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Diagnostic performance of parasitological, immunological and molecular tests for the diagnosis of *Schistosoma mansoni* infection in a community of low transmission in Venezuela

Elizabeth Ferrer[a], Berenice Villegas[a], Lapo Mughini-Gras[b,c], Diego Hernández[a], Verónica Jiménez[a], Emily Catalano[a], Renzo Nino Incani[d,*]

[a] *Instituto de Investigaciones Biomédicas "Dr. Francisco J. Triana Alonso" (BIOMED) and Departamento de Parasitología, Facultad de Ciencias de la Salud, Universidad de Carabobo, Sede Aragua, Maracay, Venezuela*
[b] *Center for Infectious Diseases Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands*
[c] *Utrecht University, Faculty of Veterinary Medicine, Institute for Risk Assessment Sciences (IRAS), Utrecht, the Netherlands*
[d] *Laboratorio de Investigaciones en Bilharzia, Departamento de Parasitología, Facultad de Ciencias de la Salud, Universidad de Carabobo, Carabobo, Sede Valencia, Venezuela*

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ABSTRACT

In Venezuela, areas endemic for schistosomiasis are of low transmission, with low parasite loads. Immunological tests often lack specificity and cannot differentiate past from present infections. Molecular tests are an alternative, although validation studies in endemic areas are needed. The aim of this study was to determine the performance of parasitological, immunological and molecular tests for the diagnosis of *Schistosoma mansoni* infection in low-transmission settings. A cross-sectional study was carried out in a rural community located in a schistosomiasis-endemic area of Venezuela to determine the prevalence and diagnostic performance of the Kato-Katz (KK) technique, Circumoval Precipitin Test (COPT), ELISA based on soluble egg antigen (ELISA-SEA) with and without treatment with sodium metaperiodate (ELISA-SEA-SMP), and PCR for amplification of the 121 bp highly repeated sequence of *Schistosoma mansoni* in faeces, urine and serum samples. The highest prevalence rates were obtained with ELISA-SEA (38.7%), COPT (33.3%), ELISA-SEA-SMP (31.5%), PCR on faeces (21.6%), and KK (17.1%), whereas PCR-based prevalence in urine was 6.2% and no positivity was detected in serum samples. Results showed that ELISA-SEA is the best method for the diagnosis of both current and former infections and that PCR on faeces is the best method for detecting recent transmission. The use of different tests that complement one another also allowed for a better diagnosis of *Schistosoma mansoni* infection, revealing a relatively high prevalence (33.8%) of schistosomiasis in a community of low transmission.

1. Introduction

Schistosomiasis is the second most important parasitic disease worldwide after malaria, affecting about 252 million people in at least 78 countries in Africa, Asia and America (Hotez et al., 2014). Moreover, schistosomiasis is a major cause of hepatointestinal and urinary morbidity, accounting for 2.5 million Disability-Adjusted Life Years (DALYs) in 2016 (WHO, 2018). In most endemic countries in the Americas, *Schistosoma mansoni* transmission is in the process of pre-elimination or already awaiting for certification of elimination.

In Venezuela, *S. mansoni* transmission has been drastically reduced to negligible prevalence rates, but there is a lack of evidence to initiate the process leading to the confirmation of elimination. To this end, highly sensitive and specific laboratory diagnosis of schistosomiasis is needed. While coprology is rather inefficient, the lateral flow kit for antigen detection in urine or the Point-Of-Care Circulating Cathodic Antigen (POC−CCA) test has been in use for some years, proving its efficacy in endemic sub-Saharan countries with moderate to high intensity of infection (Coulibaly et al., 2011; Shane et al., 2011; Tchuem Tchuenté et al., 2012). However, this test has the major drawback of having limited sensitivity in conditions of low parasite loads (van Lieshout et al., 2000; Stothard et al., 2006; van Dam et al., 2004; Legesse and Erko, 2007). There is also the option of antibody detection for the diagnosis of schistosomiasis in low or very low endemic areas (LEA or VLEA), particularly in settings where no intervention has been done for more than 10 years or where transmission

* Corresponding author.
E-mail address: rincani@uc.edu.ve (R.N. Incani).

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has been recently detected. However, due to the limitations of immunological methods to differentiate active from past infections in intervention areas, highly sensitive DNA probes have been proposed for the diagnosis of schistosomiasis (Utzinger et al., 2015).

