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MAJOR ARTICLE

Acute Schistosomiasis With a *Schistosoma mattheei* × *Schistosoma haematobium* Hybrid Species in a Cluster of 34 Travelers Infected in South Africa

Lieselotte Cnops,[1] Tine Huyse,[1,2] Ula Maniewski,[1] Patrick Soentjens,[1] Emmanuel Bottieau,[1] Marjan Van Esbroeck,[1,a] and Joannes Clerinx[1,a]

[1]Department of Clinical Sciences, Institute of Tropical Medicine Antwerp, Antwerp, Belgium, and [2]Department of Biology, Royal Museum for Central Africa, Tervuren, Belgium

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Background. Diagnosis of schistosomiasis remains elusive soon after infection. We evaluated several diagnostic methods in a cluster of travelers with simultaneous freshwater exposure in South Africa.

Methods. Eosinophil count, schistosome antibody tests, stool and urine microscopy, and serum *Dra1* PCR assays were performed at weeks 4–5 (early symptomatic phase), 7–8 (praziquantel treatment), and 13–14 (after treatment). Sequencing was done on serum samples from 3 patients to identify the species.

Results. Of the 34 travelers (16 adults and 18 children), 32 developed symptoms 2–6 weeks after exposure. A raised eosinophil count (>750/μL) was seen in 12 of 33 at weeks 4–5, and in 22 of 34 at weeks 7–8. *Schistosoma* antibodies were detected in 3 of 33 at weeks 4–5 and in 12 of 34 at weeks 7–8 and weeks 13–14. The *Dra1* PCR result was positive in 24 of 33 travelers at weeks 4–5, in 31 of 34 at weeks 7–8, in 25 of 34 at weeks 13–14, and at least once in all. Ova were absent in all urine and stool samples obtained. Sequencing identified *Schistosoma mattheei* nuclear and *Schistosoma haematobium* mitochondrial DNA, indicative of a hybrid species.

Conclusions. The *Dra1* PCR confirmed the diagnosis in all exposed travelers at a much earlier stage than conventional tests. The causative species is probably an *S. mattheei* × *S. haematobium* hybrid.

Keywords. schistosomiasis; hybrid; South Africa; travelers.

In travelers, early diagnosis of acute schistosomiasis remains difficult. Symptoms may appear 3–8 weeks after to freshwater exposure in an endemic region but are nonspecific. A raised eosinophil count is a key marker when symptoms appear, and accompanies immune sensitization to emerging schistosome antigens when the larval stadia mature into adult mating schistosomes, which usually happens 4–8 weeks after infection [1]. Serum antibody tests based on adult worm antigen and/or egg antigens are usually nonreactive during that stage, and egg shedding in stool and/or urine often starts 6–12 weeks after infection, when early symptoms have already abated.

New diagnostic techniques have recently been developed but not yet implemented routinely so far [2]. Real-time polymerase chain reaction (PCR) using either genus- or species-specific target markers has demonstrated *Schistosoma* DNA in stool or urine of travelers and people from endemic regions and has proved to be more sensitive than microscopy [3–6]. Promising developments are the detection of circulating anodic or cathodic antigens in serum and urine samples [2] and of *Schistosoma* DNA in serum samples in particular for patients in the prepatent period of infection [7, 8].

Molecular tools can help to identify the infective schistosome species, a difficult process in the case of hybridization events (ie, interbreeding between 2 species) [9]. Recently, infections with hybrids have been described in travelers returning from Mali [10], and in Corsica [11]. Hybridization can have a major impact on the host and the epidemiology of disease and may affect virulence and pathologic characteristics [9]. We describe here the follow-up of a unique cohort of travelers returning from South Africa, who were most likely infected with the hybrid species *Schistosoma mattheei* × *Schistosoma haematobium*.

