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BRIEF REPORT

Diagnosis and Clinical Management of *Schistosoma haematobium–Schistosoma bovis* Hybrid Infection in a Cluster of Travelers Returning From Mali

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Ten Belgian travelers returned from Mali with a *Schistosoma haematobium–Schistosoma bovis* hybrid infection, confirmed by DNA sequencing from eggs. Clinical symptoms and laboratory findings resembled those of classic acute schistosomiasis, but the detected eggs were morphologically unusual.

Keywords. *Schistosoma haematobium*; *Schistosoma bovis*; hybrid; travelers.

The Dogon valley in Mali is a known risk area for schistosomiasis, mainly caused by *Schistosoma haematobium* [1–5]. Molecular techniques have demonstrated hybridization between *Schistosoma* species of humans and cattle [6, 7]. Hybridization may alter the epidemiology of the disease and the spectrum of the intermediate host producing infective cercariae, and it might enhance infectivity [8, 9] and influence the clinical presentation [10]. Little is known, however, about the pathological mechanism of such hybrids in humans, particularly in nonimmune travelers. We describe herewith the clinical and laboratory characteristics in a cluster of 10 Belgian travelers with a diagnosis of hybrid *S. haematobium–Schistosoma bovis* infection after returning from Mali.

METHODS

In September 2010 the index patient received a diagnosis of acute schistosomiasis at the Institute of Tropical Medicine of Antwerp, Belgium, 5 weeks after exposure to fresh water in the Dogon Valley of Mali. Nine fellow travelers were actively traced, evaluated, treated, and followed up at the outpatient clinic.

Serum, urine, and stool samples were collected at the first visit and 6 weeks after treatment. Diagnosis of schistosomiasis was based on the presence of *Schistosoma* sp. eggs in urine and/or stool samples, or by positive results of a schistosome serum antibody test (enzyme-linked immunosorbent assay and/or indirect hemagglutination inhibition assay [11]).

Several in-house real-time polymerase chain reaction (PCR) assays for schistosomiasis were performed: genus-specific PCR, to detect schistosome DNA in urine and/or stool samples [12]; Dra PCR, to detect *S. haematobium* complex in serum samples [13]; and Sm1–7 PCR, to detect *Schistosoma mansoni* complex in serum samples [13, 14]. Genotyping was performed on DNA extracts of 2 eggs obtained from a stool sample from 1 patient by sequencing the nuclear internal transcribed spacer (ITS) ribosomal RNA region and partial *cox*1 mitochondrial DNA region [6]. In accordance with the local ethical guidelines for noninterventional studies, patient consents were obtained to perform additional diagnostic tests.

RESULTS

All 10 patients reported a 15-minute swim at the pool of a waterfall in the Dogon valley on the 31 July in 2010. Nine patients were evaluated within 4 months ("early presenters") and 1 late presenter was seen 23 months after exposure. All patients reported symptoms of reversible pruritus, suggestive of "swimmer's itch," directly after swimming. Five patients (including the index patient) developed symptoms compatible with acute schistosomiasis, presenting with respiratory symptoms [4], fever [4], fatigue [4], gastrointestinal complaints [4], headache [3], palpitations, [3] and skin problems [5], such as papules [5], rash [3], and angioedema [1]. Two patients had painful micturition and terminal hematuria at the time of diagnosis (including the late presenter). Three were asymptomatic.

At the initial visit, 8 of the 9 early presenters had eosinophilia (Table 1). Seven had already *Schistosoma* sp. eggs in stool samples and 2 of them also had eggs in urine samples; results of schistosome antibody tests were negative in 4, but 3 of these patients seroconverted later. One remained negative 7 months after exposure. The other 2 patients with initially negative parasitological examination findings had a positive schistosome antibody test result.

The 10th patient had late-stage symptoms. Initially asymptomatic and seronegative but with raised eosinophilia, this patient preferred not to be treated, against medical advice. Almost 2 years after exposure, she developed macroscopic hematuria

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Table 1. Hematological, Serological, Microscopic, and Polymerase Chain Reaction Results Before and Shortly After Initial Treatment[a]

