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An ultra-sensitive assay targeting the circulating anodic antigen for the diagnosis of *Schistosoma japonicum* in a low-endemic area, People's Republic of China

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ABSTRACT

The downward trend in prevalence and intensity of *Schistosoma japonicum* infection in the People's Republic of China (P.R. China) has reached a level where accurate methods are required for monitoring the national schistosomiasis control programme and to verify whether transmission has been interrupted. We have assessed the prevalence of active *S. japonicum* infection by use of an up-converting phosphor lateral-flow (UCP-LF) assay for determination of circulating anodic antigens (CAA) in urine and serum, and compared the findings with those of the Kato–Katz technique for egg detection in stool and an immunohaemagglutination assay (IHA) for specific antibodies in serum. The study was carried out in three villages located in a remaining *S. japonicum*-endemic area in P.R. China. Overall, 423 individuals were investigated by Kato–Katz, 395 by IHA, 371 with the UCP-LF CAA assay adapted for urine and 178 with the UCP-LF CAA assay applied on serum. The IHA showed the highest number of positive results ($n = 107$, 27.1%). The UCP-LF CAA urine assay detected 36 CAA positives (9.7%) and the serum-based CAA assay 21 positives (11.8%). The Kato–Katz technique revealed only six positive stool samples (1.4%). Among those 166 individuals with complete data records, sensitivities of the different assays were determined *versus* a combined 'gold' standard, showing the highest sensitivity for the urine CAA assay (93%), followed by the serum CAA (73%) and IHA (53%), whilst triplicate Kato–Katz thick smears had a very low sensitivity (13%). Serum CAA concentrations were about 10-fold higher than in urine and were significantly correlated. Highest prevalences as determined by CAA were found in older age groups (>40 years). Half of the CAA- or egg-positive cases were negative for antibodies by IHA, thereby revealing an important obstacle for the effectiveness of the current schistosomiasis control and elimination efforts. The significantly higher prevalence of active schistosome infections as shown by the urine and serum UCP-LF CAA assays has implications for the national control and elimination programme in P.R. China, particularly in respect to case-finding and intervention strategies.

1. Introduction

Schistosomiasis is a blood-based, trematode infection that constitutes an important part of the public health burden in tropical and sub-tropical regions of the world. Although close to 800 million people are at risk of schistosomiasis and more than 200 million

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are infected (Colley et al., 2014; Steinmann et al., 2006; Utzinger et al., 2009), schistosomiasis counts as one of the neglected tropical diseases (NTDs). The global control strategy emphasises preventive chemotherapy using praziquantel and, wherever resources allow, accompanying measures such as information, education and communication (IEC), and improvements in water, sanitation and hygiene (WASH) (Knopp et al., 2013; WHO, 2002, 2013). Accurate determination of the level of endemicity plays an important role in the selection of intervention areas in combination with the appropriate control approach (Bergquist et al., 2009; Brooker et al., 2009). For intestinal schistosomiasis (caused by *Schistosoma mansoni* and *Schistosoma japonicum*), the diagnosis is generally based on microscopy of stool samples using the Kato–Katz technique (Katz et al., 1972). The control approach for areas with ≥50% prevalence among school-aged children consists of community-wide mass drug administration (MDA), whereas school-based treatment schedules are preferably applied at prevalence levels between 10% and 50% (WHO, 2002, 2006). The Kato–Katz technique is essentially 100% specific in the hands of experienced laboratory technicians, while in stools from individuals with low intensity of infection the parasite's eggs are often missed, resulting in low sensitivities. Areas characterised by low prevalence (<10%) and low intensity of infection present a challenge to be accurately detected using the Kato–Katz method. This quandary mounts when the prevalence falls below 1% and stool examination is therefore of little use at the elimination phase. The consequential need for multiple stool examinations suppresses the already declining compliance and thus further restricts the scope and effectiveness of a diagnostic approach based on Kato–Katz thick smears (Fung et al., 2012). Hence, diagnostic tactics must be changed along with a need for high sensitivity that follows successful control. Indeed, when elimination is approached, ultra-sensitive diagnostics becomes a necessity (Bergquist et al., 2009; Knopp et al., 2013).