METHODS

Patient Observation and Management

On 26 January 2017, a woman and her 2 children were referred by their family doctor to the outpatient clinic of the Institute of Tropical Medicine Antwerp, Belgium, 3 weeks after a stay at an ecolodge in South Africa. Both children presented with headache and cough and had a high eosinophil count. The mother was feverish. Further history taking revealed that 34 adults and children in total, belonging to 8 Belgian families traveling in 2 groups, had been exposed to fresh water of the Witrivier

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[a]M. V. E. and J. C. both contributed equally.

Correspondence: L. Cnops, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium (lcnops@itg.be).

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(uMhkunyane river) at the northern border area of KwaZulu-Natal with Mpumalanga, on 2 occasions 2 days apart around 26 December 2016 (first group) and 5 January 2017 (second group). All had swum and rafted up to several hours to sections of the river with reduced water flow, downstream from a cattle crossing. Exposure, symptoms, time lapse and presence of eosinophilia were highly suspect for acute schistosomiasis.

Through the travel agency, all involved families were asked to present themselves at our outpatient clinic for a first visit and were seen 4–5 weeks after first exposure. All exposed persons were asked to participate in a previously approved prospective study to evaluate new diagnostic tests during acute schistosomiasis. A second visit was scheduled at 7–8 weeks after exposure ,when treatment with praziquantel was given in accordance with the routine treatment practice at our outpatient clinic, and a third visit at 13–14 weeks, serving as the posttreatment visit. Of note, none of the included persons had reported previous exposure to another known source of human schistosomiasis.

Diagnostic Methods

According to the prospective study protocol, the following examinations were performed at each visit: full blood count, microscopy of stool and urine samples, schistosome antibody assays (enzyme-linked immunosorbent assay [ELISA] and indirect hemagglutination inhibition assay [IHA]) and real-time PCR.

To detect *Schistosoma* eggs in feces, a single stool sample was processed for microscopic detection on a direct smear and after an ether concentration technique for ova. Detection of *Schistosoma* eggs in urine samples was performed by microscopic evaluation of the sediment of ≥20 mL of end-stream urine after centrifugation.

Schistosome antibodies were detected using 2 methods, according to the manufacturer's instructions: the Bordier *Schistosoma mansoni* ELISA kit (Bordier Affinity Products), using a mix of *S. mansoni* soluble egg and soluble adult worm antigens, and an IHA with the ELI.H.A *Schistosoma* kit (ELITechGroup Microbiology), using *S. mansoni* adult worm antigen–covered sheep red blood cells. A cutoff ratio of 1.0 and a titer of 160 were used for both tests, respectively, as recommended by the manufacturer.

Real-time PCR was performed as described elsewhere by Cnops et al [3] and Bonnefond et al [7], with minor changes. DNA was extracted from 1 mL of serum and urine and of 1 g of stool with the Maxwell RSC Blood DNA Kit (AS1400) on the Maxwell RSC automated system (Promega). Two PCR analyses were performed on serum samples, targeting the highly repetitive nuclear *Dra1* gene for detection of species of the *S. haematobium* complex (*Dra1* PCR) and the tandem repeat *Sm1–7* gene for the detection of species of the *S. mansoni* complex group (*Sm1–7* PCR) [7, 12]. The 28S PCR targeting all *Schistosoma* species (genus PCR) [3] was tested on stool and urine samples. The reactions were assessed using the LightCycler 96 PCR system (Roche Diagnostics).

Identification of the causative schistosome species was performed by sequencing both the nuclear and mitochondrial schistosome DNA markers on a selection of serum samples that had a low cycle threshold (Ct) value (<30; n = 11) with the *Dra1* PCR. For the nuclear DNA target, genus PCR was used, and of the 5 samples that were positive (Ct 36–39), the amplicons were sequenced. In 3 of those serum samples, a partial fragment of the mitochondrial DNA region (cytochrome c oxidase subunit I [COI]) could also be detected and sequenced after a nested conventional PCR approach. The first-round PCR was used as a template (1:10 dilution) for the second-round PCR that used the same forward primer but a different, nested, reverse primer (Schisto3) [13–15]. The COI amplicons were purified following the ExoSAP (Fermentas) protocol and sent to Macrogen for Sanger sequencing using BigDye chemistry.