		Before Treatment									After Treatment								
G Patient	Time From Exposure to Diagnosis, d	Eosinophil Count, $\times 10^9$/L	ELISA	IHA	Eggs in Stool, No./g	Genus PCR (Stool)	Microscopy (Urine)	Genus PCR (Urine)	Dra PCR (Serum)	Sm1–7 PCR (Serum)	Time After Treatment, d	Eosinophil Count, $\times 10^9$/L	ELISA	IHA	Eggs in Stool, No./g	Genus PCR (Stool)	Microscopy (Urine)	Genus PCR (Urine)	Dra PCR (Serum)	Sm1–7 PCR (Serum)
1	39	2.13	Positive	Negative	Negative	NA	Negative	NA	Positive	Positive	21	0.34	Negative	1/160	Negative	Negative	Negative	Negative	Positive	Positive
2	58	0.88	Negative	1/640	Negative	NA	Negative	NA	Positive	Negative	52	0.43	Negative	1/320	Negative	Negative	Negative	Negative	Positive	Negative
3	88	1.78	Negative	Negative	360	Positive	Negative	NA	Positive	Positive	31	0.49	Negative	1/160	Negative	Negative	NA	Negative	Negative	Negative
4	88	3.94	Negative	Negative	40	Positive	Negative	NA	Positive	Negative	31	0.66	Negative	Negative	Negative	Negative	NA	Positive	Positive	Negative
5	81	4.51	Negative	1/160	10	Positive	Negative	NA	Positive	Positive	61	0.27	Negative	Negative	Negative	Negative	Sh	Positive	Negative	Negative
6	95	0.67	Negative	1/1280	80	Positive	Negative	Negative	Positive	Negative	30	0.26	Negative	1/640	Negative	Negative	Negative	Negative	Negative	Negative
7	109	1.02	Negative	Negative	60	Positive	Negative	Negative	Positive	Negative	35	0.36	Positive	1/320	Negative	Negative	Negative	Negative	Negative	Negative
8	109	0.59	Negative	1/160	580	Positive	Positive	Positive	Positive	Negative	33	0.34	Negative	1/1280	Negative	Negative	Negative	Negative	Positive	Positive
9	116	0.3	Negative	Negative	140	Positive	Positive	NA	Positive	Positive	35	0.19	Negative	1/640	Negative	Negative	Negative	NA	Negative	Negative
10	719	0.95	Positive	1/160	Negative	NA	Positive	Positive	Positive	Positive	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHA, immune hemagglutination assay; NA, not applicable; PCR, polymerase chain reaction; Sh, *Schistosoma haematobium*.

[a] Genus-specific PCR (genus PCR) targets 28S ribosomal RNA (all *Schistosoma* species) [11], Dra PCR targets the Dra gene of *Schistosoma haematobium* complex species [12], and Sm1–7 PCR targets the Sm1–7 gene of *Schistosoma mansoni* complex species [13].

and lumbar pain, had persistent eosinophilia, and had a positive schistosome antibody test result.

In 7 patients with eggs in stool samples, the egg load ranged from 10 to 580 eggs per gram (Table 1). The eggs were terminal spined and similar in shape to the eggs of *S. guineensis* (formerly *Schistosoma intercalatum*) (Figure 1) and of the recently reported *S. haematobium–S.bovis* hybrids in Senegal and Corsica [6, 16].

Results of genus-specific PCR were positive in all egg-containing stool specimens with cycle threshold values ranging from 27.6 to 32.9. Dra PCR, specific for *S. haematobium complex*, was positive in serum samples from all 10 patients at first presentation, whereas Sm1–7 PCR, which detects *S. mansoni* and *S. bovis* species [13], had positive results in 5 (Table 1).

DNA sequence analysis of 2 eggs recovered from stool samples (patient 3) confirmed its *S. haematobium–S. bovis* hybrid nature. The ITS ribosomal RNA was identical to *S. haematobium* (accession No. FJ588861), whereas the 384–base pair fragment of the *cox*1 sequence was identical to those of the hybrid species found in Corsica (KT354658) and Senegal (FJ588854) and differed by 1 base pair from a Senegalese *S. bovis* isolate (AJ519521).

Nine patients whose infection was diagnosed within 4 months after exposure were treated with praziquantel (40 mg/kg once daily for 3 days, with a maximum daily dose of 2400 mg) combined with corticosteroids (methylprednisolone, 32 mg once daily for 3 days) at diagnosis and retreated with praziquantel (40 mg/kg in a single dose) 6 weeks after the initial therapy. The 10th patient, who presented late, was treated with praziquantel 2400 mg once daily for 3 days. Adverse effects and paradoxical reactions (posttreatment clinical exacerbation) were prospectively recorded [18]. In 6 patients with disease manifestations, symptoms resolved soon after treatment. Three patients mentioned dizziness, fatigue, or a metallic taste, but none of them experienced paradoxical reactions after treatment.

