



HAL
open science

Imported schistosomiasis in Paris region of France: A multicenter study of prevalence and diagnostic methods

Claire Leblanc, Sophie Brun, Olivier Bouchaud, Arezki Izri, Vichita Ok, Marion Caseris, Frédéric Sorge, Luu-Ly Pham, André Paugam, Luc Paris, et al.

► To cite this version:

Claire Leblanc, Sophie Brun, Olivier Bouchaud, Arezki Izri, Vichita Ok, et al.. Imported schistosomiasis in Paris region of France: A multicenter study of prevalence and diagnostic methods. *Travel Medicine and Infectious Disease*, 2021, 41, pp.102041. 10.1016/j.tmaid.2021.102041 . hal-03946367

HAL Id: hal-03946367

<https://hal.science/hal-03946367>

Submitted on 24 Apr 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Imported schistosomiasis in Paris region of France: a multicenter study of prevalence and diagnostic methods

Claire Leblanc^a, MD, Sophie Brun^b, MD, PhD, Olivier Bouchaud^c, MD, PhD, Arezki Izri^b, MD, PhD, Vichita Ok^b, MD, Marion Caseris^d, MD, Frédéric Sorge^e, MD, Luu-ly Pham^a, MD, André Paugam^f, MD, PhD, Luc Paris^g, MD, PhD, Stéphane Jaureguiberry^h, MD, PhD, Coralie Bloch-Queyratⁱ, MD, PhD, Marouane Boubayaⁱ, MD, Albert Faye^d, MD, PhD, Patricia Mariani^j, MD, PhD, Loïc de Pontual^a, MD, PhD

Affiliation

^a Department of Pediatrics, AP-HP University Paris 13, Jean Verdier Hospital, Bondy, France

^b Department of Parasitology-Myology, AP-HP University Paris 13, Avicenne Hospital, Bobigny, France

^c Department of Infectious and Tropical Diseases, AP-HP University Paris 13, Avicenne Hospital, Bobigny, France

^d Department of General Pediatrics and Pediatric Infectious Diseases, AP-HP University Paris Diderot, Robert Debré Hospital, Paris, France

^e Department of General Pediatrics and Pediatric Infectious Diseases, AP-HP University Paris Descartes, Necker-Enfants Malades Hospital, Paris, France

^f Department of Parasitology-Myology, AP-HP University Paris Descartes, Cochin Hospital, Paris, France

^g Department of Parasitology-Myology, AP-HP Sorbonne Université, Pitié-Salpêtrière Hospital, Paris, France

^h Department of Infectious and Tropical Diseases, AP-HP University Paris –Sud Saclay, Kremlin Bicêtre Hospital, Le Kremlin Bicêtre, France

ⁱ Department of Clinical Research, AP-HP University Paris 13, Avicenne Hospital, Bobigny, France

^j Department of Microbiology, AP-HP University Paris Diderot, Robert Debré Hospital, Paris, France

Corresponding author: Claire Leblanc, MD, Department of Pediatrics, AP-HP, Jean Verdier University Hospital, Avenue du 14 Juillet, F-93140 Bondy, France. E-mail: Claire.leblanc@aphp.fr

Key words: imported schistosomiasis; prevalence; children; diagnostic methods

Abbreviation

ICT: Immunochromatographic tests

IS: imported schistosomiasis

IHA: Indirect hemagglutination assay

LCA: Latent class analysis

NPV: Negative predictive value

OR: odds ratio

POC-CCA: Point of care circulating cathodic antigen

PPV: Positive predictive value

RT-PCR: real-time polymerase chain reaction

UMR: Unaccompanied minor refugees

WB: Western Blot

WHO: World Health Organization

Abstract

Background

There are few data on imported schistosomiasis – especially in children. The objectives of the present study were to estimate the prevalence of imported schistosomiasis in at-risk children in the greater Paris region of France and to compare diagnostic methods.

Method

Children at risk of schistosomiasis who consulted or were hospitalized in four hospitals in the greater Paris region were prospectively included. Clinical and laboratory data were collected. Urine and feces samples were screened for *Schistosoma* spp. using microscopy, a point-of-care circulating cathodic antigen and a real-time polymerase chain reaction assay. Serum samples were screened using Western blot, ELISA, indirect hemagglutination, and immunochromatographic assays. The diagnosis was characterized as confirmed (positive microscopy analysis) and as suspected (positive ELISA and Western blot assays). The prevalence of schistosomiasis and the tests' performances were estimated using the latent class method.

Results

A total of 114 children were included. Most of the children were newly arrived migrants from sub-Saharan Africa. The mean age was 13.2 years-old. There were 12 (10.5%) confirmed cases and 13 (11.4%) suspected cases. Half of the confirmed and suspected cases were asymptomatic. The prevalence was 24.3%. The ELISA and the Western blot assays presented the same sensitivity (83%) and specificity (99%). The serum immunochromatographic assay also showed good performance.

Conclusions

The high prevalence of imported schistosomiasis among at-risk children in the greater Paris region confirms the need for systematic screening. A serum immunochromatographic assay appears to be one of the most effective screening methods for a low cost.

I. Introduction

Schistosomiasis is a neglected parasitic disease that affects about 250 million people worldwide[1]. The prevalence of imported schistosomiasis (IS) among children has not been extensively characterized[2],[3]. This parasitosis is a public health issue in pediatric populations, since the prevalence in endemic countries is highest among young adolescents[4] and the later the diagnosis, the greater the risk of developing long-term comorbidities (such as urinary tract fibrosis, liver cirrhosis, and cancer)[5]. Children are often asymptomatic complicating the diagnosis for pediatricians[6].