Treatment Procedures

Patients with acute symptoms were treated with steroids (methylprednisolone, 0.5 mg/kg) for ≥3 days until symptoms subsided. At weeks 7–8, when symptoms had abated, all patients were given praziquantel, at 40 mg/kg in 2 divided doses 2 hours apart, followed by a single dose of methylprednisolone 2 hours thereafter. At weeks 13–14, a second dose of praziquantel (40 mg/kg) was administered, without steroids.

Statistical Analysis

An analysis of variance test was used to compare means of continuous values with normal distribution, and a Mann-Whitney/Wilcoxon nonparametric test was used for other continuous values. For comparison of groups and categories, a Mantel-Haenszel or Fisher exact test was used, according to sample size.

Ethical Issues

Patients were informed about the suspected diagnosis and provided written consent to be included in the ongoing observational study on acute schistosomiasis (Institute of Tropical Medicine Antwerp), for which ethical clearance had been obtained. Sampling and testing followed the standard-of-care procedure for suspected schistosomiasis, with the exception of the PCR tests, for which an additional serum sample was collected, according to the study protocol. The treatment and follow-up scheme conformed with current practice at our clinic.

RESULTS

Acute Symptomatic Phase

The cluster consisted of 34 travelers: 18 children aged 5–15 years (median, 12 years) and 16 adults aged 39–49 years (median, 43 years). All reported ≥1 bathing contact in one of the two sites of the Witrivier on day 1 and/or day 3 of exposure. Children were exposed for a longer time because they were

Table 1. Symptoms Appearing During the Acute Phase[a]

Symptom	Patients, No. (%)
Among all patients (n = 34)	
"Swimmer's itch"	16 (47)
Any acute symptoms	32 (94)
Among patients with symptoms (n = 32)	
Fever	22 (69)
Cough	16 (50)
Abdominal pain	14 (44)
Diarrhea	5 (15)
Headache	22 (69)
Muscle ache	17 (53)

[a]The onset of symptoms occurred 16–41 days after exposure (median, 25 days; interquartile range, 22–29 days). Swimmer's itch occurred within 48 hours after exposure. Overall, 32 of 34 patients had symptoms.

involved in building and sailing rafts. Within 48 hours after exposure, an itchy rash ("swimmer's itch") developed in 16 of 34 (47%) (Table 1). Symptoms of acute schistosomiasis developed 16–41 days (median, 25 days; interquartile range, 22–29 days) after exposure in 32 of 34 (94%) travelers; 22 of 32 (69%) had fever. Several patients had multiple symptoms. Most reported headache and muscle ache.

Diagnostic Evaluation

Thirty-three travelers were seen for the first time 27–37 days (median, 29 days), after exposure (weeks 4–5), and 1 adult at 50 days. At weeks 4–5, the eosinophil count was clearly elevated (>750/μL) in 12 of 33 (36%), and schistosome antibodies were detected in 3 (9%) with IHA but in none with ELISA. Lowering the eosinophil cutoff threshold to >500/μL increased the proportion to 16 of 33 (48%) (Table 2).

Ova were absent, and schistosome genus PCR results were negative in all 33 urine and 31 stool samples collected during weeks 4–5. In contrast, the *Dra1* PCR results were positive in serum samples from 24 of 33 travelers (73%), of whom one also tested weakly positive with *Sm1–7* PCR, probably owing to cross-reactivity [7].

During weeks 7–8, all 34 travelers were tested. The eosinophil count was >750/μL in 22 (65%), and >500/μL in 27 (79%). ELISA demonstrated seroconversion in 12 (35%). The IHA test result was negative in 21, and it was invalid in 13, owing to the presence of anti-sheep red blood cell antibodies, making interpretation impossible. No eggs or parasite DNA were detected in any of the 34 urine and 32 stool samples obtained. The *Dra1* PCR results were positive in 30 of 34 patients (88%), including the single patient not tested at weeks 4–5, and in 6 patients with previously negative results. The *Sm1–7* PCR results were negative in all 34 samples, and *Sm1–7* PCR was not performed with the follow-up samples from weeks 13–14.

