children were also asked to read and sign an adapted informed consent form.

References

Allam AF, Kader O, Zaki A, Shehab AY and Farag HF (2009) Assessing the marginal error in diagnosis and cure of *Schistosoma mansoni* in areas of low endemicity using Percoll and PCR techniques. *Tropical Medicine and International Health* **14**, 316–321.

Amaral RS, Tauil PL, Lima DD and Engels D (2006) An analysis of the impact of the Schistosomiasis Control Programme in Brazil. *Memórias do Instituto Oswaldo Cruz* **101**, 79–85.

Bergquist R, Johansen MV and Utzinger J (2009) Diagnostic dilemmas in helminthology: what tools to use and when? *Trends in Parasitology* **25**, 151–156.

Bergquist R, Zhou XN, Rollinson D, Reinhard-Rupp J and Klohe K (2017) Elimination of schistosomiasis: the tools required. *Infectious Diseases of Poverty* **6**, 158.

Berhe N, Medhin G, Erko B, Smith T, Gedamu S, Bereded D, Moore R, Habte E, Redda A, Gebre-Michael T and Gundersen SG (2004) Variations in helminth faecal egg counts in Kato–Katz thick smears and their implications in assessing infection status with *Schistosoma mansoni*. *Acta Tropica* **92**, 205–212.

Carneiro TR, Saramago Peralta RH, Cunha Pinheiro MC, de Oliveira SM, Peralta JM and Bezerra FS (2013) A conventional polymerase chain reaction-based method for the diagnosis of human schistosomiasis in stool samples from individuals in a low-endemicity area. *Memórias do Instituto Oswaldo Cruz* **108**, 1037–1044.

Carvalho GCD, Marques LHDS, Gomes LI, Rabello A, Ribeiro LC, Scopel KKG, Tibirica SHC, Coimbra ES and Abramo C (2012) Polymerase chain reaction for the evaluation of *Schistosoma mansoni* infection in two low endemicity areas of Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz* **107**, 899–902.

Cnops L, Tannich E, Polman K, Clerinx J and Esbroeck MV (2012) Schistosoma real-time PCR as diagnostic tool for international travellers and migrants. *Tropical Medicine and International Health* **17**, 1208–1216.

Coelho PM, Jurberg AD, Oliveira AA and Katz N (2009) Use of a saline gradient for the diagnosis of schistosomiasis. *Memórias do Instituto Oswaldo Cruz* **104**, 720–723.

Coelho PM, Siqueira LM, Grenfell RF, Almeida NB, Katz N, Almeida Á, Carneiro, NF and Oliveira, E (2016) Improvement of POC-CCA interpretation by using lyophilization of urine from patients with *Schistosoma mansoni* low worm burden: towards an elimination of doubts about the concept of trace. *PLoS Neglected Tropical Diseases* **10**, e0004778.

Colley DG, Binder S and Campell C (2013) A five-country evaluation of a point-of-care circulating cathodic antigen urine assay for the prevalence of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **88**, 426–432.

Colley DG, Bustinduy AL, Secor WE and King CH (2014) Human schistosomiasis. *The Lancet* **383**, 2253–2264.

Dean AG, Sullivan KM and Soe MM (2013) OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 3.01. Website Available at http://www.OpenEpi.com (accessed 29 july 2019).

Engels D, Sinzinkayo E and Gryseels B (1996) Day-To-Day Egg count fluctuation in *Schistosoma mansoni* infection and its operational implications. *American Journal of Tropical Medicine and Hygiene* **54**, 319–324.

Enk MJ, Lima AC, Drummond SC, Schall VT and Coelho PMZ (2008) The effect of the number of stool samples on the observed prevalence and the infection intensity with *Schistosoma mansoni* among a population in an area of low transmission. *Acta Tropica* **108**, 222–228.

Enk MJ, Oliveira e Silva G and Rodrigues NB (2012) Diagnostic accuracy and applicability of a PCR system for the detection of *Schistosoma mansoni* DNA in human urine samples from an endemic area. *PLoS ONE* **7**, e38947.