Results of genus-specific PCR analysis of urine specimens remained positive in 2 patients, at 31 and 61 days after initial treatment (Table 1; patients 4 and 5). Patient 5 still excreted viable eggs. Dra PCR still demonstrated a signal in serum samples in 4 of 9 and Sm1–7 PCR in 2 of 9 patients at a median of 42 days (range, 21–61 days) after treatment.

DISCUSSION

To our knowledge, this is the first confirmed series of *S. haematobium–S. bovis* hybrid infection in a cluster of nonimmune travelers. The combined results of the 2 species-specific PCR tests on serum, the parasitological findings (morphological features and detection of eggs in both stool and urine samples), and the confirmation of hybridization between *S. haematobium* and *S. bovis* by DNA sequencing led us to conclude that all patients in this cluster were infected with a *S. haematobium–S. bovis* hybrid.

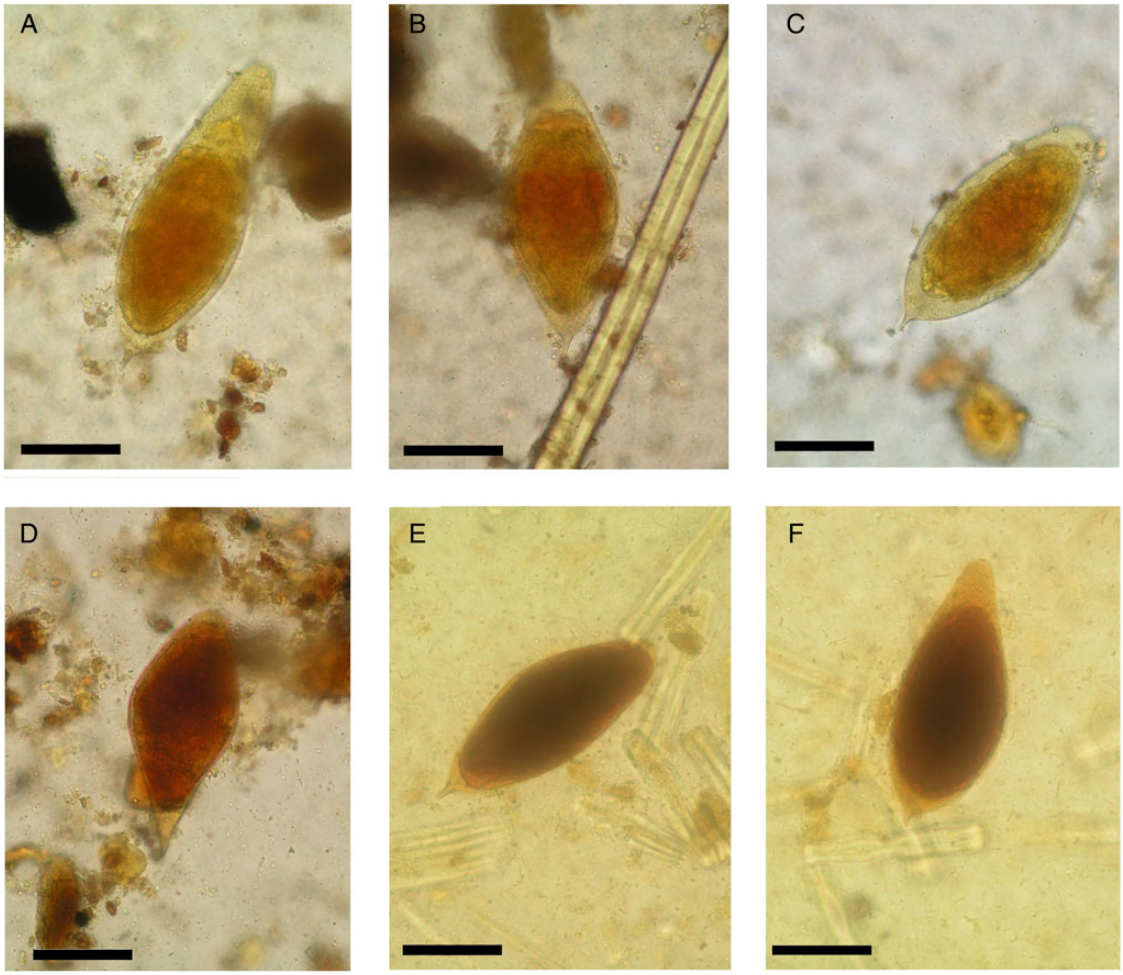

Figure 1. Polymorphic eggs seen in parasitological examination of stool sample from patient 8. The eggs looked similar to those of S*chistosoma guineensis* (formerly *Schistosoma intercalatum*) (140–240 µm; morphotype in [15–17]) but were smaller, 135–175 µm in length. Their shape was intermediate between the typical round to oval *Schistosoma haematobium* and the more elongate, spindle-shaped *Schistosoma bovis* eggs. Some eggs were more stretched, with an oval to diamond shape (*D, E*), and were larger (*A*) than the typical *S. haematobium* type, 110–170 µm long; some other eggs were smaller (*B, C*). Although *S. haematobium* has a characteristic small terminal spine (*C, F*), some eggs had a somewhat enlarged terminal spine (*A, D*). Scale bars represent 50 µm.

A similar hybrid was demonstrated in northern Senegal [6] and more recently in France (Corsica), although no eggs were found in stool samples in the latter outbreak [16]. In the past, infection with *S. haematobium* and *S. intercalatum* (since 2003 recognized as *Schistosoma guineensis* [18, 19]) has been reported in several clusters of travelers exposed in the Dogon Valley. In fact, it is not excluded that some cases of these reported clusters may have been infected with this hybrid form [2–4].

Schistosome hybridization may have important consequences in an epidemiological context, for disease prevalence, pathological characteristics, and treatment. The pathological mechanism of hybrid forms in humans is not well known. Experimental infection in laboratory animals demonstrated that hybrid forms can have a shorter maturation time, higher infectivity, and a broader spectrum of intermediate hosts [6, 9].