Reliable diagnostic assays are pivotal for monitoring the impact of interventions and for justifying potential changes in the control strategy. Tests to diagnose *Schistosoma* infections are based on (i) showing the parasite (or its eggs) directly under a microscope; (ii) detecting it through molecular means; or (iii) finding specific antibodies (Utzinger et al., 2011; van Lieshout et al., 2000). All these approaches rely on access to fresh stool, urine or serum samples, and both molecular diagnostics as well as detection of specific antibodies (serology) require adequate reagents. Although serology provides high sensitivity and specificity, this approach does not disclose the intensity of infection and, more importantly, whether the infection is active (de Jonge et al., 1990; Mott and Dixon, 1982). Reflecting the need to reach higher sensitivity, particularly for detecting low-intensity infections, research has concentrated on amplification of pathogen DNA, such as techniques based on the polymerase chain reaction (PCR) (Obeng et al., 2008; Pontes et al., 2002) and loop-mediated isothermal amplification (LAMP) (Wang et al., 2011). High sensitivities in determining active infections have also been achieved with assays for detection of the circulating cathodic antigen (CCA) and the circulating anodic antigen (CAA) in serum or urine (Colley et al., 2013; Corstjens et al., 2014; De Jonge et al., 1989; Deelder et al., 1996; van Lieshout et al., 2000). Research based on CCA has produced the point-of-care (POC) lateral flow (LF) urine cassette assay for diagnosis of *S. mansoni* infection, which has been successfully validated in a multi-country study (Colley et al., 2013). Further work using a specialised technique, the up-converting phosphor (UCP)-LF assay, to detect CAA in serum and urine at the estimated sensitivity level of infection by one single worm has recently been published (Corstjens et al., 2014).

Schistosomiasis in the People's Republic of China (PR China) goes back to antiquity (Chen, 2014). Over the past 60 years, a multifaceted integrated control programme has been implemented, which reduced the prevalence of human *S. japonicum* infections by more than 90% compared to the level in the mid-1950s (McManus et al., 2010; Utzinger et al., 2005; Zhou et al., 2010). Thus, P.R. China has reached the level where the diagnostic shortcomings of the Kato–Katz technique are becoming apparent (Zheng et al., 2013). With the country reaching the stage of transmission control and now aiming for schistosomiasis elimination (Wang et al., 2009), low-intensity infections are rapidly replacing the traditional, large worm burdens. Where interruption of transmission has been achieved a different approach is required with surveillance taking over from monitoring requiring highly sensitive diagnostic tools to confirm elimination or re-emergence of infection (Bergquist et al., 2015; Zhou et al., 2013).

In order to investigate to what extent the current diagnostic approach is limited in detecting the true infected cases, the present study was undertaken to apply the UCP-LF CAA assay on urine samples in a well-researched area still endemic for *S. japonicum* in P.R. China, but where recent control activities have resulted in a continuously diminishing rate of prevalence and intensity of infection (Balen et al., 2007). Hence, after following routine procedures in the field (i.e. triplicate Kato–Katz, miracidium hatching test and detection of serum antibodies by immunohaemagglutination (IHA)), a urine sample was obtained for the UCP-LF CAA assay. In addition, as serum samples were already collected for the serological antibody determination, the comparison and potential added value of the serum CAA assay was investigated. Anticipating further studies, implications for practical implementation within the current and future control programmes are taken into account and discussed, such as pooling of biological samples to further reduce costs.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Ethical Review Committee of the National Institute of Parasitic Diseases, Chinese Centre for Diseases Control and Prevention (Shanghai, P.R. China, no. 2014-015). Before any investigations, written consent was obtained from the city and village authorities. Oral informed consent was obtained from all individuals. Participation was voluntary, and hence withdrawal was possible at any time without further obligation. At the end of the study, participants who were identified with *S. japonicum* in their stool or had a positive CAA result in either urine or serum were treated with a single oral dose of praziquantel (40 mg/kg body weight), except for those with contraindications (such as heart problems, and serious liver and kidney dysfunctions).

2.2. Localities and sample collection

The study was conducted in an area in close proximity to the Dongting Lake in Hunan province, P.R. China (Fig. 1). Individuals (aged 10 years and above) with oral informed consent were invited for stool, urine and blood sample collection. Overall transmission is low, but for a more detailed analysis the villages can also be divided in two different transmission settings, i.e. agricultural villages (stool prevalence by microscopy <1%) and fishing villages (stool prevalence 1–5%) (Zhou et al., 2011). Sample size calculations (Fleiss, 1981), using a significance level of 5% and a power of 80%, based on the assumption of an observed overall prevalence by Kato–Katz of 1.5% and a 4-fold higher prevalence by the urine UCP-LF CAA assay indicated a required number of 322 individuals. In total, 423 individuals from two agricultural villages (Huhua Zhou and Shizi Geng, $n = 303$) and one fishing village (Minglang Shan, $n = 120$) provided samples for the study. Stool samples (approximately 30 g) were obtained from all participants, whilst 18 and