Previously we adapted the protocol of Hamburger et al. (1998a, 1998b) used by others (Pontes et al., 2002, 2003; Abreu et al., 2010) for the 121 bp highly repeated sequence of *Schistosoma* and tested its efficiency in detecting DNA from isolated eggs of *S. mansoni* using PCR. The PCR detected parasite DNA in the range of attograms, and diagnosis of active infection was achieved in two cases from an old focus of schistosomiasis (Ferrer et al., 2015). In the present study, we compared the diagnostic performance of the PCR based on the 121 bp highly repeated sequence of *S. mansoni* on faeces, urine and serum samples *versus* coprology and serology (ELISA and Circumoval Precipitin Test or COPT) in a relatively undisturbed rural community in Venezuela characterized by low transmission of *S. mansoni*.

2. Material and methods

2.1. Study population

A cross-sectional study was conducted in a rural community called "Los Toros" in the Manuare Valley, which is located in the central-north region of Venezuela. This community is part of the municipal sector "Parroquia Belen" (9°59´18´´N; 67°41´10´´W) at about 50 km southeast from the city of Valencia. The whole community (122 people) was invited to participate in this study. In order for a participant to be included in the study, a sample of faeces, urine and blood was requested, which resulted in 112 people enrolled in the study (92% participation). The only known potential exposure leading to *S. mansoni* infection amongst the participants was direct contact with the water of the Manuare Valley river, mostly during barefoot crossings to the main road (on the other side of the river), which is the only mean of land connection for the community. There are indeed no functional bridges to cross the river at the community. The main source of income amongst the participants comes from working outside the community as manual workers. Springs and small mountain creeks provide water for drinking and domestic use.

2.2. Sampling

Stool specimens were collected in *ad hoc* containers and split in three parts: one to be examined using the Kato-Katz (KK) technique (50 mg kit) (Katz et al., 1972), one to be fixed in MIF (Merthiolate, Iodine, Formalin) (Sapero and Lowless, 1953) for diagnosis of integral ova and cysts, and one to be fixed in ethanol (70%) for DNA extraction. Urine and serum samples were collected at the same moment and stored at −20 °C pending analysis. From the 122 people enrolled, sera could be obtained from 112 people, faecal samples for coprology examination from 76 people and for PCR from 96 people, and urines for PCR from 96 people. Not all participants provided the amount of faeces required for both PCR and coprology.

2.3. Microscopy

Two KK slides per sample per person were examined within 1 h from processing. Slides were kept covered in the refrigerator while waiting to be examined. Faecal samples were examined the following day of collection. Two KK slides were examined per person. For the MIF samples (2 slides/faecal sample) with 100 µl of sample were covered with a 24 × 50 mm coverslip and sealed with melted paraffin before examination. The whole slide was examined for eggs and larvae of helminths, and cysts/trophozoites of protozoan parasites.

2.4. Serology

2.4.1. Circumoval precipitin test (COPT)

COPT was carried out as described by Oliver-Gonzalez (1954). The COPT was considered positive when the proportion of positive eggs (with precipitates in their surfaces) was >15%, weakly positive when it was between 10 and 15% (also considered positive in this paper), and negative when <10%.

2.4.2. ELISA-Soluble-egg-antigen (SEA)

The preparation of SEA of *S. mansoni* was done according to Noya et al. (1995). Protease inhibitors included TLCK (Tosyl-L-Lysine-Chloromethyl Ketone) 10 mg/mL in distilled water, TPCK (Tosyl-L-Phenylalanine-Chloromethyl Ketone) 10 mg/mL in Isopropanol and PMSF (Phenyl Methyl Sulfonyl Fluoride) 35 mg/mL in Isopropanol. Eggs were homogenised manually in a glass tissue grinder and the resulting material was centrifuged at 900 g, 8 °C for 30 min. After adjusting the protein concentration, the supernatant was aliquoted and stored at −20 °C until use. The conditions established for the ELISA were as follows: 96-well polystyrene microplates, optimal concentration of protein used 6 µg/mL, optimum dilution of serum 1:100, the conjugate was an anti-IgG-peroxidase diluted 1:10.000 (Pierce®) ABTS as the substrate and the plates read at 405 nm. ELISAs were also prepared by pre-treating the SEA with sodium-metha-periodate, a treatment that has been reported to reduce cross-reacting sugar epitopes (Noya et al., 1995; Alarcon de Noya et al., 2000). This ELISA will be called hereafter as ELISA-SEA-SMP.