Espirito-Santo MCC, Alvarado-Mora MV, Dias-Neto E, Botelho-Lima LS, Moreira JP, Amorim M, Pinto PL, Heath AR, Castilho VL, Goncalves EM, Luna EJ, Carrilho FJ, Pinho JR and Gryschek RC (2014) Evaluation of real-time PCR assay to detect *Schistosoma mansoni* infections in a low endemic setting. *BMC Infectious Diseases* **14**, 558.

Ferrari MLA, Coelho PMZ, Antunes CMF, Tavares CA and Da Cunha AS (2003) Efficacy of oxamniquine and praziquantel in the treatment of *Schistosoma mansoni* Infection: a controlled trial. *Bulletin of the World Health Organization* **81**, 190–196.

GBD (2017) Causes of death collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the global burden of disease study 2016. *Lancet (London, England)* **390**, 1151–1210.

Gomes AL, Melo FL, Werkhauser RP and Abath FG (2006) Development of a real time polymerase chain reaction for quantitation of *Schistosoma mansoni* DNA. *Memórias do Instituto Oswaldo Cruz* **101**, 133–136.

Gomes LI, Marques LH, Enk MJ, Coelho PM and Rabello A (2009) Further evaluation of an updated PCR assay for the detection of *Schistosoma mansoni* DNA in human fecal samples. *Memórias do Instituto Oswaldo Cruz* **104**, 1194–1196.

Gomes LI, Dos Santos Marques LH, Enk MJ, de Oliveira MC, Coelho PM and Rabello A (2010) Development and evaluation of a sensitive PCR-ELISA system for detection of schistosoma infection in feces. *PLoS Neglected Tropical Diseases* **4**, e664.

Gordon CA, Acosta LP, Gobert GN, Olveda RM, Ross AG, Williams GM, Gray DJ, Harn D, Li Y and McManus DP (2015) Real-time PCR demonstrates high prevalence of *Schistosoma japonicum* in the Philippines:

implications for elimination programs. *PLoS Neglected Tropical Diseases* **9**, e0003483.

Gryseels B, Polman K, Clerinx J and Kestens L (2006) Human schistosomiasis. *Lancet (London, England)* **368**, 1106–1118.

Hamburger J, Turetski T, Kapeller I and Deresiewicz R (1991) Highly repeated short DNA sequences in the genome of *Schistosoma mansoni* recognized by a species-specific probe. *Molecular and Biochemical Parasitology* **44**, 73–80.

Hamburger J, Xu YX, Ramzy RM, Jourdane J and Ruppel A (1998*a*) Development and laboratory evaluation of a polymerase chain reaction for monitoring *Schistosoma mansoni* infestation of water. *American Journal of Tropical Medicine and Hygiene* **59**, 468–470.

Hamburger J, Xu YX, Ramzy RM, Jourdane J and Ruppel A (1998*b*) A polymerase chain reaction assay for detecting snails infected with bilharzia parasites (*Schistosoma mansoni*) from very early prepatency. *American Journal of Tropical Medicine and Hygiene* **59**, 872–876.

He P, Gordon CA, Williams GM, Li Y, Wang Y, Hu J, Gray DJ, Ross AG, Harn D and McManus DP (2018) Real-time PCR diagnosis of *Schistosoma japonicum* in low transmission areas of China. *Infectious Diseases of Poverty* **7**, 8.

Katz N (2018) *Inquérito Nacional de Prevalência da Esquistossomose mansoni e Geo-Helmintoses*, 22nd Edn. Belo Horizonte, BR: Editora Fiocruz.

Kongs A, Marks G, Verlé P and Van Der Stuyft P (2001) The unreliability of the Kato-Katz technique limits its usefulness for evaluating *S. mansoni* Infections. *Tropical Medicine and International Health* **6**, 163–169.

Landis JR and Koch GG (1977) The measurement of observer agreement for categorical data. *Biometrics* **33**, 159. http://dx.doi.org/10.2307/2529310

Lier T, Simonsen GS, Haaheim H, Hjelmevoll SO, Vennervald BJ and Johansen MV (2006) Novel real-time PCR for detection of *Schistosoma japonicum* in stool. *Southeast Asian Journal of Tropical Medicine and Public Health* **37**, 257–264.

