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False-Positive *Plasmodium falciparum* Histidine-Rich Protein 2 Immunocapture Assay Results for Acute Schistosomiasis Caused by *Schistosoma mekongi*[▽]

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We report seven cases of false-positive *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) malaria assay results in patients with acute schistosomiasis caused by *Schistosoma mekongi*. PfHRP2 assays were negative in travelers infected with *Schistosoma mansoni* or *Schistosoma haematobium* ($n = 13$). Malaria was ruled out and rheumatoid factor was negative in all patients.

Acute schistosomiasis is a transient hypersensitivity reaction associated with tissue migration of schistosomula in nonimmune individuals. Fever, cough, rash, and other symptoms typically appear 2 to 12 weeks postexposure. Malaria is the most common cause of acute nonspecific febrile illness in travelers and has a geographic distribution similar to that of schistosomiasis (12).

Microscopic examination of stained blood smears is the gold standard for malaria diagnosis. In recent years, rapid malaria antigen detection assays were developed to assist in malaria diagnosis. These assays are increasingly used in regions in which malaria is nonendemic, due to high rates of false-negative results in blood smears (5). Moreover, these assays are regarded as sensitive (83 to 100%), specific (87 to 99%), and simple to perform (9).

We have recently reported seven cases of acute schistosomiasis caused by *Schistosoma mekongi* infection acquired in Laos (7). Four of these febrile travelers were initially evaluated for malaria by *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) assays. The assays were positive; however, malaria was ruled out by repeated negative blood smears and real-time PCR (RT-PCR). Consequently, we suspected that acute schistosomiasis may cause false-positive results in PfHRP2 assays. We investigated samples of patients with schistosomiasis caused by different species to evaluate the rate of false positivity in malaria antigen detection assays.

Study. A total of 23 patients were included: 16 patients with acute schistosomiasis and 7 patients with chronic schistosomiasis (Table 1). Acute schistosomiasis was defined by compatible clinical features in a traveler exposed to freshwater at an area in which *Schistosoma* infection is endemic. Cases were confirmed by species-specific serology (Falcon screening enzyme-linked immunosorbent assay [ELISA]), performed at the Centers for Disease Control and Prevention, Atlanta, GA (7, 11), or by stool or urine ovum detection performed at the Reference Parasitology Laboratory, Israel Ministry of Health, Jerusalem. Currently, there is no widely available species-specific serology for *S. mekongi*. Therefore, *S. mekongi* diagnosis is inferred by place of exposure and (cross-) reactivity in the *S. japonicum* assay. All samples of patients with acute schistosomiasis were examined using a PfHRP2 assay (Now Malaria; Binax Inc., ME), and *Plasmodium falciparum* lactic dehydrogenase (PfLDH) and panmalarial LDH were investigated by a PfLDH-based assay (OptiMAL; Flow Inc., Portland, OR). In most patients with acute schistosomiasis (14/16), both whole blood and serum were available and immediately tested. In patients with chronic schistosomiasis, serum samples which were immediately frozen at −20°C were tested by PfHRP2 assays 1 to 3 years later. The assays were performed according to the manufacturers' instructions (10). Malaria RT-PCR was conducted at the Reference Parasitology Laboratory, Israel Ministry of Health, Jerusalem. Samples of patients suffering from acute schistosomiasis were drawn 4 to 8 weeks postexposure during the acute illness, and sera of the seven patients with chronic infection were drawn 1 to 7 years postexposure. The study was approved by the institutional review board.

Acute schistosomiasis caused by *S. mekongi* ($n = 7$). All serum ($n = 7$) and whole-blood ($n = 5$) samples drawn during the acute illness were positive by PfHRP2 assay (Table 1). Repeated malaria smears and malaria RT-PCR as well as PfLDH-based assays were negative in all patients. All patients received praziquantel 3 months postexposure and were asymptomatic at follow-up (12 months). Serum samples from two patients drawn 7 months after exposure were still positive by PfHRP2 assay.