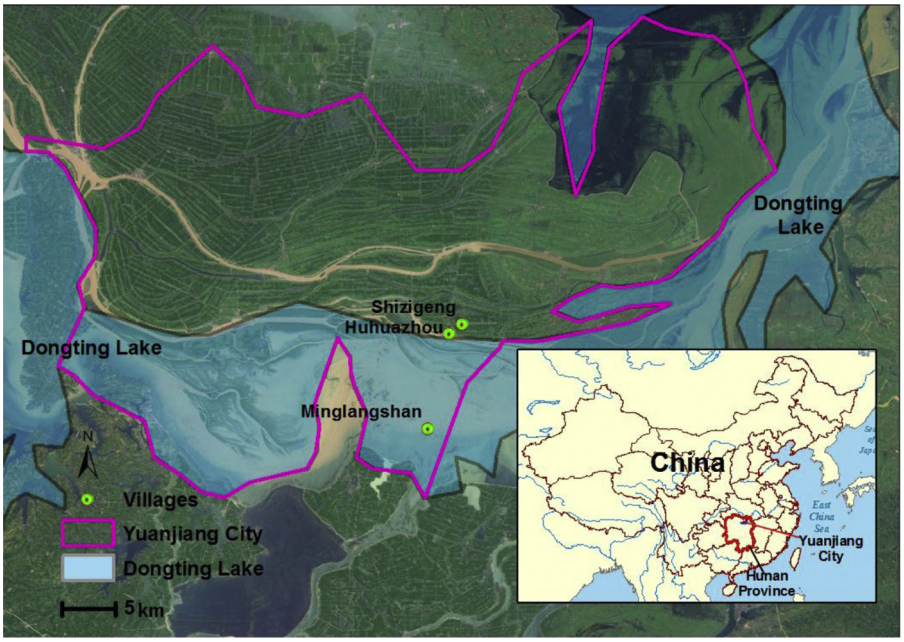

Fig. 1. Study area in Hunan province on the Dongting Lake and its location in P.R. China.

28 individuals failed to provide urine and blood samples, respectively, and hence, the number of tests carried out with each method varied.

2.3. On-site sample processing

Stool samples were subjected to microscopy using triplicate Kato–Katz thick smears (Katz et al., 1972). Standard 41.7 mg Kato–Katz templates were used and the slides were quantitatively examined for *S. japonicum* eggs under a microscope by one of two experienced laboratory technicians 24 h after preparation. The results were recorded as egg counts per slide and multiplied by a factor of 24 to convert it into eggs per 1 g of stool (EPG) to be used for subsequent data analysis. Additionally, the hatching test for detection of miracidia was performed in the field as described by Qiu and Xue (1990), using about 30 g of homogenised faeces suspended in distilled water and subsequently filtered through a nylon tissue-bag. Filtrates were transferred to volumetric flasks and kept at 26–30 °C in an illuminated incubator. Miracidia were observed 4, 8 and 12 h later, by two trained laboratory technicians.

Simultaneously, urine samples were obtained from each participant in 50 ml centrifuge tubes flasks, transported to Shanghai, and stored at 4 °C in a fridge. At least 2 ml venous blood was taken, serum was prepared in the field and samples were tested on the spot by an IHA kit targeting human antibodies against the schistosome soluble egg antigen (Anhui Anji Pharmaceutical Technology Co., Hefei, P.R. China; catalogue no. 20120618) according to the manufacturer's instructions. Briefly, after pipetting 25 μl serum dilutions (in normal saline, 1:5, 1:10 and 1:20, respectively) into V-bottom plates, 25 μl sensitized red blood cells (RBC) were added to each well, plates were gently shaken and kept at 37 °C for 30 min. Positive and negative control serum samples provided by the company were tested simultaneously on each plate. The dilution showing a clear, sharp dark spot similar to that in the negative control wells was recorded as the sample titre, where ≥10 values were considered as positive. The remaining serum was transported to Shanghai and stored frozen at −20 °C for further examination.

2.4. UCP-LF CAA assays

Urine and serum samples were examined by the UCP-LF assay for CAA at the National Institute of Parasitic Diseases, Chinese Centre for Diseases Control and Prevention in Shanghai. Two ml urine samples were tested after concentration as described by Corstjens et al. (2014), UCAA2000 format with dry reagents. The samples were diluted with an equal volume of 4% (w/v) tri-chloro-acetic acid (TCA) and centrifuged, after which the clear supernatants were reduced to amounts of 20–30 μl using an Amicon Ultra-4 device (EMD Millipore; Billerica, MA, USA). A limited number of samples was also evaluated using up to 8 ml urine and multiple loading of the concentration devices. After incubation with the UCP-antibody conjugate, strips were added and the samples allowed to run as described elsewhere (van Dam et al., 2013). Following overnight drying, the strips were scanned for bound UCP, using a portable ESE-Quant reader (Qiagen; Lake Constance, Germany), readily connected to a laptop computer.