International guidelines recommend that adult migrants from endemic areas should be systematically screened for schistosomiasis, regardless of whether they are symptomatic or not [7],[8],[9]. However, the lack of studies of pediatric IS means that there are no specific guidelines on screening and diagnostic methods in children.

Furthermore, the lack of a “reference standard” diagnostic test for schistosomiasis is associated with underdiagnosis of this disease, especially in individuals with a low worm burden [10],[11]. Combining several laboratory assays can nevertheless increase the reliability of diagnosis. According to the current French guidelines, a diagnosis of schistosomiasis should be confirmed by combining serologic assays (screening by an ELISA and/or an indirect hemagglutination (IHA) assay and confirmation by a Western blot (WB) assay if one (or two) of the previous test is positive) and microscopic detection of eggs in urine or feces samples[12]. A urine rapid diagnostic test for the circulating cathodic antigen (CCA) produced by adult *Schistosoma* has been used in endemic areas for several years (Point of care (POC) – CCA)[13]. These POC-CCAs give immediate results, are inexpensive, and require little analytical material but their accuracy is very controversial depending on the studies, with a low specificity for IS [14],[15]. Moreover, it has been reported that rapid immunoglobulin G and M immunochromatographic tests (ICTs) of serum samples are a highly sensitive tool and help not to miss cases of IS [16],[17]. It appears to be a suitable screening tests, even when used alone but its lower specificity compared to microscopy cannot confirm diagnosis[18]. Finally, the value of molecular biology techniques (such as real-time polymerase chain reaction (RT-PCR) assays) relative to other diagnostic tools is currently being studied but has not yet been clearly determined[19],[20].

In view of the lack of (i) epidemiological and clinical data on IS in children in Europe and (ii) data on the performance and feasibility of the various diagnostic tests in this population, we performed a prospective and multicenter study of confirmed and suspected cases, the

prevalence of IS and the diagnosis test performance using latent class method (LCA) in an at-risk pediatric population in Paris area, France.

II. Methods

2.1 Study design

Between June 16th, 2017, and June 5th, 2018, children were prospectively included during general pediatric consultation or hospitalization for a different pathology by four hospitals in the greater Paris region of France (Delafontaine, Jean Verdier, Necker-Enfant Malades and Robert Debré hospitals). Clinicians screened every patient for study inclusion criteria during consultation or hospitalization. These centers were selected because they treat a large number of immigrants or people having visited friends and relatives in regions where schistosomiasis is endemic.

2.2 Inclusion and exclusion criteria

The main inclusion criteria were (i) age over 3 and under 18, and (ii) arrival within the previous 12 months from an endemic area (defined by the 2012 World Health Organization (WHO) Schistosomiasis World Map) or, for children born in France, return from a stay of more than 3 months in an endemic area with at least one skin contact with freshwater during the trip. The exclusion criteria were (i) previous diagnosis and treatment of schistosomiasis in France and no subsequent at-risk fresh water contacts after treatment (for children born in France), (ii) lack of consent or (iii) missing clinical or diagnostic data. Due to ethical considerations, children who did not need a blood sample could not be included in the study.

2.3 Collection of clinical and laboratory data

When corresponding to inclusion criteria, demographic and clinical data (gender, age, comorbidities, country of origin or endemic regions visited, and hematuria) were collected anonymously on an online case report form. Weight and height were recorded and compared to the WHO growth standards. Four mL of venous blood was collected in an anticoagulant-free, and 7 mL in an EDTA tube for a blood count. An eosinophil count above 0.5 G/L was considered abnormal.

For each child, the parents or legal guardians were also asked to collect (i) three samples of fresh feces several days apart and (ii) a 24-hour urine sample or a post-exercise full bladder urine sample. All samples were sent to the Parasitology and Mycology Laboratory at Avicenne Hospital (Bobigny, France) for centralized analysis. After sedimentation or centrifugation of the 24-hour or full-bladder urine sample, the whole pellet was examined

under the microscopic. *Schistosoma* eggs in feces samples were detected by direct microscopic observation after centrifugation in merthiolate-iodine-formaldehyde saline reagent

Serologic assays were carried out according to the respective manufacturer's instructions: an IHA assay (Fumouze Diagnostics, Signes, France), an ELISA assay (Bordier Affinity products, Crissier, Switzerland), a serum immunochromatographic assay for anti-*Schistosoma* IgG/IgM (LDBIO Diagnostics, Lyon, France), a WB assay for anti-*Schistosoma* IgG (SCHISTO II, LDBIO Diagnostics) and a cassette-based POC-CCA (Rapid Medical Diagnostics, Pretoria, South Africa). The results of the POC-CCA were scored as “negative”, “trace” or “positive” when the test line was absent, faint or a clear, respectively.

DNA from one gram of feces or from 1 ml of urine was extracted with the MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume (Roche), after incubation with proteinase K and Bacteria Lysis Buffer (Roche) for 10 min at 65°C and then 10 min at 95°C. A genus-specific RT-PCR assay targeted the 28S ribosomal RNA gene of the most common *Schistosoma* species in humans. The forward and reverse primers were those described previously by Cnops *et al.* [19]. An internal positive control (Taqman Exogenous IPC, Life Technologies) was used to detect the possible presence of PCR inhibitors. The reaction was run on a QuantStudio7™ system (Thermo Fisher Scientific).

2.4 Ethics statement

Except in the case of unaccompanied minor refugees (UMRs), each child's parents or legal guardians gave their written, informed consent to the child's participation in the study. The children also gave their verbal, informed consent. Assistance from a translator was provided when necessary. Given the absence of parents or legal guardians for UMRs, the latter were considered to be emancipated minors and so were asked to give their written, informed consent directly.