2.5. DNA extraction

The DNA extraction was performed according to Incani et al. (2017), with some modifications. In brief, 300 µL of ethanol preserved faeces suspension (or parasite ova suspension) was centrifuged for 1 min (5600 g) and the pellet washed with 1 mL PBS. After centrifugation, the pellet was re-suspended into 200 µL of freshly prepared lysis buffer (2% polyvinylpolypyrrolidone, 10% SDS, 0.5 M EDTA) and heated for 10 min at 100 °C. After proteinase K (Roche, Switzerland) treatment (2 h at 55 °C), DNA was extracted using the salt precipitation technique. After centrifugation (10,000 g), the supernatant was transferred to a new tube to precipitate the DNA by adding 2 vol of cold 95% ethanol and one tenth volume of 3 M sodium acetate at pH 7. This was mixed gently and incubated overnight at −80 °C. After precipitation, it was centrifuged at 10,000 g for 30 min, and the ethanol was carefully removed, and then the precipitate was washed by adding 1 mL of cold 70% ethanol. Finally, it was centrifuged at 10,000 g for 15 min, and the ethanol was carefully removed, to allow the tube to dry and to re-suspend the DNA in 50 µL of sterile distilled water. The samples of extracted DNA were kept at −20 °C until use. DNA extraction from serum and urine samples was performed using Chelex 100® resin (BioRad) following manufacturer's instructions. Briefly, 800 µL of sterile distilled water was added to 200 µL of serum or urine and incubated at room temperature for 30 min. Subsequently, 200 µL of Chelex® 100 at 5% was added and incubated 30 min at 56 °C, mixed and incubated for 10 min at 100 °C. Then, it was centrifuged for 5 min at 10,000 g to separate the resin and the denatured proteins and the supernatant containing the DNA was taken, which was stored at −20 °C until use.

2.6. PCR for amplification of the 121 bp highly repetitive sequence of S. mansoni

The amplification reactions was carried out according to protocol described by Pontes et al. (2002) and modified by Ferrer et al. (2015), using the forward primer 5′-GATCTGAATCCGACCAACCG-3′ and reverse primer 5′-ATATTAACGCCCACGCTCTC-3′. The conditions for the PCR were; 2.5 mM $MgCl_2$, 150 mM dNTPs, 0.4 µM of each primer,

0.75 U of GoTaq® Flexi DNA Polymerase (Promega, Madison, USA), 5 μL (5 ng, approximately) of DNA template and water untill a 25 μL total volume. The amplification program used was; initial denaturation 94 °C × 5 min, 35 cycles (denaturation 94 °C × 60 s, hybridization 63 °C × 60 s, extension 72 °C × 60 s) and final extension 72 °C × 5 min, using a BIORAD Cycler (Bio-Rad Laboratories, Philadelphia, USA). Positive (DNA extracted from parasite eggs obtained from experimentally infected hamsters) and negative controls (reaction mix without DNA) were included for each reaction. The PCR products were analysed by electrophoresis on a 2% agarose gels, visualized by ethidium bromide staining and compared with molecular size markers.

Validation of the PCR protocol was carried out using samples (serum, urine and faeces) of individuals with schistosomiasis, with other parasitic infections and individuals apparently uninfected. *S. mansoni* DNA was subsequently detected by PCR in serum, urine and faecal samples. PCR products were visualized in 2% agarose gels.

2.7. Statistical analysis

We first calculated *S. mansoni* prevalence as determined by each diagnostic test and stratified it by gender (male or female) and age group (≤17 years or >18 years, according to median split). Differences in prevalence over gender and age groups were tested for statistical significance using multivariable logistic regression models, including the infection status (infected or non-infected) derived from each test as binary dependant variable, and gender and age group as independent variables, adjusting for clustering (*i.e.* non-independence) of individuals living in the same households using cluster-robust standard errors. A separate model for each diagnostic test was used. Associations were expressed as Odds Ratios (OR) and corresponding 95% Confidence Intervals (95%CI).

