Early diagnosis and follow-up of acute schistosomiasis in a cluster of infected Belgian travellers by detection of antibodies and circulating anodic antigen (CAA) a diagnostic evaluation study

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Original article

Early diagnosis and follow-up of acute schistosomiasis in a cluster of infected Belgian travellers by detection of antibodies and circulating anodic antigen (CAA): A diagnostic evaluation study

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ABSTRACT

Background: In order to evaluate the diagnostic value of schistosome circulating anodic antigen (CAA) detection, serum and urine CAA-levels were determined in a single cluster of 34 Belgian tourists at three timepoints within a period of 14 weeks following proven *Schistosoma* exposure in South Africa and compared with two in-house antibody assays.

Methods: Samples were collected 4–5 and 7–8 weeks post-exposure and subsequently 5–6 weeks following praziquantel treatment. *Schistosoma* antibodies were detected by an adult worm antigen-immunofluorescence assay (AWA-IFA) and a soluble egg antigen-enzyme-linked immunosorbent assay (SEA-ELISA), while CAA concentrations were determined by the Up-Converting reporter Particle labelled Lateral Flow (UCP-LF) test.

Results: Antibodies were detected in 25/34 (73%) travellers pre-treatment and in 27/34 (79%) post-treatment, with the AWA-IFA showing better performance than the SEA-ELISA. Pre-treatment, CAA was detected in 13/34 (38%) and 33/34 (97%) of the travellers in urine and serum, respectively. Post-treatment, all except one traveller became serum CAA negative. This in contrast to the detected antibodies, as well as the previously reported diagnostic results of this cluster.

Conclusions: The UCP-LF CAA serum assay has been demonstrated as the most sensitive method for the diagnosis of early *Schistosoma* infections and post-treatment monitoring in travellers.

1. Introduction

Schistosomiasis, a disease caused by parasitic blood flukes of the *Schistosoma* genus, affects more than 230 million people worldwide [1]. Diagnosing acute schistosomiasis in travellers returning from endemic areas is a known challenge: in non-endemic regions the number of infections might be underdiagnosed due to lack of proper diagnostic procedures. Clinical symptoms might appear before egg production has started or before specific antibodies can be demonstrated [2], implying that these methods are not really suitable for confirming acute *Schistosoma* infections. Treatment at this stage of the disease includes some important considerations. The anti-schistosomal drug praziquantel (PZQ) is known to be less effective against juvenile worms and may sometimes even aggravate symptoms, for which additional corticosteroids need to be given either concomitantly or prior to PZQ treatment [1, 3].

Abbreviations: AWA, (Adult Worm Antigen); CAA, (Circulating Anodic Antigen); ELISA, (Enzyme-Linked ImmunoSorbent Assay); IFA, (ImmunoFluoresence Assay); IHA, (Indirect Hemagglutination inhibition Assay); ITM, (Institute for Tropical Medicine, Antwerp, Belgium); LUMC, (Leiden University Medical Center, Leiden, The Netherlands); PZQ, (Praziquantel); SCAA, (Serum Circulating Anodic Antigen); SEA, (Soluble Egg Antigen); UCAA, (Urine Circulating Anodic Antigen); UCP-LF, (Up-Converting reporter Particle Lateral Flow).

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Classic diagnosis of *Schistosoma* infections is often still based on microscopic detection of parasite eggs in stool or urine, depending on the species [4]. However, this approach lacks sensitivity in low-burden infections [5], which is often the case in infected travellers. Schistosome antibody detection is a far more sensitive method for confirming infection in exposed travellers [4,6] and it is therefore the most commonly used method for diagnosing schistosomiasis in non-endemic routine diagnostic laboratories. Antibodies usually develop within a few weeks to months after infection, generally before *Schistosoma* eggs can be detected, but usually after the first clinical symptoms [4]. However, detection of antibodies is not suitable for monitoring treatment efficacy as they remain present after treatment [7,8]. Furthermore, sensitivity of these methods may vary considerably depending on the specific method and on the targeted *Schistosoma* biomarkers [9,10]. Detection of *Schistosoma* DNA in serum is a recent development that allows both for early detection after infection and for species identification [11]. However, individuals remain DNA-positive in serum after treatment, making this method unsuitable to monitor treatment efficacy [11,12].