In line with the French legislation on non-interventional, observational research, the study was registered with the French National Data Protection Commission (*Commission nationale de l'informatique et des libertés* (Paris, France); reference: 2043391) and approved by the local institutional review board (CLEA, Avicenne Hospital, Bobigny, France; reference: CLEA-2015-020). Personal data were anonymized by assigning a unique identifier to each study participant. The laboratory data were anonymized after the child and/or the parents or legal guardians had been informed of the diagnosis. Children with positive serology and/or

positive microscopy were treated with praziquantel (40-60mg per kg) and a follow up was planned according to the French guidelines (microscopy and ultra-sound follow up every 3 months for at least a year)[12].

2.5 Study primary and secondary endpoints

The study's primary endpoint was to estimate prevalence of imported schistosomiasis in children using the LCA. The secondary endpoints were to describe children suspected cases (positive ELISA and WB serologic assays) and confirmed cases (urine or feces microscopic analysis with positive egg search) [21] of schistosomiasis and describe the diagnostic performance (sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV)) of the various tests: the WB, ELISA, IHA, and immunochromatographic serologic assays, the egg microscopy analysis of urine and feces samples, and the urine POC-CCA. In all cases, blood and urine samples were also screened for eosinophilia and hematuria, respectively.

2.6 Sample size calculation

We expected to see a prevalence rate of 10% for IS. For a 95% confidence interval (CI) with a precision of $\pm 3.5\%$, it is necessary to include 300 children using exact Clopper Pearson Method.[22].

2.7 Statistical analysis

The data collected were described using the number and the percentage (%) for the qualitative variables. In the other cases, the mean and standard error (SD) or the median and interquartile range (IQR) were used for the quantitative variables. With a view to identifying clinical factors associated with IS, we performed a logistic regression. All factors with a p-value <0.25 were included in the multivariate model with stepwise selection based on the Akaike information criterion (AIC).

In the absence of a reference standard, a statistical model based on latent class analysis (LCA) was used to combine multiple test results and prevalence (the WB, ELISA, and IHA assays, the urine microscopic analysis, the POC-CCA, and the ICT) in order to construct a reference standard outcome [23],[24]. Feces microscopic analysis, feces and urine RT-PCR were excluded due to the large proportion of missing data. Three models presented a variable containing 2, 3 and 4 latent classes. The latent two-class model was selected using the AIC

and Bayesian Information Criterion (BIC). Then for each of the diagnostic tests, we calculated sensitivity, specificity, positive and negative predictive values (PPV and NPV).

All tests were two-tailed, and the results were considered statistically significant when $p < 0.05$. All analyses were performed using R software (version 3.3.3, The R Foundation for Statistical Computing [25]).

III. Results

3.1 Study population characteristics

A total of 123 children of 300 planned were included. Nine children were excluded, due to missing clinical or laboratory data. The study population's demographic and clinical characteristics are summarized in Table 1.

One hundred and eight of the 114 children (95%) had been born outside of France. The main countries of birth were Mali (n=34, 31.6%), Republic of Guinea (n=21, 19.3%), Côte d'Ivoire (n=16, 14.9%) and Democratic Republic of Congo (n=14, 13.1%). For the six patients born in France, the at-risk areas visited were Benin, Democratic Republic of Congo, Côte d'Ivoire, Mali, and Senegal. Male to female sex ratio was 2.9.

Forty-eight children (42.1%) were UMRs; on average, the UMR were older than the non-UMRs (mean age: 15.5 ± 1.73 vs. 11.6 ± 4.46 , respectively; $p < 0.001$). New arrivals were asked if they had already been treated with praziquantel (in mass treatment campaigns) but the answers were often uncertain and could not be reliably analyzed.

3.2 Parasitological and biological data

The patients' laboratory data are summarized in Table 2. Of the nine patients with a positive urine microscopy assay for *Schistosoma haematobium*, six had a negative POC-CCA, two had a positive POC-CCA, and the ninth patient was not tested with a POC-CCA). Of the three patients with a positive *Schistosoma* feces microscopic analysis, 2 had a positive POC-CCA test (one positive microscopy for *Schistosoma mansoni* and the other for *Schistosoma haematobium*). In 15 patients, the feces microscopic analysis revealed the presence of other parasites (*Giardia intestinalis*, *Entamoeba histolytica/dispar*, *Entamoeba. coli*, *Endolimax nanus*, *Ankylostomidea*, *Hymenolepis nana*, *Blastocystis hominis*). Twenty-nine of the 114 patients had a positive WB assay. Twenty-two patients had positive results for two or more serologic tests: 15 were positive according to the WB, the IHA and the ELISA assays, and 13 were positive according to the WB and the ELISA assays only. Thirty-seven patients had a positive ICT, 20 corresponding to a "trace" result (considered as positive). Only five patients

had a negative ICT and a positive WB assay. Twenty-four children had a positive ICT, WB and ELISA assays. One child was tested positive for *S. haematobium* in microscopy, with positive ELISA, IHA and ICT assays but with a negative WB assay.

3.3 Prevalence and burden of schistosomiasis

There were 12 confirmed cases (12/114, 10%) and 13 (13/114, 11.4%) suspected cases. The estimated prevalence of IS using LCA analysis was 24.3% (23/93, 95CI%: 16.4-34.8). Our initial screening target (n=300) was based on a hypothetical prevalence of 10%. However, the final analysis revealed a prevalence above 20% meaning the precision of this prevalence is now wider ($\pm 8.5\%$) with 114 children included [22]. The clinical data on schistosomiasis-positive (confirmed and suspected patients) are summarized in Table 3. Nine out of the 25 positive patients (36.0%) had underlying conditions – mostly infectious diseases, such as active tuberculosis, malaria, typhoid and scabies. The child born in France had been infected for schistosomiasis in Mali. Thirteen of the 25 children (52%) were asymptomatic.