We used the outcome of the COPT as the 'gold' standard for serology, and the combined outcomes of KK and PCR (on serum, urine, and/or faeces) as diagnostic 'gold' standard for parasite detection. For this latter composite standard, a patient to be positive had to have all these four tests performed and to have tested positive to at least one of them, whereas to be negative, a patient needed to have all these four tests performed and to have tested negative to all of them. It follows, therefore, that for parasite detection, we assumed 100% specificity, and hence the complete absence of false-positive results for KK and PCR on the basis of unambiguously identifiable *S. mansoni* eggs under a microscope by experienced technicians and unambiguously detectable *S. mansoni* DNA by PCR. The same approach has been used by others (Utzinger et al., 2008; Knopp et al., 2011; Periago et al., 2015). The sensitivity (proportion of true-positives detected) was then calculated for KK and PCR in relation to this composite 'gold' standard, whereas the sensitivity and specificity for the ELISAs were calculated in relation to the COPT as 'gold' standard. Using these standards as reference, we also calculated the Negative Predictive Value (NPV), Positive Predictive Value (PPV), and the Receiver Operating Characteristic (ROC) curve. The agreement between test results was assessed using Cohen's kappa (κ) statistic and percentage agreement. The following cut-offs were used to calculate the degree of agreement: κ < 0, poor agreement; κ between 0 and 0.20, slight agreement; κ between 0.21 and 0.40, fair agreement; κ between 0.41 and 0.60, moderate agreement; κ between 0.61 and 0.80, substantial agreement; and, κ between 0.81 and 1.0, almost perfect agreement (Landis and Koch, 1977). All these test performance statistics were also provided by gender and age group. Statistical analysis was performed using STATA 15.1 (StataCorp, College Station, Texas, USA).

3. Results

3.1. Prevalence

Prevalence rates varied according to the method used (Fig. 1). The highest prevalence was obtained with the ELISA-SEA (38.7%, 95%CI 28.9–49.6%), followed by COPT (33.0%, 95%CI 22.1–46.2%) and ELISA-SEA-MPI (31.5%, 95%CI 22.7–41.9%). For pathogen detection, coprology (KK) produced a prevalence of 17.1% (95%CI 7.9–33.1%) (<50 eggs/gr faeces), while the prevalence obtained with PCR on faeces was higher (21.6%, 95%CI 10.5–39.4%) and the one based on PCR on urine was lower (6.2%, 95%CI 2.2–16.2%), with PCR on serum detecting no positivity. The composite gold standard for parasite detection in which at least one method detecting parasite eggs or parasite DNA was positive in any of the 4 samples (PCR on faeces, urine and serum, and coprology) resulted in a prevalence of 33.8% (95% CI 19.7–51.5%) (Fig. 1).

Age was divided into children (≤ 17 years) and adults (> 18 years). All three serological tests showed significantly higher prevalence rates in adults as compared to children, whereas coprology (KK) did not show significantly different prevalence rates between age groups (Table 1). PCR in faeces, however, did show significant differences, with a higher prevalence in children (Table 1). There were no significant differences in prevalence between males and females (Table 2).

3.2. Test performance

The ELISAs were compared to COPT to assess their performance, while KK and PCR were compared to the composite 'gold' standard for parasite detection. ELISA-SEA-SMP performed slightly better in terms of specificity than ELISA-SEA, but otherwise both tests performed similarly. PCR on faeces, on the other hand, showed the best performance, with a sensitivity of 69.6%, 89.7% agreement, 86.5% NPV, a kappa index of 0.75 and a ROC of 0.85 (Table 3). Regarding age, there were significant differences in the performance of serological tests, being this better in adults where the prevalence was also higher, whereas for the PCR in faeces, performance was better in children where the prevalence was also higher (Table 4).

3.3. Agreement between tests

The best agreements of tests was found between coprology (KK) and COPT ($p < 0.05$), ELISA-SEA and ELISA-SEA-SMP ($p < 0.001$), and between PCR in faeces and KK ($p < 0.01$) (Table 5).

4. Discussion

This study revealed a relatively high prevalence (33.8%) of schistosomiasis in a community of low transmission in Venezuela as determined by different immunological, parasitological and molecular tests. Improvements in diagnostic accuracy by combining different tests has been shown in several works (Weerakoon et al., 2015; Ogongo et al., 2018). The inadequacy of current diagnostics for the detection of low parasite loads in humans (when one or a few diagnostic tests are used) means that schistosomiasis is likely to be even more widespread than thought (Ogongo et al., 2018).

A difficulty in determining the sensitivity and specificity of molecular-based diagnostic techniques is the lack of an universally agreed-upon gold standard against which to compare these techniques, given the generally low sensitivity of stool-based microscopic techniques that are commonly used, particularly for low-intensity infections. Some studies have attempted to deal with the lack of an official gold standard by considering the true positives to be a combination of the positives found at microscopy and/or PCR (Nicolay et al., 2014; Werakoon et al., 2015; Ogongo et al., 2018), as performed in the present study as well.