There clearly is a need for a diagnostic marker which can be accurately detected from the early infection stages onwards and which is cleared soon after treatment. Circulating cathodic antigen (CCA) and circulating anodic antigen (CAA) are two well-studied *Schistosoma* antigens which are regurgitated by living *Schistosoma* worms, and therefore indicative of an active infection [13]. While the field-applicable point-of-care CCA (POC-CCA) test is being used extensively in *S. mansoni* endemic settings, it has demonstrated inconsistent performance in imported cases in non-endemic routine clinical settings [14,15]. Detection of CAA, present within weeks after infection as observed recently in a controlled human schistosomiasis infection model [16], seems to be a promising alternative for diagnosing (acute) schistosomiasis in returning travellers [17,18]. Furthermore, CAA-levels decline rapidly after treatment with PZQ [17,19], allowing monitoring of treatment efficacy. Using an ultrasensitive reporter technology (Up-Converting reporter Particles, UCP) in combination with lateral flow (LF) immunochromatography, CAA can be accurately measured in urine and in serum [20,21]. This UCP-LF CAA assay has demonstrated high sensitivity and specificity in detecting the four major *Schistosoma* species (*S. mansoni*, *S. haematobium*, *S. japonicum* and *S. mekongi*) in endemic areas [21–26] as well as in a non-endemic routine diagnostic setting [17].

In a cluster of Belgian travellers, who all were most likely infected with a *S. mattheei* x *S. haematobium* hybrid in South Africa, the performance of two commercial schistosome antibody assays and a serum schistosome real-time PCR assay was evaluated as described previously [11]. Here, we compare these previously described diagnostics with two in-house antibody assays and the schistosome UCP-LF CAA assay for antigen detection in urine and serum, before and after PZQ treatment.

2. Materials and methods

Samples were available from a cluster of 34 Belgian travellers (17 males, 17 females) with a PCR-confirmed *Schistosoma* infection, determined shortly after exposure during a holiday in a known *Schistosoma*-endemic region in South Africa. All participated in a prospective study evaluating new diagnostic tests for acute schistosomiasis at the Institute of Tropical Medicine (ITM) in Antwerp, Belgium, where ethical clearance had been obtained [11]. Informed consent included storage of samples for future use in studies evaluating new diagnostic tests for schistosomiasis. At LUMC, all samples were tested anonymously.

In total, 16 parents (8 males) aged 39–49 years (median, 43 years) and 18 children (9 males) aged 5–15 years (median, 12 years) were seen at ITM and samples collected at three moments during the course of infection (Fig. 1). Firstly at 4–5 weeks post-exposure, where 32/34 (94%) had developed symptoms of acute schistosomiasis and who were treated with corticosteroids accordingly; secondly at 7–8 weeks post-exposure when symptoms had abated and all received PZQ (40 mg/kg) according to current practice at ITM; and thirdly in a final post-treatment visit 13–14 weeks post-exposure (5–6 weeks after treatment) receiving a second treatment with PZQ.

Serum samples were tested at the ITM using two commercially available schistosome antibody assays, an enzyme-linked immunosorbent assay (*S. mansoni* ELISA, Bordier Affinity Products) and an indirect hemagglutination inhibition assay (IHA, ELITechGroup Microbiology), as well as an in-house real-time PCR assay detecting Dra-1, a target which is specific for the *S. haematobium* complex [11,27,28]. Sequencing of schistosome DNA obtained from serum of 3 travellers with very low Ct-values in the serum PCR demonstrated a hybrid infection with *S. mattheei* x *S. haematobium* [11]. No eggs or parasite DNA were detected in any of the collected urine and stool samples [11].

At the Leiden University Medical Center (LUMC), samples from the three visits were subjected to serum antibody testing and urine and serum CAA detection. Serum antibody assays comprised the adult worm antigen-immunofluorescence assay (AWA-IFA) and the soluble egg antigen-enzyme-linked immunosorbent assay (SEA-ELISA) as used in our routine clinical setting. *Schistosoma*-specific IgM antibodies against adult worm gut antigens were determined by AWA-IFA using sections of Rossmann's fixed adult male *S. mansoni* worms and IgG antibodies against *S. mansoni* SEA were detected by SEA-ELISA. Both assays are ISO 15189:2012-certified and have been used for the routine clinical diagnosis of schistosomiasis at the LUMC since the 1990s [17,29,30]. Additional details of these antibody assays can be found elsewhere [16,31].