The serum samples were examined in a similar way using the UCP-LF assay with the 500 μl concentration assay utilising 500 μl of serum (SCAA500 with dry reagents). We followed an analogous procedure to the UCAA2000 method, with 500 μl serum-TCA supernatant being concentrated over an Amicon Ultra-0.5 device to about 20–30 μl.

Samples were processed taking 64 in each batch; at least one standard curve of a concentration series of partly purified antigen, i.e. the TCA-soluble fraction of *S. mansoni* adult worm antigen (AWA) containing 3% CAA in urine/serum, was included for calculation of the CAA concentration in the original sample as described by Polman et al. (2000). The cut-offs of the assays were decided in accordance with (Corstjens et al., 2014) using 0.3 pg/ml urine and 3 pg/ml serum as the lower limits of detection (LOD), and 0.15 pg/ml urine and 1.5 pg/ml serum as the LOD if the assay would have been performed with multiple samples under ideal laboratory conditions. The region between the two LOD's was designated 'indecisive', indicating that samples are suspected to be positive but to truly ascertain the positive or negative score, the samples would need to be retested, preferable at higher sample volume.

Table 1
General characteristics of the study population ($n = 423$).

	All villages	Fishing village	Agricultural villages
Gender (no. males/no. females)	236/187	72/48	164/139
Age in years (median/range)	49/12–83	49/24–78	49/12–83
Kato–Katz (no. pos/% prevalence)	6/1.4%	6/5.0%	0/0%
IHA (no. pos/% prevalence)*	107/27%	42/46%	65/21%

* $n = 395$–92–303, respectively, for all cases, fishing and agricultural villages.

2.5. Data entry and processing

Data were entered manually in a spreadsheet (Excel 2010™), while the UCP-LF CAA data were entered into Excel by copying directly from the ESE-Quant reader. Statistical analysis was done using Excel and SPSS version 20 (IBM Corp.; Armonk, NY, USA). Non-parametric statistics were used to assess the correlation between urine and serum CAA concentrations. The sensitivities of the diagnostic tests were calculated and used for indication of test performance. For this reason, 'true positive' infection status ('gold' standard) was defined as a sample from an individual presented with *S. japonicum* eggs at least once, as well as being positive in the UCP-LF CAA assay (serum or urine) (Glinz et al., 2010). This approach implied that, by definition, the specificities of the individual tests were 100%. This might be a slight overestimation, but is considered validated because of the very high specificities of the circulating antigen assays (Midzi et al., 2009). In addition, an 'indecisive' category is defined and analysed separately, following the approach described by Coulibaly et al. (2013)..

In order to investigate to what extent a potential pooling schedule would reduce costs, the urine data were statistically grouped into sets that could practically be processed. To supply programme managers with more exact information in which part of the populations most infected individuals were located, the data were also analysed across age-groups (10–19, 20–39, 40–49, 50–59 and ≥60 years).

3. Results

3.1. Individual test results

All 423 participants in the study were subjected to triplicate Kato–Katz thick smears, 395 had a single IHA test performed, whilst UCP-LF CAA assays were done on 371 urine and 178 serum samples. Overall, 383 individuals were tested with one or both UCP-LF CAA assays. Table 1 shows the general characteristics of our study population.

Only six out of the 423 stool samples were found positive by Kato–Katz thick smears and miracidium hatching test, owing to a prevalence of 1.4%. Meanwhile, we found 107 IHA-positives (27.1%), 36 urine CAA positives (9.7%), and 21 serum CAA positives (11.8%). Additionally, in the urine CAA assay, 21 cases were scored indecisive (5.7%), and six cases (3.4%) in the serum CAA. These indecisive samples should be retested, preferably with higher sample volumes, in order to get a definite signal. For the urine assay this was done with 8 ml urine for two indecisive cases, with one of them returning positive. Of the 21 urine CAA indecisive cases, 11 were also tested by serum CAA and found negative, while all six serum CAA indecisive cases were tested with the urine CAA and four were found positive, indicating that the LOD of the serum assay might be underestimating the true CAA positives. The total number of urine CAA positives in relation to stool microscopy using Kato–Katz and IHA is shown in Table 2 for all cases and stratified into the two different transmission settings.