In this work the prevalence based on PCR faeces was higher than the one based on KK. This is because PCR can detect DNA traces of broken eggs, whereas KK only detects intact eggs. The composite gold standard for parasite detection showed substantial recent (active) transmission in the area. On the other hand, we found that the prevalence measured by our standardized ELISA is close to the COPT one. Difficulties in

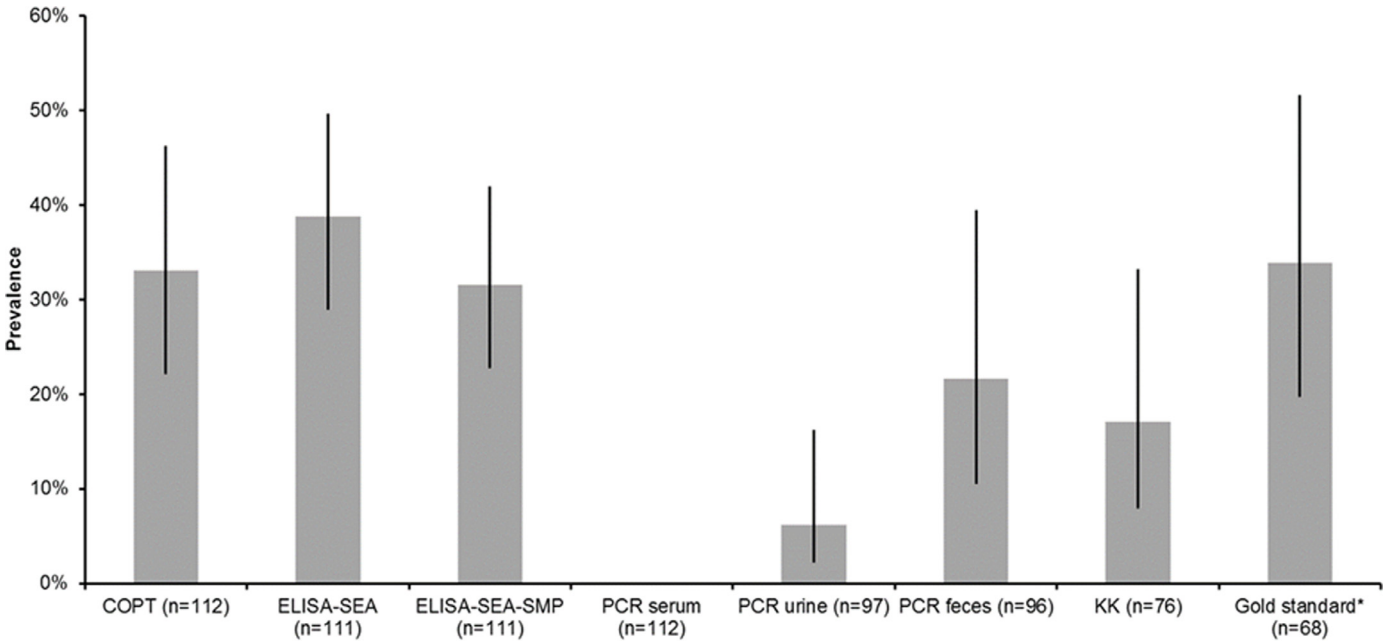

Fig. 1. Prevalence of schistosomiasis in Los Toros community, Carabobo State, Venezuela, according to the different diagnostic tests. Prevalence is adjusted for clustering of individuals at the household level. Error bars represent 95% confidence intervals. COPT = Circumoval Precipitin Test, KK = Kato-Katz technique, ELISA-SEA = ELISA based on soluble egg antigen, ELISA-SEA-SMP = ELISA based on soluble egg antigen with treatment with sodium metaperiodate. * Composite 'gold' standard = PCR serum + PCR urine + PCR feces + KK. To be positive, a participant had to have all these 4 tests performed and to have tested positive to at least 1 of them. To be negative, a patient needed to have all these 4 tests performed and have tested negative to all of them.

Table 1
Prevalence of schistosomiasis in Los Toros community, Carlos Arvelo Municipality, Carabobo State, Venezuela, according to age group and diagnostic test.