At weeks 13–14 (posttreatment visit), the eosinophil count remained elevated to >750/μL in 3 of 34 patients (9%), and antibodies were detected in 11 of 34 (32%) with ELISA. IHA results were positive in 1, negative in 31 and could not be interpreted in 2 because of a nonspecific reaction (see above). The *Dra1* PCR test still showed a positive signal in serum samples from 23 of 34 patients (71%).

By weeks 13–14, the cumulative numbers for diagnostic confirmation were 15 of 34 patients (44%) for ELISA and all 34 (100%) for *Dra1* PCR. In patients with positive *Dra1* PCR results, Ct values were lowest (corresponding to the highest amount of parasite DNA) at weeks 4–5, with a mean Ct value of 30.48, and increased during follow-up to mean values of 33.29 at weeks 7–8 and 35.97 at weeks 13–14.

Acute Schistosomiasis in Children and Adults

Children reported swimmer's itch more frequently than adults (12 of 16 [75%] vs 6/18 [33%]; $P = .02$).There was no difference

Table 2. Diagnostic Test Results in a Cluster of Patients With Acute Schistosomiasis

	Patients, No./Total No. (%)			
Result	Weeks 4–5	Weeks 7–8	Weeks 13–14	Cumulative
Eosinophil count				
>500/μL	16/33 (48)	27/34 (79)	7/34 (21)	28/34 (82)
>750/μL	12/33 (36)	22/34 (65)	3/34 (9)	24/34 (71)
>1000/μL	9/33 (27)	21/34 (62)	2/34 (6)	23/34 (68)
Positive Ab results				
ELISA	0/33 (0)	12/34 (35)	11/34 (32)	15/34 (44)
IHA	3/33 (9)	0/34[a] (0)	1/34[a] (3)	4/34[a] (12)
Schistosoma ova in samples				
Urine	0/33 (0)	0/34 (0)	0/34 (0)	0/34 (0)
Stool	0/31 (0)	0/27 (0)	0/28 (0)	0/34 (0)
Positive serum PCR results				
Dra1	24/33 (73)	30/34 (88)	24/34 (71)	34/34 (100)
Sm1–7	1/34 (3)	0/34 (0)	ND	1/34 (3)

Abbreviations: Ab, antibody; ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination inhibition assay; ND, not done; PCR, polymerase chain reaction.
[a]No interpretation was possible in 13 of 34 serum samples at weeks 7–8 and in 2 of 34 at weeks 13–14), because of the presence of interfering hemolytic antisheep antibodies.

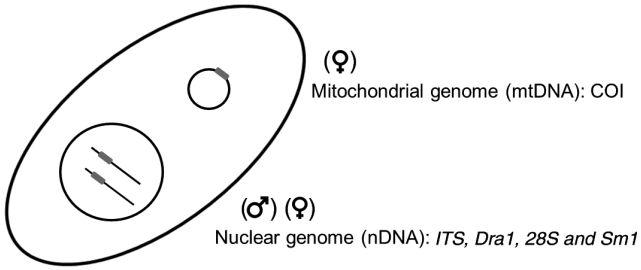

Figure 1. Detection of schistosome hybrids with polymerase chain reaction (PCR). Hybrid detection of schistosomes requires a multilocus approach, analyzing both nuclear and mitochondrial DNA simultaneously from individual specimens. Nuclear DNA (nDNA) can be detected by several markers (*ITS, Dra1, 28S*, and *Sm1–7*) and is inherited by male (♂) and female (♀) worms. Mitochondrial DNA (mtDNA), inherited by the female worm only, can be detected by the cytochrome c oxidase subunit I (COI) marker. When nDNA and mtDNA from different species are detected in a single egg, this is indicative of a hybrid species. In the hybrid name, the male species is mentioned first, followed by a times sign (×) and the female species. In this cluster, we detected nDNA of *Schistosoma mattheei* with 28S PCR and mtDNA of *Schistosoma haematobium* with COI PCR in serum samples 3 individuals, indicating an *S. mattheei* × *S. haematobium* hybrid.