3.3.a. Confirmed IS

Of the nine patients with positive urine microscopic analyses [9/111], two were asymptomatic. Three of the 5 patients with macroscopic hematuria had a positive urine dipstick test for hematuria. The two patients with negative dipstick for hematuria had intermittent macroscopic hematuria. Two patients had abdominal pain, one had a positive urine dipstick test for hematuria and the other was negative.

Three out of 66 tested patients had a positive feces microscopic analysis (two for *S. mansoni* and one for *S. haematobium*). The two patients harboring *S. mansoni* were asymptomatic, and the patient harboring *S. haematobium* had abdominal pain and a negative urine dipstick test.

3.3.b Suspected/confirmed IS

Fifteen schistosomiasis suspected/confirmed cases out of 25 (60%) had undergone an ultrasound assessment after diagnosis, the rest being lost to follow up. Eight of the 15 tested patients (53.3%) had an abnormal abdominal ultrasound result (bladder calcification or hepatomegaly). It is of interest to note that 3 asymptomatic patients had an abnormal abdominal ultrasound result (including a confirmed case positive for *S. mansoni*).

The results of bivariate and multivariable logistic regression analyses comparing schistosomiasis suspected/confirmed cases with negative cases are summarized in Table 4.

3.4 Diagnostic test performance

Ninety-three of 114 patients (81.6%) had available data for six diagnostic tests (the WB, ELISA, and IHA assays, the urine microscopic analysis, the POC-CCA, and the ICT), and were included in the LCA (Table 5). The two latent classes are composed of 23 patients determined to be positive and 70 patients determined to be negative.

ICT had the highest sensitivity (100%), microscopy and IF had the highest specificity (100%). WB and ELISA assays had the equivalent performances.

IV. Discussion

The present prospective study is the first to highlight a high prevalence of IS (24.3%) in at-risk children in the greater Paris region of France, according to the LCA method. Twenty-four of the 25 patients diagnosed with schistosomiasis were boys (96%). This very strong male predominance was not found in other studies but might be due (at least in part) to selection bias, with the inclusion of a high proportion of UMRs[26]. In 2017, it was estimated that UMRs in France were predominantly boys (95.8%) from sub-Saharan, north and east Asia [27]. The UMRs were older than the non-refugees in the study; an older age was found to be a confounding factor and not a risk factor. Most of the children diagnosed with schistosomiasis in the present study were from Mali or Republic of Guinea – both of which are schistosomiasis high-prevalence countries [28]. Of the three patients previously treated for schistosomiasis, two still had one or more positive serologic tests, but a negative microscopy. These two children were still symptomatic even though they reported previous treatment in the endemic country. Neither of the two patients was able to specify the date or the name of the treatment received in the endemic area. One can hypothesize that treatment in the endemic zone was ineffective (depending on the number of doses received)[29], that children were reinfected after treatment (since they were symptomatic), or that children retained a “serological scar”. We consider that all patients with a history of schistosomiasis and previous treatment in an endemic area should be screened for recontamination or relapse and should be provided with appropriate care.

In line with the literature data[6],[30], our results showed that of the patients with suspected/confirmed IS were asymptomatic. Four confirmed out of 12 and nine suspected cases out of 13 were asymptomatic. Children who had positive diagnostic tests were nevertheless more symptomatic than those with negative tests (adjusted OR for IS = 3.32). One can hypothesize that children were asymptomatic because they did not have the time to

develop symptoms or because they were ancient cases and not active schistosomiasis (especially for adolescents), even though this second theory seems less plausible since three asymptomatic patients had an abnormal ultrasound. Among confirmed IS with signs and symptoms, hematuria was the most common feature (5 out of 8 cases) but did not always match with a positive urine dipstick, since hematuria can be intermittent. Clinicians should be aware that macroscopic hematuria is not the only sign of *S. haematobium* infection. Though, in the study, the urinary dipstick test for hematuria showed to be a useful predictor of IS (adjusted OR = 10.47) and clinicians should use it easily. Considering all children included, there were 13 out of 114 with hematuria but only 6 were confirmed/suspected of IS, though 2 of the 7 considered negative for IS had a positive WB and immunochromatography assay, but were negative for the rest of the tests. These results show how difficult is the diagnosis of IS even when children are symptomatic. Given the high prevalence of IS observed in children at risk in the greater Paris region, systematic praziquantel treatment of all at-risk patients (even without screening) could be considered, since the treatment is well tolerated and cheap (€ 4 per course). However, the fact that three asymptomatic patients (including one tested positive for *S. mansoni*) had abnormal ultrasound results confirms the importance of systematic screening and regular follow-up of at-risk patients. In order to avoid missing complicated, asymptomatic forms of schistosomiasis, pediatricians should screen children at risk of schistosomiasis, especially UMRs who present an adjusted OR for IS of 3.09 in our study.

In the literature, the prevalence of IS varies markedly from one study to another, depending on the diagnostic method used[18],[31],[32]. Our initial screening target was 300 children with a hypothetical prevalence of 10% but unfortunately we were able to include 114 children and the study found a greater prevalence of 23%. The reasons of this lower inclusion number were mainly because most blood tests were not planned (exclusion criteria) for the child outside the study or parental refusal of consent. UMRs were the most included patients because as new arrivals, a blood test was planned to screen at least for tuberculosis, HIV, hepatitis B and C. The high prevalence of IS found here is slightly higher than the literature value for serologic studies, probably because of a selection bias with a large proportion of UMRs[33]. When considering microscopic analyses alone, the prevalence here would have been 8% for urine samples and 4.5% for feces samples, in line with the literature data. One of the limitations of the present study was that some of the included patients had not undergone all the diagnostic tests (primarily the feces microscopy analysis, and the urine and feces RT-PCR assays). This study highlights the difficulties of feces collection for children and the

need for easier diagnostic methods. We were therefore only able to perform the LCA on 93 patients (81.6%) with data from the other 6 diagnostic tests.