	Age ≤ 17 years			Age > 17 years			OR[1,*]	95%CI	P-value
	N	Prevalence[1]	95% CI	N	Prevalence[1]	95% CI			
COPT	56	21.7%	9.5–33.9%	56	44.2%	28.9–59.4%	2.86	1.46–5.63	0.002
ELISA-SEA	56	17.6%	5.8–29.4%	55	60.5%	47.8–73.1%	7.23	2.76–18.94	0.000
ELISA-SEA-SMP	56	15.8%	3.68–27.8%	55	47.8%	36.4–59.3%	4.95	1.76–13.92	0.002
PCR serum	56	0.0%	–	56	0.0%	–	–	–	–
PCR urine	50	7.6%	0.0–16.4%	47	4.5%	0.0–10.4%	0.56	0.11–2.82	0.486
PCR feces	48	32.1%	11.1–53.1%	49	11.9%	3.7–20.1%	0.28	0.11–0.71	0.007
KK (all < 50 egg/g)	41	17.0%	0.34–33.7%	35	17.2%	6.5–28.0%	1.02	0.32–3.18	0.980
Composite gold standard**	34	38.2%	16.3–66.3%	34	29.4%	17.5–45.1%	0.64	0.22–1.86	0.416

COPT = Circumoval Precipitin Test, KK = Kato–Katz technique, ELISA-SEA = ELISA based on soluble egg antigen, ELISA-SEA-SMP = ELISA based on soluble egg antigen with treatment with sodium metaperiodate.

[1] Adjusted for gender and clustering of individuals at the household level.

* Age group ≤ 17 years is the reference category.

** Composite 'gold' standard for parasite detection = PCR serum + PCR urine + PCR feces + KK. To be positive, a participant had to have all these 4 tests performed and to have tested positive to at least 1 of them. To be negative, a patient needed to have all these 4 tests performed and have tested negative to all of them.

differentiating between current and formerly infected individuals is a limitation of conventional antibody detection methods (Weerakoon et al., 2015). However, we also found significant age effects on the prevalence and performance of serological tests, being the prevalence and performance greater in adults than in children, as older people are more likely to have already experienced the infection. Conversely, for PCR on faeces, prevalence and performance were greater amongst those under 17 years of age, suggesting that the prevalence measured in adults mainly consists of old cases, treated (immunological memory), treated and not cured, or not treated (chronic) but still detectable serologically. In this sense, COPT has shown to become negative between 3–12 months after successful chemotherapy (Garcia et al., 2006). Conversely, the prevalence in children appears to be mainly due to recent infections (symptomatic, acute or asymptomatic), as PCR detects DNA from the parasite itself. The lower sensitivity of KK as compared to PCR did not allow us to observe this age difference. Greater concordance was identified for PCR on faeces with respect to the composite 'gold' standard for parasite detection, with an ROC of 0.988 in those under 17 years of age, highlighting the diagnostic value of this test as described elsewhere (Pontes et al., 2003; Gomes et al., 2009; Abreu et al., 2010; Carvalho et al., 2012). The absence of differences in terms of gender, neither in prevalence nor in performance, has been reported by other authors as well (Pinot de Moira et al., 2010; Hofstede et al., 2014; Bakuza et al., 2017) and suggests that the risk of transmission is similar and that the tests perform in a homogeneous way.

In general, the ELISA with SMP treatment agrees more with the COPT than the one without such treatment. Since COPT is considered a very specific immunological test, this suggests that treatment with SMP improves the specificity of ELISA, as has been described in other studies (Noya et al., 1995; Alarcón de Noya et al., 2000). As expected, the PCR in faeces agrees more with the 'gold' standard for parasite detection, and it also has a greater ROC, suggesting good diagnostic value as also described in other studies (Pontes et al., 2003; Abreu et al., 2010;

Table 2
Prevalence of schistosomiasis in Los Toros community, Carlos Arvelo Municipality, Carabobo State, Venezuela, according to gender and diagnostic test.