Detection of CAA in urine and serum samples was done using the laboratory-based, ultra-sensitive and highly specific UCP-LF CAA assay:

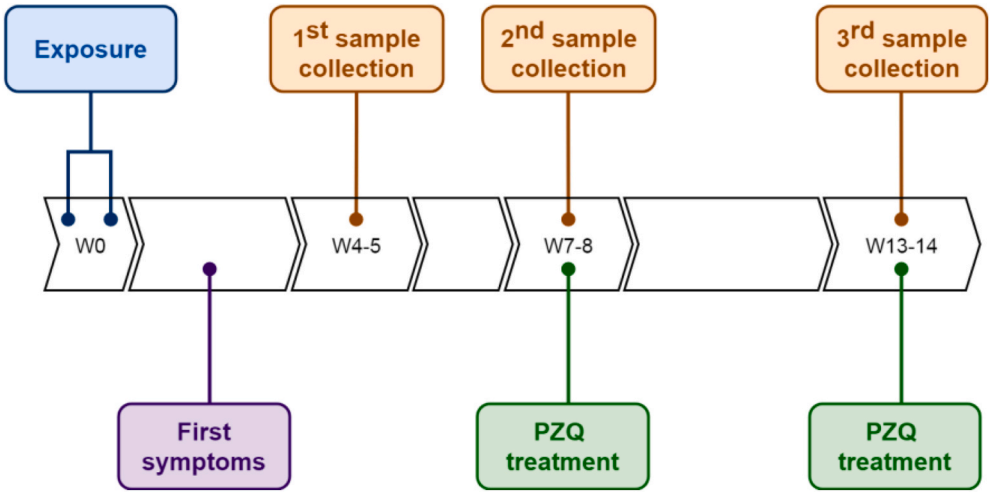

Fig. 1. Timeline of exposure, first appearance of symptoms, sample collection and treatment in a cluster of 34 infected Belgian travellers.

the UCAA2000 and the SCAA500, respectively, as described previously [18,20,22,32]. Urine samples were subjected to the user friendly dry-format assay, while for serum samples the wet-format assay was used, which includes the additional step of UCP-sonication [32]. Briefly, 500 μl of serum (or 2 ml of urine) was mixed with an equal volume of 4% trichloroacetic acid, incubated and centrifuged. Of the clear supernatant 500 μl of serum (or 2 ml of urine) was concentrated to 20 μL using a 0.5 ml (or 4 ml) centrifugal filter device (Amicon Ultra-4, Millipore). The concentrates were added to microtiter plate wells containing UCP reporter particles labelled with anti-CAA antibodies hydrated in 100 μL LF assay buffer and incubated on a shaker at 37 °C. After 1 h, LF strips were added to the wells and incubated overnight, followed by scanning the strips using a Packard FluoroCount microtiter plate reader [20]. Samples with a known CAA concentrations were included as a reference standard to quantify individual CAA-levels and to validate the cut-off of the assay. A predefined cut-off level was used; 0.3 pg/ml for the urine UCAA2000 assay and 1 pg/ml for the serum SCAA500 assay [20]. Samples with a CAA concentration above these cut-offs were considered positive, whereas samples below these cut-offs were considered negative. Data were entered into a Microsoft Excel spreadsheet and analysed using GraphPad Prism 8.1.1 (GraphPad Software Inc.; California, USA) and SPSS version 25 (IBM Corp.; Armonk, USA). Statistical analysis was performed using descriptive statistics.

3. Results

In Table 1 an overview is given of the outcomes of the diagnostic tests that were performed in the current study. At the first post-exposure visit, schistosome antibodies were detected in 13 travellers (39%), all positive by AWA-IFA with two (6%) by both AWA-IFA and SEA-ELISA. Positivity increased substantially at the second post-exposure (pre-treatment) visit where 68% of travellers tested positive by AWA-IFA compared to 32% by SEA-ELISA. After treatment, the number of AWA-IFA-positive travellers further increased to 74%, while the number of SEA-ELISA-positive travellers decreased to 12%. For comparison, the outcomes of the diagnostic tests that were previously performed at ITM [11] have also been included in the table. Results of the SEA-ELISA at LUMC were comparable to results with the commercial ELISA at ITM.

CAA was detected in urine of 8 (27%) and 9 (28%) travellers 4–5 weeks and 7–8 weeks post-exposure, respectively, with a total of 13 (39%) travellers being positive on at least one of these time points. All travellers became urine CAA negative after PZQ treatment. In serum, CAA was detected in samples from 30 travellers of 4–5 weeks (91%) and 7–8 weeks (88%) post-exposure. Cumulatively, 33 (97%) travellers were serum CAA positive pre-treatment, with all but one turning negative after treatment. When compared to the previously published serum PCR results, the serum CAA showed a higher number of positives at the first post-exposure visit. One the other hand, all 34 travellers had detectable Dra-1 by PCR in at least one of the serum samples, while 33 tested positive for CAA. The traveller who tested negative for CAA also remained negative in all antibody detecting tests performed. No association was observed between CAA-levels and gender or age of the host.