between children and adults in the frequency of any individual symptom of acute schistosomiasis, nor in the prepatent time lapse. Children tended to have a higher eosinophil count than adults soon after infection at weeks 4–5 (median 790/μL vs 250/μL; $P = .001$) and at weeks 13–14 after treatment (median 390/μL vs 175/μL; $P = .002$) but the difference was not significant at weeks 7–8 (median, 1.440/μL vs 880/μL; $P = .06$). Likewise, the proportion of children with a positive *Dra1* PCR result was significantly higher than that of adults at weeks 4–5 (16 of 18 [88%] vs 8 of 15 [53%]; $P = .046$), but no difference was noted at weeks 7–8 (17 of 18 [94%] vs 13 of 16 [81%]; $P = .25$) or weeks 13–14 (12 of 18 [67%] vs 12 of 16 [75%]; $P = .69$). There was no significant difference in median Ct values between children and adults at any of the 3 visits.

Infective Species Determination

The sequencing of schistosome nuclear and mitochondrial DNA extracted from serum samples identified an *S. mattheei* × *S. haematobium* hybrid species (Figure 1). The genus PCR results was positive in 5 of the 11 serum samples with the lowest Ct values at *Dra1* PCR. In all 5, the 28S amplicon (264 base pairs) showed identity scores of 95%–100% with *S. mattheei* (accession no. AY157265), with a coverage of 98%–100%. For 3 of these samples, the nested COI PCR assay produced positive results. The resulting sequences (377 base pairs) were all identical to *S. haematobium* (accession no. JQ397397).

DISCUSSION

Clinical Aspects

We described herein a unique cohort of travelers simultaneously infected in South Africa with diagnosis during the early infective stage. Although almost all patients reported extensive and repeated exposure on 2 occasions at river sections with slow-moving water, the infection rate in this cluster was still exceptionally high. As in our cluster, swimmer's itch has been reported much more frequently after infection with schistosome hybrids than in nonhybrid cluster observations [9, 16–18]. *Acute schistosomiasis* refers to the clinical and diagnostic events that may appear within 3 months after infection. In most observations, patients rarely report only a single exposure, which renders the timing of infection and subsequent clinical events imprecise. Data from clusters like this are helpful to fine-tune the time frame of acute symptoms to 2–6 weeks after exposure [16]. It remains unclear whether symptoms result from hypersensitivity to antigens from maturing schistosomules, from oviposition, or from a combination of both [1].

Fever was the most prominent symptom, raising suspicion of acute schistosomiasis when a history of recent freshwater exposure emerged. Headache and muscle ache were reported unusually frequently, whereas most cluster studies report cough and abdominal pain as predominant symptoms, often concomitant with fever [16, 19]. Contrary to what we observed in a cluster of *S. mansoni* infection, urticaria and angioedema were notably absent [17]. This may be due to distinct species-specific antigens.

Diagnostic Performance in Acute Schistosomiasis

A raised eosinophil count is a prominent, but not a sensitive, feature of a recent schistosome infection [1, 10, 16, 17]. It is not specific for primary infection [17]. The current findings confirm that the eosinophil count gradually increases from symptom onset until symptoms subside 6–8 weeks after exposure, and that it decreases rapidly after mature schistosomes are eliminated with praziquantel. There is still debate about the best cutoff eosinophil count level to raise suspicion in travelers. Lowering the cutoff to >500/μL raises the sensitivity for (acute) schistosomiasis substantially, but probably at the expense of specificity when applied indiscriminately to travelers visiting an outpatient clinic [17, 20].