In a recent study, the use of RT-PCR improved *Schistosoma* detection rate in urine and fecal samples by a factor of 2.6 for *S. haematobium* and of 1.4 for *S. mansoni*, relative to microscopy[20]. In the present study, a molecular diagnosis of IS using RT-PCR was not relevant. Relatively few of the 114 patients were tested with this technique (51 for feces and 77 for urine), and the RT-PCR assay did not revealed any additional cases (relative to the serologic or microscopy analysis). This RT-PCR assay should be improved by studying a larger cohort of patients and performing an extraction control.

The POC-CCA gave rather disappointing results in our study, even though the literature data clearly show that POC-CCA is less effective at detecting *S. haematobium* than *S. mansoni* - probably because *S. mansoni* secretes more CCA[34]. This diagnostic method is probably effective for the detection of *S. mansoni* in endemic areas[35] but is not well suited to the detection of IS[18],[36]. It has been reported that a lateral flow test for CAA was highly sensitive for diagnosing low-level schistosomiasis, such as IS [37]. However, this recently developed technique was not available at the time of our study.

Our serologic assay results showed that ELISA and WB assays achieved the same level of diagnostic performance. Beltrame *et al.* found that WB assay was more sensitive but that ELISA assay had higher PPV and specificity; the researchers were unable to account for this finding[18]. In Europe, the WB assay main disadvantage is its price (€48.60 per test, *vs.* €13.50 for an ELISA assay). In our study, the IHA assay had a very low sensitivity (48%) but a very high specificity making this method less interesting. The serological approach is a valuable screening tool but its interpretation is only possible in a relatively late stage of infection and does not allow the differentiation between acute and previous infections or between persisting infection and re-infection and cannot be used for the follow up[38]. Only the combination of diagnostic techniques (e.g. serologic assays with microscopic analyses) increases the sensitivity/specificity of schistosomiasis screening but also increases the cost.

In the present study, the serum ICT gave the best results in terms of sensitivity and the NPV. Beltrame *et al.* found similar results[18]. This method has many advantages, such as its low cost, rapidity (with a result in 30 minutes) and lack of requirement for material. The relatively low specificity observed in the present study was probably caused by the qualitative nature of the test result (i.e. positive or negative only). The test was difficult to read because the test line was faintly positive for 20 of the 37 tested patients –making the interpretation reader-dependent. However, the ICT gave promising results for mass screening for new diagnostic of

schistosomiasis and for a low-cost, and should be considered for use in non-endemic areas. Other interesting, rapid point-of-care diagnostic techniques (such as blood immunochromatography) are currently being developed[39].

V. Conclusion

This study showed a high prevalence of IS in at-risk children in the greater Paris region of France. Moreover, this is the first prospective study to have compared ten diagnostic methods for IS in at-risk children. The serum ICT appears to be an attractive screening method in terms of high sensitivity, low cost, and short turnaround time; it might usefully replace less sensitive methods such as IHA and help the clinicians to provide personalized care, rather than mass treatment, to children at risk of schistosomiasis in non-endemic areas.

Acknowledgements

We are very grateful to Dr Denis Limonne (LD-BIO Diagnostics, Lyon, France) for donating the Western blot and serum immunochromatographic assays, and to Chantel Lamon (Rapid Medical Diagnostics, Pretoria, South Africa) for donating the POC-CCA.

We would like to thank Maya Husain, Alice Bergevin, Simon Escoda, Alexis Mandelcwajg for all their help with the acquisition of data.

Conflict of Interest: The authors have indicated they have no potential conflicts of interest to disclose