Supplementary Table 1 gives an overview of all collected data per traveller, indicating that among the diagnostic tests performed, only the UCP-LF CAA assay demonstrated a significant decline following praziquantel treatment. Individual CAA-levels over time are also shown in Fig. 2. Between the first and second post-exposure visit, thus before treatment, CAA-levels increased in urine of 6 travellers (20%) and in serum of 11 travellers (33%). In addition, a spontaneous reduction in CAA-levels was observed during the same time period in urine of 6 travellers (19%), four of them even becoming negative, and in serum of 20 travellers (59%), with three of them becoming negative. From two travellers, who were urine CAA positive before treatment, a post-treatment urine sample was missing. However, no CAA was detected in the post-treatment serum sample of these two individuals. Overall, CAA-levels in urine were lower compared to CAA-levels in serum. All urine CAA-positive cases were also serum CAA positive.

4. Discussion

Accurate diagnosis of acute schistosomiasis in travellers is challenging as in this early stage of the infection egg detection methods are often not reliable. Antibody detection assays are commonly applied in non-endemic routine diagnostic laboratories, especially for those travellers who have been exposed for a relatively short period. This, despite the fact that these assays have several known limitations, some of which are confirmed again in the current study. In this cluster of acutely infected travellers, the serum CAA assay was the most sensitive test to confirm active *Schistosoma* infection as well as to assess cure. The serum CAA assay also showed an overall better performance compared to a real-time serum PCR assay which has been considered by some authors as the most accurate diagnostic reference test for this specific target population [12].

Even though almost all travellers showed symptoms of acute schistosomiasis, no eggs were detected in stool or urine at any of the pre-treatment visits [11]. It was hypothesized that the infective species in this cluster, a *S. mattheei* x *S. haematobium* hybrid as demonstrated by schistosome DNA sequences extracted from serum of three travellers, is

Table 1
Number of positive cases at the three time points according to the different diagnostic tests in a cluster of 34 infected Belgian travellers.

	Pre-treatment			Post-treatment
	4–5 wks PE n (%)	7–8 wks PE n (%)	Cumulative positive pre- treatment n (%)	13–14 wks PE n (%)
Diagnostic tests performed at LUMC				
AWA-IFA	13/33 (39%)	23/34 (68%)	23/34 (68%)	25/34 (74%)
SEA-ELISA	2/33 (6%)	11/34 (32%)	11/34 (32%)	4/34 (12%)
Urine CAA	8/30 (27%)	9/32 (28%)	13/33 (39%)	0/30
Serum CAA	30/33 (91%)	30/34 (88%)	33/34 (97%)	1/34 (3%)
Diagnostic tests performed at ITM[a]				
ELISA	0/33	12/34 (35%)	12/34 (35%)	11/34 (32%)
IHA	3/33 (9%)	0/34[b]	3/34 (9%)	1/34[c] (3%)
Serum PCR	24/33 (73%)	30/34 (88%)	31/34 (91%)	24/34 (71%)

Abbreviations: AWA-IFA; adult worm antigen-immunofluorescence assay, CAA; circulating anodic antigen, IHA; indirect hemagglutination inhibition assay, ITM; Institute for Tropical Medicine, Antwerp, Belgium, LUMC; Leiden University Medical Center, PCR; polymerase chain reaction, PE; post-exposure, PZQ; praziquantel, SEA-ELISA; soluble egg antigen-enzyme-linked immunosorbent assay.

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[b] Uninterpretable in 13 cases.

[c] Uninterpretable in 2 cases.