Etiologic diagnosis of acute schistosomiasis in its early phase (by week 5) has been elusive hitherto [16]. Eggs are usually not yet excreted when symptoms appear, and antibody tests lack sensitivity during the early infection phase [19, 21]. Sensitivity does not depend so much on the type of antigen used, whether adult worm or egg antigen, or even cercarial antigen, which showed promise, however, in acute infection [22, 23].

Molecular tests have been sensitive diagnostic tools in several cluster studies, in acute *S. mansoni* as well as in *S. haematobium* infection [7, 10, 17, 23]. However, in almost all studies, PCR tests were performed rather late in the course of acute infection, when symptoms had already subsided or had been suppressed with steroids. In travelers with acute schistosomiasis, PCR tests using different target sequences have shown high sensitivity with stool, urine, blood/serum, and semen samples, biopsy specimens, and, recently, cerebrospinal fluid samples

[7, 24]. In the current study, PCR tests successfully demonstrated schistosomiasis in serum but not in stool or urine samples, with the lowest Ct values seen in the early acute phase. This is consistent with the hypothesis that parasitic DNA from maturing schistosomules and adult schistosomes is already present in the bloodstream in sufficient quantities during the prepatent phase. At that time, parasite DNA levels are highest, and they decline during follow-up, even before anthelmintic treatment.

Both eosinophil counts and the amount of schistosome DNA in serum were higher in children than in adults early after infection. However, similar Ct values suggest that children do not acquire a higher parasite load at primary exposure.

Diagnosing Schistosome Hybrids

The current cluster provided more insight into the epidemiology and hybridization of *S. mattheei* with *S. haematobium*. Schistosomiasis is endemic in South Africa, particularly in the province of KwaZulu-Natal, and *S. haematobium* is the most common species, with a prevalence of 37.5% in uMkhanyakude District in 2015 [25]. The prevalence and intensity of *S. haematobium* infections in rural regions can be higher during the hot than during the cold season [26]. These seasonal fluctuations are also seen for *S. mansoni* and *S. mattheei* in South Africa, but their prevalence is lower than that of *S. haematobium* [27]. It is known that the nonhuman schistosome species *S. mattheei* can infect humans, but only as a hybrid with *S. haematobium* [28]. Since 1948, potential hybrids between *S. haematobium* and *S. mattheei* have reported in Zimbabwe and South Africa among patients with urogenital schistosomiasis [29], and these were first confirmed using isoelectric focusing of enzymes by Wright and Ross in 1980 [30]. More recently, *S. haematobium* × *S. mattheei* hybrids were retrieved from children in Malawi [31].

In the current study, classic species determination based on morphologic characteristics of ova was impossible, because ova were simply not found. Schistosome infection was confirmed in all patients by *Dra1* PCR of serum samples indicating the presence of circulating immature or mature schistosomes of the *S. haematobium* complex group. Additional typing by sequencing the nuclear DNA with genus PCR and the mitochondrial DNA with COI PCR (Figure 1) identified the parasite as an *S. mattheei* × *S. haematobium* hybrid. Mitochondrial DNA is maternally inherited. As a consequence this hybrid had to be a crossbreed between a male *S. mattheei* and a female *S. haematobium*.

Given the absence of eggs, we hypothesize that this crossbreed was not fertile. The reverse crossbreed, male *S. haematobium* × female *S. mattheei*, has been found to produce viable eggs [29–32]. Although crossbreeds between male *S. haematobium* and females from other species are usually viable, the reverse crossbreeds are not [33]. Because we performed the analysis on serum samples and not on the individual eggs, a mixed infection of *S. mattheei* males, which do not usually reach sexual maturity in humans, with *S. haematobium* is less likely but could not be excluded [34]. Although in 1990 the prevalence of human hybrid infections with the bovine parasite, *S. mattheei*, was reported to be relatively high in children from Eastern Transvaal (Mpumalanga) Lowveld, human infection with a hybrid between a male *S. mattheei* and a female *S. haematobium* has not been reported before [34].