References

- [1] WHO | Schistosomiasis. Available at <https://www.who.int/schistosomiasis/en/>. Accessed August 21, 2019.
- [2] Redditt VJ, Janakiram P, Graziano D, Rashid M. Health status of newly arrived refugees in Toronto, Ont: Part 1: infectious diseases. *Can Fam Physician* 2015;61:e303-309.
- [3] Mutch RC, Cherian S, Nemba K, Geddes JS, Rutherford DM, Chaney GM, et al. Tertiary paediatric refugee health clinic in Western Australia: analysis of the first 1026 children. *J Paediatr Child Health* 2012;48:582–7. <https://doi.org/10.1111/j.1440-1754.2012.02429.x>.
- [4] Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. *Lancet* 2014;383:2253–64. [https://doi.org/10.1016/S0140-6736\(13\)61949-2](https://doi.org/10.1016/S0140-6736(13)61949-2).
- [5] Abdou A, Tligui M, Le Loup G, Raynal G. Bilharziose urinaire : une série française. *Progrès en Urologie* 2012;22:598–601. <https://doi.org/10.1016/j.purol.2012.03.004>.
- [6] Lingscheid T, Kurth F, Clerinx J, Marocco S, Trevino B, Schunk M, et al. Schistosomiasis in European Travelers and Migrants: Analysis of 14 Years TropNet Surveillance Data. *Am J Trop Med Hyg* 2017;97:567–74. <https://doi.org/10.4269/ajtmh.17-0034>.
- [7] Chaves NJ, Paxton GA, Biggs B-A, Thambiran A, Gardiner J, Williams J, et al. The Australasian Society for Infectious Diseases and Refugee Health Network of Australia recommendations for health assessment for people from refugee-like backgrounds: an abridged outline. *Med J Aust* 2017;206:310–5. <https://doi.org/10.5694/mja16.00826>.
- [8] CDC - Schistosomiasis - Resources for Health Professionals 2019. https://www.cdc.gov/parasites/schistosomiasis/health_professionals/index.html (accessed October 29, 2019).
- [9] Gray DJ, Ross AG, Li Y-S, McManus DP. Diagnosis and management of schistosomiasis. *BMJ* 2011;342. <https://doi.org/10.1136/bmj.d2651>.
- [10] Ogongo P, Kariuki TM, Wilson RA. Diagnosis of schistosomiasis mansoni: an evaluation of existing methods and research towards single worm pair detection. *Parasitology* 2018;145:1355–66. <https://doi.org/10.1017/S0031182018000240>.
- [11] Toor J, Alsallaq R, Truscott JE, Turner HC, Werkman M, Gurarie D, et al. Are We on Our Way to Achieving the 2020 Goals for Schistosomiasis Morbidity Control Using Current World Health Organization Guidelines? *Clin Infect Dis* 2018;66:S245–52. <https://doi.org/10.1093/cid/ciy001>.
- [12] Haute Autorité de Santé - Actualisation des actes de biologie médicale relatifs au diagnostic de la schistosomose (bilharziose). Available at: https://www.has-sante.fr/portail/jcms/c_2678400/fr/actualisation-des-actes-de-biologie-medicale-relatifs-au-diagnostic-de-la-schistosomose-bilharziose?xtmc=&xtcr=1. Accessed 7 March 2018
- [13] El-Ghareeb AS, Abd El Motaleb GS, Waked NM, Osman Hany Kamel N, Aly NS. Circulating cathodic antigen cassette test versus haematuria strip test in diagnosis of urinary schistosomiasis. *J Parasit Dis* 2016;40:1193–8. <https://doi.org/10.1007/s12639-015-0648-2>.
- [14] Marti H, Halbeisen S, Bausch K, Nickel B, Neumayr A. Specificity of the POC-CCA urine test for diagnosing *S. mansoni* schistosomiasis. *Travel Med Infect Dis* 2020;33:101473. <https://doi.org/10.1016/j.tmaid.2019.101473>.
- [15] Neumayr A, Chernet A, Sydow V, Kling K, Kuenzli E, Marti H, et al. Performance of the point-of-care circulating cathodic antigen (POC-CCA) urine cassette test for follow-up after treatment of *S. mansoni* infection in Eritrean refugees. *Travel Med Infect Dis* 2019;28:59–63. <https://doi.org/10.1016/j.tmaid.2018.09.004>.

- [16] Coulibaly JT, N’Goran EK, Utzinger J, Doenhoff MJ, Dawson EM. A new rapid diagnostic test for detection of anti-Schistosoma mansoni and anti-Schistosoma haematobium antibodies. *Parasit Vectors* 2013;6:29. <https://doi.org/10.1186/1756-3305-6-29>.
- [17] Nausch N, Dawson EM, Midzi N, Mduluza T, Mutapi F, Doenhoff MJ. Field evaluation of a new antibody-based diagnostic for Schistosoma haematobium and S. mansoni at the point-of-care in northeast Zimbabwe. *BMC Infectious Diseases* 2014;14:165. <https://doi.org/10.1186/1471-2334-14-165>.
- [18] Beltrame A, Guerriero M, Angheben A, Gobbi F, Requena-Mendez A, Zammarchi L, et al. Accuracy of parasitological and immunological tests for the screening of human schistosomiasis in immigrants and refugees from African countries: An approach with Latent Class Analysis. *PLoS Negl Trop Dis* 2017;11:e0005593. <https://doi.org/10.1371/journal.pntd.0005593>.
- [19] Cnops L, Soentjens P, Clerinx J, Van Esbroeck M. A Schistosoma haematobium-specific real-time PCR for diagnosis of urogenital schistosomiasis in serum samples of international travelers and migrants. *PLoS Negl Trop Dis* 2013;7:e2413. <https://doi.org/10.1371/journal.pntd.0002413>.
- [20] Guegan H, Fillaux J, Charpentier E, Robert-Gangneux F, Chauvin P, Guemas E, et al. Real-time PCR for diagnosis of imported schistosomiasis. *PLoS Negl Trop Dis* 2019;13:e0007711. <https://doi.org/10.1371/journal.pntd.0007711>.
- [21] Marchese V, Beltrame A, Angheben A, Monteiro GB, Giorli G, Perandin F, et al. Schistosomiasis in immigrants, refugees and travellers in an Italian referral centre for tropical diseases. *Infect Dis Poverty* 2018;7:55. <https://doi.org/10.1186/s40249-018-0440-5>.
- [22] Clopper CJ, Pearson ES. The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. *Biometrika* 1934;26:404–13. <https://doi.org/10.2307/2331986>.
- [23] Rutjes AWS, Reitsma JB, Coomarasamy A, Khan KS, Bossuyt PMM. Evaluation of diagnostic tests when there is no gold standard. A review of methods. *Health Technol Assess* 2007;11:iii, ix–51. <https://doi.org/10.3310/hta11500>.
- [24] Reitsma JB, Rutjes AWS, Khan KS, Coomarasamy A, Bossuyt PM. A review of solutions for diagnostic accuracy studies with an imperfect or missing reference standard. *J Clin Epidemiol* 2009;62:797–806. <https://doi.org/10.1016/j.jclinepi.2009.02.005>.
- [25] R Development Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2019. <https://www.R-project.org>, n.d.
- [26] Herbringer K-H, Drerup L, Alberer M, Nothdurft H-D, Sonnenburg F von, Löscher T. Spectrum of imported infectious diseases among children and adolescents returning from the tropics and subtropics. *J Travel Med* 2012;19:150–7. <https://doi.org/10.1111/j.1708-8305.2011.00589.x>.
- [27] MMNA-RAA2019.pdf. Available at http://www.justice.gouv.fr/_telechargement/MMNA_RAA2019.pdf. Accessed 26 september 2020.
- [28] Lai Y-S, Biedermann P, Ekpo UF, Garba A, Mathieu E, Midzi N, et al. Spatial distribution of schistosomiasis and treatment needs in sub-Saharan Africa: a systematic review and geostatistical analysis. *Lancet Infect Dis* 2015;15:927–40. [https://doi.org/10.1016/S1473-3099\(15\)00066-3](https://doi.org/10.1016/S1473-3099(15)00066-3).
- [29] King CH, Olbrych SK, Soon M, Singer ME, Carter J, Colley DG. Utility of repeated praziquantel dosing in the treatment of schistosomiasis in high-risk communities in Africa: a systematic review. *PLoS Negl Trop Dis* 2011;5:e1321. <https://doi.org/10.1371/journal.pntd.0001321>.