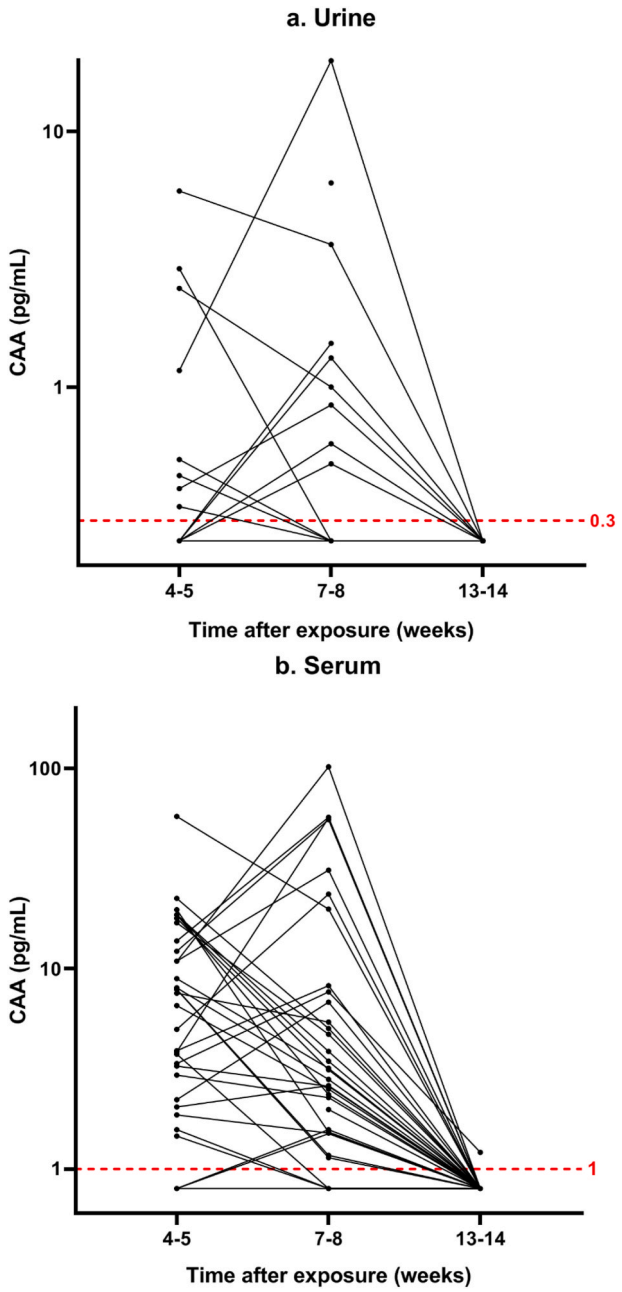

Fig. 2. Individual circulating anodic antigen (CAA) concentrations in urine (a) and serum (b) in a cluster of 34 infected Belgian travellers at three different time points.

sterile [10]. In such a setting, microscopic examination of stool or urine, even after further sample concentration or repeated sample collection, will never be reliable for accurately detection of schistosome infections. Alternative explanations for the absence of eggs in stool or urine could be a mono-sexual infection, immaturity of the worms which do not yet produce eggs or a very low worm burden as often found in travellers [33]. The finding that around 32% of the travellers had detectable antibodies against egg antigen (e.g. positive SEA-ELISA) does not prove any deposition of eggs, as antibodies against the schistosome egg antigen have also been detected in volunteers following infection with male parasites only [16].

Serum antibody tests for schistosomiasis come in many assay formats and use a variety of schistosome antigen extracts: from adult worm, eggs or even cercariae. ELISA is the most commonly used format in commercial serological tests, and therefore considered as a benchmark test in established schistosome infection. The rationale for including our in-house antibody test in the current diagnostic test evaluation rests on our overall positive experiences with these tests. In particular the AWA-IFA assay, detecting schistosome IgM, has been found to be highly sensitive in early detection of schistosomiasis in returning travellers, as well as in a controlled human *S. mansoni* infection model, showing 100% seroconversion within 4–6 weeks after exposure [16,17,31]. In the current study, however, the performance of the AWA-IFA, although much better than the SEA-ELISA, remained inferior to serum CAA and serum PCR detection, possibly because of the *S. haematobium* hybrid nature of the present infection. Our in-house SEA-ELISA format, based on soluble egg antigen, did not perform better than a commercial ELISA using a mixture of egg and adult worm antigen [11]. Antibody tests are particularly useful for detecting infections in asymptomatic travellers, and they are often used for screening purposes [4,31,34]. However, for diagnosing acute schistosomiasis within a limited number of weeks after exposure, antibody tests seem to be less suitable. In addition, our results confirmed that antibody tests are not suitable to monitor the effect of PZQ treatment, as the majority of individuals remained or even became positive after successful treatment.