Symptoms may differ as well [20]. In our cohort, cutaneous lesions were predominantly present: first as swimmer's itch in all patients soon after exposure and in a second stage as papules, itching, and angioedema in 5 patients with acute schistosomiasis. Eosinophilia was present in virtually all cases, as expected in acute schistosomiasis, but egg shedding was surprisingly frequent (in 7 of 9 patients) for such an early stage. Moreover, contrary to what is usually seen in *S. haematobium* infection and what was observed in the Corsica "hybrid" outbreak [16], eggs were shed in stool rather than in urine. However, 2 patients, including the late presenter, had clinical symptoms of urinary schistosomiasis, as expected in *S. haematobium* infections. This observation suggests that the adult *S. haematobium–S. bovis* hybrid worms may have a slightly different anatomic localization, maybe closer to the perirectal venous plexus, or that their eggs may follow more erratic trajectories.

In acute schistosomiasis the current standard diagnostic techniques lack sensitivity and species specificity at first presentation [20, 21]. Diagnosis in nonimmune travelers is routinely performed by means of serology, because egg loads are usually low. In our cohort, however, more than one-third of the patients did not demonstrate schistosome antibodies at initial presentation [20, 21]. This observation highlights the need to systematically examine urine and samples in addition to performing serology, to increase the diagnostic yield even in acute schistosomiasis. More sensitive tools are necessary, however. Recently developed real-time PCR techniques for the detection of *Schistosoma* species in serum have shown promising results for early diagnosis of acute schistosomiasis in nonimmune travelers [12–14]. In this *S. haematobium–S. bovis* cluster, Dra PCR analysis of serum samples detected all cases at initial presentation, demonstrating very high sensitivity for this hybrid combination. This finding is especially important for those patients with initially negative serological and microscopic results. Genus-specific real-time PCR results correlated very well with egg shedding detected with microscopy of a concentrated stool or urine sample, but the study was not designed to assess sensitivity.

When used to monitor treatment response, genus-specific real-time PCR results were universally negative when eggs were absent in follow-up samples. In contrast, the serum Dra PCR test seemed less useful for monitoring treatment response, with findings still positive in 4 of the 9 patients 21–52 days after drug administration. In a posttreatment assessment of nonimmune infected travelers, performing stool microscopy and/or genus-specific PCR on stool and urine samples (preferably 6 weeks after treatment) may be the best approach currently available to ascertain cure.

In conclusion, hybrid schistosome infections in humans may be more common than previously thought. Diagnostic tools are readily available to confirm such infections. In combination with extensive clinical data from clusters of patients such as the cluster reported here, these tools will provide better insights into the epidemiological and pathological mechanisms of these hybrids.

Notes

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