	Males			Females			OR[1,*]	95%CI	P-value
	N	Prevalence[1]	95% CI	N	Prevalence[1]	95% CI			
COPT	53	29.7%	13.9- 45.5%	59	35.9%	23.2- 48.6%	0.74	0.34–1.61	0.449
ELISA-SEA	52	41.6%	32.1–51.3%	59	36.3%	24.2–48.5%	1.32	0.66–2.66	0.434
ELISA-SEA-SMP	52	35.2%	23.3–47.1%	59	28.6%	17.1–40.1%	1.41	0.59–3.40	0.440
PCR serum	53	0.0%	–	59	0.0%	–	–	–	–
PCR urine	43	9.0%	0.0- 8.7%	54	3.8%	0.0–18.9%	2.51	0.58–10.77	0.216
PCR feces	44	16.9%	4.8–29.2%	53	25.9%	8.9–42.9%	0.56	0.22–1.47	0.241
KK (all < 50 egg/g)	34	17.7%	3.8–31.6%	42	16.7%	3.4–29.9%	1.07	0.42–2.78	0.884
Composite gold standard**	30	30.0%	12.9–55.3%	38	36.8%	19.7–58.1%	0.69	0.21–2.26	0.545

COPT = Circumoval Precipitin Test, KK = Kato–Katz technique, ELISA-SEA = ELISA based on soluble egg antigen, ELISA-SEA-SMP = ELISA based on soluble egg antigen with treatment with sodium metaperiodate.

[1] Adjusted for age and clustering of individuals at the household level.

* Age group ≤ 17 years is the reference category.

** Composite 'gold' standard for parasite detection = PCR serum + PCR urine + PCR feces + KK. To be positive, a participant had to have all these 4 tests performed and to have tested positive to at least 1 of them. To be negative, a patient needed to have all these 4 tests performed and have tested negative to all of them.

Table 3
Test performance for the diagnosis of schistosomiasis in Los Toros community, Carlos Arvelo Municipality, Carabobo State, Venezuela.

	N	Sensitivity	Specificity	Agreement	PPV	NPV	Kappa	p-value Kappa	ROC	95%CI ROC
ELISA-SEA*	111	47.22%	65.33%	59.46%	39.53%	72.06%	0.1195	0.1018	0.5628	0.46–0.66
ELISA-SEA-SMP*	111	38.89%	72.00%	61.26%	40.00%	71.05%	0.1097	0.1239	0.5544	0.46–0.65
PCR serum**	68	–	–	66.18%	–	66.18%	–	–	–	–
PCR urine**	68	8.70%	100%	69.12%	100%	68.18%	0.1119	0.0223	0.5435	0.48–0.60
PCR feces**	68	69.57%	100%	89.71%	100%	86.54%	0.7516	0.0000	0.8478	0.75–0.94
KK**	68	47.83%	100%	82.35%	100%	78.95%	0.5482	0.0000	0.7391	0.63–0.84

COPT = Circumoval Precipitin Test, KK = Kato–Katz technique, ELISA-SEA = ELISA based on soluble egg antigen, ELISA-SEA-SMP = ELISA based on soluble egg antigen with treatment with sodium metaperiodate.
KK (all < 50 egg/g).

* Serology COPT as gold standard.

** vs. composite 'gold' standard for parasite detection = PCR serum + PCR urine + PCR feces + KK. To be positive, a participant had to have all these 4 tests performed and to have tested positive to at least 1 of them. To be negative, a patient needed to have all these 4 tests performed and have tested negative to all of them.

Table 4
Test performance for the diagnosis of schistosomiasis in Los Toros community, Carlos Arvelo Municipality, Carabobo State, Venezuela, according to age.

	N	Sensitivity	Specificity	Agreement	PPV	NPV	Kappa	p-value Kappa	ROC	95%CI ROC
Age ≤ 17 years										
ELISA-SEA*	56	8.33%	79.55%	64.29%	10.00%	76.09%	−0.1290	0.8344	0.4394	0.33789 0.54090
ELISA-SEA-SMP*	56	0.00%	79.55%	62.50%	0.00%	74.47%	−0.2250	0.9564	0.3977	0.33745 0.45801
ELISA-SEA*	34	–	–	61.76%	–	61.76%	–	–	–	–
ELISA-SEA-SMP*	34	7.69%	100%	64.71%	100%	63.64%	0.0933	0.0985	0.5385	0.46308 0.61384
PCR serum**	34	84.62%	100%	94.12%	100%	91.30%	0.8717	0.0000	0.9231	0.82101 1.00000
PCR urine**	34	38.46%	100%	76.47%	100%	72.41%	0.4357	0.0010	0.6923	0.55468 0.82994
Age >17 years										
ELISA-SEA*	55	66.67%	45.16%	54.55%	48.48%	63.64%	0.1135	0.1873	0.5591	0.42796 0.69032
ELISA-SEA-SMP*	55	58.33%	61.29%	60.00%	53.85%	65.52%	0.1944	0.0741	0.5981	0.46491 0.73132
ELISA-SEA*	34	–	–	70.59%	–	70.59%	–	–	–	–
ELISA-SEA-SMP*	34	10.00%	100%	73.53%	100%	72.73%	0.1356	0.0579	0.5500	0.45200 0.64800
PCR serum**	34	50.00%	100%	85.29%	100%	82.76%	0.5854	0.0001	0.7500	0.58667 0.91333
PCR urine**	34	60.00%	100%	88.24%	100%	85.71%	0.6792	0.0000	0.8000	0.63997 0.96003