No correlation was observed between the level of parasite DNA and CAA and while CAA in serum and urine became negative after treatment in almost all individuals, the majority remained PCR positive. These findings suggest that the output of this specific PCR assay is not a reflection of the actual worm burden, but a measure of the overall amount of parasite DNA present, probably released from surrounding tissues, including trapped eggs. Notable is the single traveller who showed a discrepancy between PCR and serum CAA results. Based on previously described data from this cluster, it was assumed that all individuals of this cluster had been infected with *Schistosoma* [11]. However, the single individual showing no serum CAA did not present any clinical signs of acute schistosome infection, no eosinophilia, nor any positive serological test result at any of the three time points. The PCR test of this CAA-negative individual showed a high Ct-value of 43.8, which corresponds to a very low level of parasite DNA, suggesting that the PCR result might be false positive and that most likely this individual was not infected.

Overall, in this cluster of travellers, the serum CAA assay performed better than the urine CAA assay. CAA-levels in serum seem to be higher and more stable, as has been observed in some previous studies [13,35–37]. All detected urine CAA-levels were above but very close to the assay cut-off, indicating that testing with even larger urine volumes would have been more beneficial. Although urine CAA-levels were low, all urine CAA positive individuals became negative after treatment, indicating that the infection has been cleared. In some individuals, urine CAA-levels already decreased to undetectable levels even before treatment. A similar trend was observed in serum: in some individuals serum CAA-levels decreased at the second follow-up visit before treatment while in others serum CAA-levels still increased. These fluctuations indicate that the course of infection as well as intensities of infection varies from individual to individual in the first weeks after exposure. The growth/maturation of (young) worms will cause an increase in CAA-levels over time, while the decrease in CAA-levels indicate that non-matured worms die before treatment is given. After treatment, CAA-levels in serum decreased significantly to below the cut-off in all but one traveller, indicating that they cleared the infection. Even though in this cluster the infecting species turned out to be a *S. mattheei* x *S. haematobium* hybrid [11], the serum CAA assay was able to detect all infected individuals, confirming again that CAA is a *Schistosoma*-genus specific antigen [21,38].

The current study confirms the usefulness of CAA detection for diagnosis of schistosomiasis in travellers and has several advantages over existing methods. First and foremost, the presence of CAA indicates an active *Schistosoma* infection [35,39]. Furthermore, CAA is excreted by all schistosome species, making the UCP-LF CAA assay generally applicable in all *Schistosoma* endemic areas [20], and as shown in this

study also for detection of hybrid infection. Serum CAA has shown to be an excellent marker for demonstrating active *Schistosoma* infections at a very early stage, as the majority of travellers had detectable CAA-levels in serum already 4–5 weeks after exposure. Clearance of CAA from the host circulation appears to be relatively fast, within 5–6 weeks after treatment, as also previously observed [40]. Unlike PCR and serological tests, CAA is the only reliable marker to assess treatment efficacy soon after administration of PZQ. Furthermore, the better sensitivity of detecting CAA in serum compared to urine fits nicely with clinical routine diagnostic settings where, in contrast to endemic countries, the use of serum is often preferred over urine.

A limitation of the UCP-LF CAA assay is its inability to differentiate between *Schistosoma* species, as CAA is excreted by all *Schistosoma* species. However, if needed, known geographical distribution of *Schistosoma* could be of use to indicate the suspected species, especially to decide whether and how individuals should be treated. Another limitation is the availability of the UCP-LF CAA assay. Efforts are ongoing to implement the serum CAA assay in our clinical routine diagnostic laboratories as well as to make a CAA detection test generally available.

5. Conclusions

This study confirms the accuracy and usefulness of the UCP-LF CAA serum assay for diagnosing active *Schistosoma* infections in (recently) exposed travellers and its ability to assess efficacy of treatment. The UCP-LF CAA serum assay is the only test that fulfils the criteria for correctly determining cure.

CRediT authorship contribution statement

Pytsje T. Hoekstra: Project administration, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Marjan van Esbroeck:** Resources, Basic Data Compilation, Writing – review & editing. **Claudia J. de Dood:** Investigation. **Paul LAM. Corstjens:** Writing – review & editing. **Lieselotte Cnops:** Writing – Resources, Basic Data Compilation, Writing – review & editing. **Christel JG. van Zeijl-van der Ham:** Investigation. **Linda J. Wammes:** Investigation, Writing – review & editing. **Govert J. van Dam:** Writing – review & editing. **Jan Clerinx:** Resources, Basic Data Compilation, Writing – review & editing. **Lisette van Lieshout:** Conceptualization, Methodology, Data curation, Supervision, Writing – review & editing.

Declaration of competing interest

All authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2021.102053.

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