Lier T, Simonsen GS, Wang T, Lu D, Haukland HH, Vennervald BJ, Hegstad J and Johansen MV (2009) Real-time polymerase chain reaction for detection of low-intensity *Schistosoma japonicum* infections in China. *American Journal of Tropical Medicine and Hygiene* **81**, 428–432.

Meurs L, Brienen E, Mbow M, Ochola EA, Mboup S, Karanja DMS, Secor WE, Polman K and van Lieshout L (2015) Is PCR the next reference standard for the diagnosis of schistosoma in stool? A comparison with microscopy in Senegal and Kenya. *PLoS Neglected Tropical Diseases* **9**, e0003959.

Oliveira LM, Santos HL, Gonçalves MM, Barreto MG and Peralta JM (2010) Evaluation of polymerase chain reaction as an additional tool for the diagnosis of low-intensity *Schistosoma mansoni* infection. *Diagnostic Microbiology and Infectious Diseases* **68**, 416–421.

Oliveira WJ, Magalhães FDC, Elias AMS, de Castro VN, Favero V, Lindholz CG, Oliveira AA, Barbosa FS, Gil F, Gomes MA, Teixeira CG, Enk MJ, Carneiro M, Negrão-Correa D and Geiger SM (2018) Evaluation of diagnostic methods for the detection of intestinal schistosomiasis in endemic areas with low parasite loads: saline gradient, Helmintex, Kato-Katz and rapid urine test. *PLoS Neglected Tropical Diseases* **12**, e0006232. doi: 10.1371/journal.pntd.0006232

Pontes LA, Dias-Neto E and Rabello A (2002) Detection by polymerase chain reaction of *Schistosoma mansoni* DNA in human serum and feces. *American Journal of Tropical Medicine and Hygiene* **66**, 157–162.

Pontes LA, Oliveira MC, Katz N, Dias-Neto E and Rabello A (2003) Comparison of a polymerase chain reaction and the Kato-Katz technique for diagnosing infection with *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **68**, 652–656.

Rabello A, Pontes LA and Dias-Neto E (2002) Recent advances in the diagnosis of *Schistosoma* Infection: the detection of parasite DNA. *Memórias do Instituto Oswaldo Cruz* **97**(Suppl.I), 71–172.

Sandoval N, Siles-Lucas M, Perez-Arellano JL, Carranza C, Puente S, Lopez-Aban J and Muro A (2006) A new PCR-based approach for the specific amplification of DNA from different Schistosoma species applicable to human urine samples. *Parasitology* **133**, 581–587.

Senra C, Gomes LI, Siqueira LMV, Coelho PMZ, Rabello A and Oliveira E (2018) Development of a laboratorial platform for diagnosis of schistosomiasis mansoni by PCR-ELISA. *BMC Research Notes* **11**, 455.

Siqueira LMV, Gomes LI, Oliveira E, de Oliveira ER, de Oliveira AA, Enk MJ, Carneiro NF, Rabello A and Coelho PMZ (2015) Evaluation of parasitological and molecular techniques for the diagnosis and assessment of cure of schistosomiasis mansoni in a low transmission área. *Memórias do Instituto Oswaldo Cruz* **110**, 209–214.

Siqueira LM, Couto FF, Taboada D, Oliveira ÁA, Carneiro NF, Oliveira E, Coelho PMZ and Katz N (2016) Performance of POC-CCA in diagnosis of schistosomiasis mansoni in individuals with low parasite burden. *Journal of the Brazilian Society of Tropical Medicine* **49**, 341–347.

Standley CJ, Lwambo NJS, Lange CN, Kariuki HC, Adriko M and Stothard JR (2010) Performance of circulating cathodic antigen (CCA) urine-dipsticks for rapid detection of intestinal schistosomiasis in schoolchildren from shoreline communities of Lake Victoria. *Parasites and Vectors* **3**, 7. doi: 10.1186/1756-3305-3-7.