Chronic *S. mekongi* ($n = 3$). These seropositive patients were evaluated 1 to 3 years after exposure. None of them described symptoms of acute schistosomiasis, and serum was drawn prior

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TABLE 1. Rates of false-positive malaria antigen assays in travelers suffering from schistosomiasis ($n = 23$)

Clinical syndrome	Specimen (no. of samples)	Malaria assay[a]	No. of positive samples/total no. of samples tested		
			S. mekongi	*S. mansoni*	*S. haematobium*
Acute schistosomiasis	Whole blood (14)	PfHRP2 assay (Binax)	5/5	0/6	0/3
		PfLDH assay (OptiMAL)	0/5	0/6	0/3
	Sera (16)	PfHRP2 assay (Binax)	7/7	0/6	0/3
		PfLDH assay (OptiMAL)	0/7	0/6	0/3
Past infection[b]	Sera (7)	PfHRP2 assay (Binax)	0/3	0/3	0/1

[a] PfHRP2, *Plasmodium falciparum* histidine-rich protein 2-based assay (Now Malaria; Binax Inc. ME); PfLDH, *Plasmodium falciparum* lactic dehydrogenase (PfLDH) and panmalarial LDH-based assay (OptiMAL; Flow Inc., Portland, OR).
[b] Samples were drawn 1 to 3 years following exposure to *S. mekongi* and 1 to 7 years postexposure to *S. mansoni or S. haematobium*.

to praziquantel treatment. All three serum samples were negative by PfHRP2 assay.

Schistosomiasis caused by *S. mansoni* or *S. haematobium* ($n = 13$). Nine patients suffered from acute schistosomiasis, and four suffered from chronic infection. All samples were negative by PfHRP2 assay.

In summary, we describe a previously unreported phenomenon of false-positive PfHRP2 malaria assays occurring in patients with acute schistosomiasis caused by *S. mekongi*. Malaria was ruled out in all febrile patients by clinical and laboratory features (negative blood smear, PfLDH assay, and RT-PCR).

PfHRP2 assays identify HRP2, a water-soluble protein unique to *Plasmodium falciparum*, which is localized in the parasite cytoplasm and on parasitized erythrocyte membrane. False-positive results of PfHRP2 assays may occur in patients with autoimmune diseases, positive rheumatoid factor, and/or antinuclear antibodies (1–4, 6, 8). It was hypothesized that nonspecific attachment occurs between rheumatoid factor and monoclonal antibodies of the malaria assay (8). One study described false-positive PfHRP2 results in 2/50 patients suffering from schistosomiasis (species were not specified); however, in both cases PfHRP2 assays were negative after rheumatoid factor was absorbed (4). We tested our patients for rheumatoid factor, and all were negative.

One possible reason that our observation was not previously described is that *S. mekongi* was only recently reported to cause acute schistosomiasis (7). Moreover, other species that commonly cause acute schistosomiasis in travelers (*S. mansoni* or *S. haematobium*) do not cause false-positive PfHRP2 assays. Thus, our results suggest that this phenomenon might be species specific. We did not have any cases of acute schistosomiasis caused by *S. japonicum*; therefore, it remains to be determined whether acute *S. japonicum* infection causes false-positive PfHRP2 assays.

We could not determine why acute schistosomiasis caused by *S. mekongi* resulted in false-positive PfHRP2 assays while disease caused by other species did not. We speculate that sera of patients with acute *S. mekongi* infection contains a soluble factor that cross-reacts with *Plasmodium falciparum* HRP2. Sera from patients suffering from acute *S. mansoni* or *S. haematobium* infection probably does not contain this factor. Moreover, this reaction does not occur in PfLDH-based assays.

It is possible that false positivity exists only during symptomatic acute schistosomiasis and several months later. This was demonstrated by persistent false-positive assays 7 months after acute infection but negative assays 1 to 3 years later in chronic patients. An antigen (originating from young forms of the parasite) eliciting a time-limited antibody response that fades over time may explain this phenomenon.

In conclusion, in travelers presenting with febrile illness after returning from Laos, acute schistosomiasis must be ruled out and a malaria diagnosis based on positive PfHRP2 assays must be considered with caution. Further research is needed to identify the cause of this phenomenon.

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