- [30] Coltart C, Whitty CJM. Schistosomiasis in non-endemic countries. *Clin Med (Lond)* 2015;15:67–9. <https://doi.org/10.7861/clinmedicine.15-1-67>.
- [31] Chernet A, Kling K, Sydow V, Kuenzli E, Hatz C, Utzinger J, et al. Accuracy of Diagnostic Tests for *Schistosoma mansoni* Infection in Asymptomatic Eritrean Refugees: Serology and Point-of-Care Circulating Cathodic Antigen Against Stool Microscopy. *Clin Infect Dis* 2017;65:568–74. <https://doi.org/10.1093/cid/cix366>.
- [32] Chernet A, Utzinger J, Sydow V, Probst-Hensch N, Paris DH, Labhardt ND, et al. Prevalence rates of six selected infectious diseases among African migrants and refugees: a systematic review and meta-analysis. *Eur J Clin Microbiol Infect Dis* 2018;37:605–19. <https://doi.org/10.1007/s10096-017-3126-1>.
- [33] Theuring S, Friedrich-Jänicke B, Pörtner K, Trebesch I, Durst A, Dieckmann S, et al. Screening for infectious diseases among unaccompanied minor refugees in Berlin, 2014–2015. *Eur J Epidemiol* 2016;31:707–10. <https://doi.org/10.1007/s10654-016-0187-x>.
- [34] Colley DG, Andros TS, Campbell CH. Schistosomiasis is more prevalent than previously thought: what does it mean for public health goals, policies, strategies, guidelines and intervention programs? *Infect Dis Poverty* 2017;6:63. <https://doi.org/10.1186/s40249-017-0275-5>.
- [35] Danso-Appiah A, Minton J, Boamah D, Otchere J, Asmah RH, Rodgers M, et al. Accuracy of point-of-care testing for circulatory cathodic antigen in the detection of schistosome infection: systematic review and meta-analysis. *Bull World Health Organ* 2016;94:522-533A. <https://doi.org/10.2471/BLT.15.158741>.
- [36] Grenfell RFQ, Pedrosa ML, Couto FFB, Almeida A, Coelho PMZ, Katz N. Suitability of commercially available POC-CCA tests for schistosomiasis: Considerations for efficiency, reproducibility and decision making criteria for field application in areas of low endemicity. *J Immunol Methods* 2019;472:1–6. <https://doi.org/10.1016/j.jim.2019.06.006>.
- [37] van Grootveld R, van Dam GJ, de Dood C, de Vries JJC, Visser LG, Corstjens PLAM, et al. Improved diagnosis of active *Schistosoma* infection in travellers and migrants using the ultra-sensitive in-house lateral flow test for detection of circulating anodic antigen (CAA) in serum. *Eur J Clin Microbiol Infect Dis* 2018;37:1709–16. <https://doi.org/10.1007/s10096-018-3303-x>.
- [38] Hinz R, Schwarz NG, Hahn A, Frickmann H. Serological approaches for the diagnosis of schistosomiasis - A review. *Mol Cell Probes* 2017;31:2–21. <https://doi.org/10.1016/j.mcp.2016.12.003>.
- [39] Buonfrate D, Rodari P, Brunelli D, Degani M, Ragusa A, Tais S, et al. Diagnostic study on an immunochromatographic rapid test for schistosomiasis: comparison between use on serum and on blood spot from fingerprick. *BMJ Open* 2018;8:e019228. <https://doi.org/10.1136/bmjopen-2017-019228>.

Tables

Variable	Characteristics (n = 114)
Age (years), mean \pm sd	13.2 \pm 4
Male:female sex ratio	2.9 (85/29)
Unaccompanied minor refugees, n (%)	48 (42.1%)
Underlying conditions, n(%)	50 (43.8%)
None	
<i>Declared by the participants</i>	17 (14.9%)
Malaria	3 (2.6%)

Schistosomiasis	
<i>Confirmed diagnosis during the study</i>	5 (4.4%)
Sickle cell disease	6 (5.3%)
Active tuberculosis	3 (2.6%)
HIV	3 (2.6%)
VHB	1(0.9%)
Syphilis	
	30 (26.3%)
Other conditions*	
Time interval between arrival in France and consultation (years), mean ± sd	0.6 ± 1.3
Signs and symptoms, n (%)	
None	84 (73.6%)
Urinary (hematuria)	13 (11.4%)
Intestinal:	17 (14.9%)
Abdominal pain	7
Diarrhea	5
Bloody stools	2
Rectal bleeding	1
Vomiting	2
Urine dipstick test for hematuria (positive), n (%)	10 (9.2%)