In conclusion, in the cluster of travelers with acute schistosomiasis that we report, the schistosome infection rate was very high and caused acute-phase symptoms in almost all infected. PCR-based diagnosis based on a blood sample was by far the most sensitive diagnostic test soon after infection, and may become available as a standard test for travel clinics. The *Dra1* PCR test detects *S. haematobium* and its hybrid forms. In the absence of ova, sequencing of schistosome DNA from a blood sample can reveal the infective schistosome species. A sterile hybrid infection with male *S. mattheei* × *female S. haematobium* was demonstrated in 3 patients.

Notes

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References

1. Langenberg MCC, Hoogerwerf MA, Janse JJ, van Lieshout L, Corstjens PLAM, Roestenberg M; CoHSI clinical trial team. Katayama syndrome without *Schistosoma mansoni* eggs. Ann Intern Med **2019**; 170:732–3.
2. Utzinger J, Becker SL, van Lieshout L, van Dam GJ, Knopp S. New diagnostic tools in schistosomiasis. Clin Microbiol Infect **2015**; 21:529–42.
3. Cnops L, Tannich E, Polman K, Clerinx J, Van Esbroeck M. *Schistosoma* real-time PCR as diagnostic tool for international travelers and migrants. Trop Med Int Heal **2012**; 17:1208–16.
4. Meurs L, Brienen E, Mbow M, et al. Is PCR the next reference standard for the diagnosis of *Schistosoma* in stool? a comparison with microscopy in Senegal and Kenya. PLoS Negl Trop Dis **2015**; 9:e0003959.
5. Aryeetey YA, Essien-Baidoo S, Larbi IA, et al. Molecular diagnosis of *Schistosoma* infections in urine samples of school children in Ghana. Am J Trop Med Hyg **2013**; 88:1028–31.
6. Guegan H, Fillaux J, Charpentier E, et al. Real-time PCR for diagnosis of imported schistosomiasis. PLoS Negl Trop Dis **2019**; 13:e0007711.
7. 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.
8. Bonnefond S, Cnops L, Duvignaud A, et al. Early complicated schistosomiasis in a returning traveller: key contribution of new molecular diagnostic methods. Int J Infect Dis **2019**; 79:72–4.
9. Leger E, Webster JP. Hybridizations within the genus *Schistosoma*: implications for evolution, epidemiology and control. Parasitology **2017**; 144:65–80.
10. Soentjens P, Cnops L, Huyse T, et al. Diagnosis and clinical management of *Schistosoma haematobium-Schistosoma bovis* hybrid infection in a cluster of travelers returning from Mali. Clin Infect Dis **2016**; 63:1626–9.
11. Oleaga A, Rey O, Polack B, et al. Epidemiological surveillance of schistosomiasis outbreak in Corsica (France): are animal reservoir hosts implicated in local transmission? PLoS Negl Trop Dis **2019**; 13:e0007543.
12. Wichmann D, Panning M, Quack T, et al. Diagnosing schistosomiasis by detection of cell-free parasite DNA in human plasma. PLoS Negl Trop Dis **2009**; 3:e422.
13. Huyse T, Webster BL, Geldof S, et al. Bidirectional introgressive hybridization between a cattle and human schistosome species. PLoS Pathog **2009**; 5:e1000571.
14. Lockyer AE, Olson PD, Ostergaard P, et al. The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of *Schistosoma* Weinland, 1858. Parasitology **2003**; 126:203–24.