Steinmann P, Keiser J, Bos R, Tanner M and Utzinger J (2006) Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infectious Diseases* **6**, 411–425.

Stothard JR, Sousa-Figueiredo JC, Standley C, Van Dam GJ, Knopp S, Utzinger J, Ameri H, Khamis AN, Khamis IS, Deelder AM, Mohammed KA and Rollinson D (2009) An evaluation of urine-CCA strip test and finger prick blood SEA-ELISA for detection of urinary schistosomiasis in schoolchildren in Zanzibar. *Acta Tropica* **111**, 64–70.

ten Hove RJ, Verweij JJ, Vereecken K, Polman K, Dieye L and van Lieshout L (2008) Multiplex real-time PCR for the detection and quantification of *Schistosoma mansoni* and *S. haematobium* Infection in fecal samples collected in northern Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102**, 179–185.

Utzinger J, N'Goran EK, N'Dri A, Lengeler C and Tanner M (2000) Efficacy of praziquantel against *Schistosoma mansoni* with particular consideration for intensity of infection. *Tropical Medicine and International Health* **5**, 771–778.

Utzinger J, Booth M, N'goran EK, Muller I, Tanner M and Lengeler C (2001) Relative contribution of day-to-day and intra-specimen variation in faecal egg counts of *Schistosoma mansoni* Before and after treatment with praziquantel. *Parasitology* **122**, 537–544.

Van Dam GJ, Bergwerff AA, Thomas-Oates JE, Rotmano JP, Kamerling JP, Vliegenthard JF and Deelder AM (1994) The immunologically reactive O-linked polysaccharide chains derived from circulating cathodic antigen isolated from the human blood fluke *Schistosoma mansoni* have Lewis x as repeating unit. *European Journal Biochemistry* **225**, 467–482.

Van Dorssen CF, Gordon CA, Li Y, Williams GM, Wang Y, Luo Z, Gobert GN, You H, McManus DP and Gray DJ (2017) Rodents, goats, and dogs – their potential role in ongoing transmission of schistosomiasis in China. *Parasitology* **144**, 1633–1642.

Weerakoon KG, Gordon CA, Gobert GN, Cai P and McManus DP (2016) Optimisation of a droplet digital PCR assay for the diagnosis of *Schistosoma japonicum* infection: a duplex approach with DNA binding dye chemistry. *Journal of Microbiological Methods* **125**, 19–27.

Weerakoon KG, Gordon CA, Williams GM, Cai P, Gobert GN, Olveda RM, Ross AG, Olveda DU and McManus DP (2017) Droplet digital PCR diagnosis of human schistosomiasis: parasite cell-free DNA detection in diverse clinical samples. *Journal Infectious Diseases* **216**, 1611–1622.

Weerakoon KG, Gordo CA and McManus DP (2018) DNA Diagnostics for Schistosomiasis control. *Tropical Medicine and Infectious Disease* **3**, 81.

Wichmann D, Panning M, Quack T, Kramme S, Burchard GD, Grevelding C and Drosten C (2009) Diagnosing schistosomiasis by detection of cell-free parasite DNA in human plasma. *PLoS Neglected Tropical Diseases* **3**, e422.

World Health Organization (1993) *The Control of Schistosomiasis Second Report of the WHO Expert Committee. WHO Technical Report Series No. 830*. Geneva, Switzerland: World Health Organization.

World Health Organization (2002) *Prevention and Control of Schistosomiasis and Soil-Transmitted Helminthiasis WHO Technical Report Series No. 912*. Geneva, Switzerland: World Health Organization.

World Health Organization (2016) *World Health Statistics 2016: Monitoring Health for the SDGs*. World Health Organization. Retrieved from Global Health Observatory (GHO) data. Geneva, Switzerland: World Health Organization. website https://www.who.int/gho/publications/world_health_statistics/2016/en/ (accessed 20 may 2018).

https://doi.org/10.1017/S003118202000089X Published online by Cambridge University Press