Table 1. Demographic and clinical characteristics of the children population studied

*surgical, auto-immune diseases (lupus, sclerodermia), traumatic(fracture,burn), scabies, nephrotic syndrome, typhoid

Diagnostic method	N (%)
WB	
Tests performed	114 (100%)
Positive	29 (25.4%)
ELISA	
Tests performed	114 (100%)
Positive	28 (25.0%)
IHA	
Tests performed	112 (98.2%)
Positive	15 (13.4%)
Serum ICT	
Tests performed	105 (92.1%)
Positive	37 (35.2%)
Feces microscopy analysis	
Tests performed	66 (57.9%)
Positive for parasites	18 (27.3%)
<i>S. mansoni</i>	2 (11%)
<i>S. haematobium</i>	1 (5.6%)
Other	15 (83.4%)
Urine microscopy analysis	
Tests performed	111 (97.4%)
Positive (<i>S. haematobium</i>)	9 (8.1%)
POC-CCA	
Tests performed	102 (89.5%)
Positive	14 (13.7%)
Feces RT-PCR	
Tests performed	51 (44.7%)
Positive	2 (3.9%)
Urine RT-PCR	
Tests performed	77 (67.5%)
Positive	1 (1.3%)
Eosinophilia	
Tests performed	114 (100%)
Positive (>500/mm ³)	14 (12.3%)
Mean ± SD	1,270 ± 1,011.2

Table 2: Laboratory test data

Variable	Categories		
	Confirmed (n=12)	Suspected (n=13)	Confirmed and suspected (n=25)
Country of birth N (%)	12	13	25

Table 3: Clinical characteristics of children with suspected and/or confirmed diagnostic of schistosomiasis

Mali	8	7	15
Guinea	2	5	7
Burkina Faso	1	0	1
Côte d'Ivoire	0	1	1
France	1	0	1
Male: female sex ratio	12/0	12/1	24/1
Age on the day of consultation (years)			
N (%)	12 (100%)	13 (100%)	25 (100%)
Mean ± SD	13.9± 2.8	15.8 ± 0.95	14.9±2.3
Unaccompanied minor refugees (n%)	5 (42%)	11 (85%)	16 (64%)
Weight percentile			
N (%)	12 (100%)	13 (100%)	25 (100%)
Mean ± SD	46,6± 23.1	57 ±21.5	59.6 ±23.25
Height percentile			
N (%)	11 (92%)	11 (85%)	22 (88%)
Mean ± SD	55 ±24.6	62,3 ±17.2	61,6 ±20.1
Signs and symptoms			
N(%)	12 (100%)	13 (100%)	25 (100%)
None	4 (36.4%)	9 (69%)	13 (52%)
Urinary (hematuria)	5 (45.5%)	1 (7.7%)	6 (24%)
Intestinal:	3 (25%)	3 (23%)	6 (24%)
Abdominal pain	3 (100%)	1 (33%)	4 (66.7%)
Bloody stools	0	1 (33%)	1 (16.7%)
Other	0	1 (33%)	1 (16.7%)
Duration of urinary tract symptoms (months)			
N (%)	4 (80%)	1 (100%)	5 (83%)
Mean ± SD	36 ±36.9	48	38±33.4
Duration of intestinal symptoms (months)			
N (%)	3 (100%)	3 (100%)	6 (100%)
Mean ± SD	9.3±3.8	20,6±19.7	15±15.3
Urine dipstick test for hematuria			
N (%)	11 (92%)	13 (100%)	24 (96%)
(n%)	4 (36,4%)	2 (15.3%)	6 (25%)
Time interval between arrival in France and consultation (years)			
N (%)	11 (92%)	13 (100%)	24 (96%)
Mean ± SD	0.7±1.5	0.4±0.4	0.5±0.9

Variable	Category	Studied population		p-value	Crude OR [95% CI]	Adjusted OR [95% CI]
		Suspected/ confirmed IS (n=25)	Negative (n=89)			
Age on the day of consultation (years) Mean ± SD		14.9±2.3	12.7±4	<0.001	2.4 [1.16-4.36]	1.98 [0.85-4.67]
Unaccompanied minor refugees (n%)	Yes	16 (64%)	32 (35.9%)	0.006	2.84 [1.16-7.1]	3.09 [1.04-9.42]
Symptoms (n%)	Yes	12 (48%)	18 (20.2%)	0.003	3.74 [1.48-9.79]	3.32 [1.16-10.51]
Urine dipstick test for hematuria (n%)	Yes	6 (25%)	4 (4.5%)	0.004	7.48 [1.81-32.12]	10.47 [2.14-56.21]

Table 4: Bivariate and multivariate analyses comparing suspected/confirmed IS cases and negative children

Diagnostic method	Sensitivity % [95% CI]	Specificity % [95% CI]	PPV [95% CI]	NPV [95% CI]
WB	83 [61-95]	99 [92-100]	95 [75-100]	95 [87-98]
ELISA	83 [61-95]	99 [92-100]	95 [75-100]	95 [87-98]
IHA	48 [27-69]	100 [95-100]	100 [72-100]	85 [76-92]
Urine microscopy analysis	35 [16-57]	100 [95-100]	100 [63-100]	82 [73-90]
POC-CCA	26 [10-48]	90 [80-96]	46 [19-75]	78 [68-87]
ICT	100 [85-100]	89 [79-95]	74 [55-88]	100 [94-100]

Table 5: Diagnostic performances of six schistosomiasis diagnostic tests from results of LCA