COPT = Circumoval Precipitin Test, KK = Kato–Katz technique, ELISA-SEA = ELISA based on soluble egg antigen, ELISA-SEA-SMP = ELISA based on soluble egg antigen with treatment with sodium metaperiodate.

* Serology COPT as gold standard.

** vs. composite 'gold' standard for parasite detection = PCR serum + PCR urine + PCR feces + KK. To be positive, a participant had to have all these 4 tests performed and to have tested positive to at least 1 of them. To be negative, a patient needed to have all these 4 tests performed and have tested negative to all of them.

Carvalho et al., 2012). Although the serum samples can be used for molecular diagnosis of schistosomiasis through PCR, several authors report a lower sensitivity of PCR on serum *vs.* stool to detect *S. mansoni* due to the limited amount of circulating DNA in the serum (Pontes et al., 2003; Cnops et al., 2012; Werakoon et al., 2015; Ogongo et al., 2018). Yet, it has been shown that free nucleic acids circulating in the blood are partially excreted in the urine, especially those with a relatively low molecular size (100–200 bp) (Enk et al., 2012); and in some studies it was possible to detect DNA from *S. mansoni* in urine samples (Enk et al., 2010, 2012; Lodh et al., 2013), as demonstrated in our work where the sensitivity of PCR on urine was higher than that obtained with PCR on serum. Possibly, using more

Table 5
Agreement and Kappa values of the diagnostic tests in the detection of schistosomiasis in Los Toros community, Carlos Arvelo Municipality, Carabobo State, Venezuela.

	Serology COPT	ELISA SEA	ELISA SEA SMI	PCR serum	PCR urine	PCR feces	Coprology K-K
Serology COPT		59.46%	61.26%	66.96%	63.92%	53.76%	68.49%
ELISA SEA	0.1195		92.79%	61.26%	60.42%	54.35%	58.33%
ELISA SEA SMI	0.1097	0.8428***		68.47%	64.58%	59.78%	65.28%
PCR serum	0.0000	0.0000	0.0000		93.81%	79.57%	82.19%
PCR urine	−0.0553	−0.0133	−0.0543	0.0000		74.07%	81.43%
PCR feces	−0.0673	−0.0946	−0.0799	0.0000	−0.1038		75.68%
Coprology K-K	0.2266*	−0.0355	0.0302	0.0000	−0.0508	0.3239**	

% Agreement (upper panel) and Kappa values (lower panel).
KK (all < 50 egg/g).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

advanced PCR techniques, such as Real Time PCR or Droplet Digital PCR (Cnops et al., 2012; Cai et al., 2019), would increase the sensitivity in serum samples. However, these techniques are more expensive and difficult to implement in most schistosomiasis-endemic areas. In our study, testing the urine improved the diagnosis of schistosomiasis, in addition to the application of different diagnostic methods on the same samples.

5. Conclusion

The use of different diagnostic tests that may complement one another allowed for a better diagnosis of *S. mansoni* infection, revealing a relatively high prevalence of schistosomiasis in a low-transmission setting in Venezuela. This concerned both old and recent infections depending on the age group in question. Advances in diagnosis will play a central role in the control and eventually elimination of schistosomiasis, especially for low intensity infections in lowly endemic areas.

Ethics approval and consent to participate

The project was approved by the Committee of Bioethics of the Institute for Biomedical Research of the University of Carabobo (BIOMED-UC), Venezuela, following the guidelines for humans and animals care by the Commission of Bioethics of the Venezuelan Ministry of Science and Technology and the "Operational Guidelines for Ethics Committees that Review Biomedical Research (TDR/PRD/ETHICS/2000.1). Written informed consent was obtained from all adults and parents/caretakers of children. All participants received complete medical care and children were given antiparasitic and specific treatments for other diseases as required.

Declaration of Competing Interest

The authors declare that they have no competing interest.

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