15. Littlewood DT, Rohde K, Clough KA. Parasite speciation within or between host species? phylogenetic evidence from site-specific polystome monogeneans. Int J Parasitol **1997**; 27:1289–97.
16. Grandière-Pérez L, Ansart S, Paris L, et al. Efficacy of praziquantel during the incubation and invasive phase of *Schistosoma haematobium* schistosomiasis in 18 travelers. Am J Trop Med Hyg **2006**; 74:814–8.
17. Clerinx J, Bottieau E, Wichmann D, Tannich E, Van Esbroeck M. Acute schistosomiasis in a cluster of travelers from Rwanda: diagnostic contribution of schistosome DNA detection in serum compared to parasitology and serology. J Travel Med **2011**; 18:367–72.
18. Lachish T, Tandlich M, Grossman T, Schwartz E. High rate of schistosomiasis in travelers after a brief exposure to the high-altitude Nyinambuga crater lake, Uganda. Clin Infect Dis **2013**; 57:1461–4.
19. Jauréguiberry S, Paris L, Caumes E. Acute schistosomiasis, a diagnostic and therapeutic challenge. Clin Microbiol Infect **2010**; 16:225–31.
20. Logan S, Armstrong M, Moore E, et al. Acute schistosomiasis in travelers: 14 years' experience at the Hospital for Tropical Diseases, London. Am J Trop Med Hyg **2013**; 88:1032–4.
21. Van Gool T, Vetter H, Vervoort T, Doenhoff MJ, Wetsteyn J, Overbosch D. Serodiagnosis of imported schistosomiasis by a combination of a commercial indirect hemagglutination test with *Schistosoma mansoni* adult worm antigens and an enzyme-linked immunosorbent assay with *S. mansoni* egg antigens. J Clin Microbiol **2002**; 40:3432–7.
22. Hayunga EG, Möllegård I, Duncan JF, Sumner MP, Stek M Jr, Hunter KW Jr. Development of circulating antigen assay for rapid detection of acute schistosomiasis. Lancet **1986**; 2:716–8.
23. Smith H, Doenhoff M, Aitken C, et al. Comparison of *Schistosoma mansoni* soluble cercarial antigens and soluble egg antigens for serodiagnosing schistosome infections. PLoS Negl Trop Dis **2012**; 6:e1815.
24. van Delft F, Visser L, Polderman A, van Lieshout L. Cough and alterations in semen after a tropical swim. Neth J Med **2007**; 65:304–6.
25. Kabuyaya M, Chimbari MJ, Mukaratirwa S. Infection status and risk factors associated with urinary schistosomiasis among school-going children in the Ndumo area of uMkhanyakude District in KwaZulu-Natal, South Africa two years post-treatment. Int J Infect Dis **2018**; 71:100–6.
26. Christensen EE, Taylor M, Zulu SG, et al. Seasonal variations in *Schistosoma haematobium* egg excretion in school-age girls in rural KwaZulu-Natal Province, South Africa. S Afr Med J **2018**; 108:352–5.
27. Donnelly FA, Appleton CC. Observations on the field transmission dynamics of *Schistosoma mansoni* and *S. mattheei* in southern Natal, South Africa. Parasitology **1985**; 91(pt 2):281–90.
28. Appleton C, Miranda N. Locating bilharzia transmission sites in South Africa: guidelines for public health personnel. South African J Infect Dis **2015**; 30:95–102.
29. Kruger FJ. Frequency and possible consequences of hybridization between *Schistosoma haematobium* and *S. mattheei* in the Eastern Transvaal Lowveld. J Helminthol **1990**; 64:333–6.
30. Wright CA, Ross GC. Hybrids between Schistosoma haematobium and S. mattheei and their identification by isoelectric focusing of enzymes. Trans R Soc Trop Med Hyg **1980**; 74:326–32. PMID: 7434427.
31. Webster BL, Alharbi MH, Kayuni S, et al. Schistosome interactions within the *Schistosoma haematobium* group, Malawi. Emerg Infect Dis **2019**; 25:1245–7.
32. Van Wyk JA. The importance of animals in human schistosomiasis in South Africa. S Afr Med J **1983**; 63:201–3.
33. Southgate VR, Jourdane J, Tchuenté LA. Recent studies on the reproductive biology of the schistosomes and their relevance to speciation in the Digenea. Int J Parasitol **1998**; 28:1159–72.
34. Kruger FJ, Evans AC. Do all human urinary infections with *Schistosoma mattheei* represent hybridization between *S. haematobium* and *S. mattheei*? J Helminthol **1990**; 64:330–2.