3.2. Correlation of serum and urine CAA levels

A total of 166 individuals had both serum and urine samples subjected to CAA testing and we found a significant correlation (Spearman's rho 0.52, $p < 0.001$) between the concentrations (Fig. 2). Serum CAA concentrations were significantly higher than those in urine (Wilcoxon signed rank test, $p < 0.01$).

3.3. Sensitivities of the tests versus 'gold' standard

Table 3 shows the results obtained using a composite reference ('gold') standard based on study subjects either positive according to triplicate Kato–Katz thick smears or for the UCP-LF CAA assay (assuming 100% specificity of the CAA result). A total of 166 cases had data from all assays with 30 positive by Kato–Katz and/or urine or serum CAA. Ultimate (100%) sensitivity was obtained by the combination of urine and serum CAA findings, while, taken separately, urine CAA showed a higher sensitivity compared to serum CAA (93%

Table 2
Number of urine CAA positive cases, compared to Kato–Katz egg positives and IHA serum antibody positives for the respective *S. japonicum*-endemic settings in P.R. China.

	Parasitology			**Serology**		
	Total	Positive	Negative	Total	Positive	Negative
			All cases			
CAA positive	36	5	31	32	17	15
CAA indecisive	21	0	21	20	3	17
CAA negative	314	1	313	301	75	226
Total tested	371	6	365	353	95	258
Prevalence	**10%**	**2%**		**9%**	**27%**	
			Fishing village			
CAA positive	19	5	14	15	8	7
CAA indecisive	7	0	7	6	0	6
CAA negative	75	1	74	62	26	36
Total tested	101	6	95	83	34	49
Prevalence	**19%**	**6%**		**18%**	**41%**	
			Agricultural village			
CAA positive	17	0	17	17	9	8
CAA indecisive	14	0	14	14	3	11
CAA negative	239	0	239	239	49	190
Total tested	270	0	270	270	61	209
Prevalence	**6%**	**0%**		**6%**	**23%**	

Table 3
Diagnostic characteristics of various assays used for the diagnosis of *S. japonicum* against a standard of infection-positivity by either Kato–Katz or UCP-LF CAA assay[#].

Diagnostic assay	True positives	False positives	False negatives	True negatives	Sensitivity (%)	Specificity (%)	Negative predictive value (%)	Positive predictive value (%)
Kato–Katz	4	0	26	136	13	b.d.[@]	84	b.d.
IHA	16	71	14	65	53	48	82	18
Urine CAA	28	0	2	136	93	b.d.	99	b.d.
Serum CAA	23	0	7	136	77	b.d.	95	b.d.
CAA[*]	30	0	0	136	100	b.d.	100	b.d.

[#] i.e. combined 'gold standard', assuming 100% specificity of the egg detection and CAA results (total $n = 30$ positives).
[@] b.d. = by definition; specificity and positive predictive values are 100%.
[*] CAA in urine and/or serum.

versus 77%). Both methods applied in the field (Kato–Katz and IHA) resulted in much lower sensitivities, with the former being only 13% sensitive.

3.4. Effect of pooling (statistical exercise)

The 371 UCP-LF urine CAA data (with 36 positives, i.e. 9.7% prevalence) were statistically grouped into sets of seven (based on the practical approach of using a 7×2 ml urine samples treated with 30% TCA and concentrated using an Amicon Ultra-15 device) and the average CAA concentration calculated. From these 53 pools, 18 had CAA levels above the cut-off level (34%). In order to identify the positive individuals, these would have to be tested again individually using the UCAA2000 procedure, so in total 53 plus 126 tests would have to be performed, compared to the 371 individually tested, thereby reducing costs and workload by about half. In case the overall prevalence would be lower than 10%, the total number of tests would decrease (e.g. if the same exercise were to be carried out for a small village with, say 270 individuals and a prevalence of 6%, the reduction in the number of tests would be about 60%).

3.5. Age-dependent prevalences of urine CAA and IHA

Fig. 3 shows the prevalence of the urine CAA positives, according to different age-groups. The graph shows that the highest prevalence occurred in adults (≥20 years of age), with a particularly pronounced pattern in the fishing village. Of note, no cases in the age group 10–20 years tested positive for CAA in urine from the fishing village. The ages of the egg-positive individuals ranged from 43 to 64 years.

Fig. 4 compares the serologically positive cases over the different age-groups. In the overall group, the prevalence rates according to serology were found to be stable over the age of 20 years, but for the fishermen they appeared to decrease.

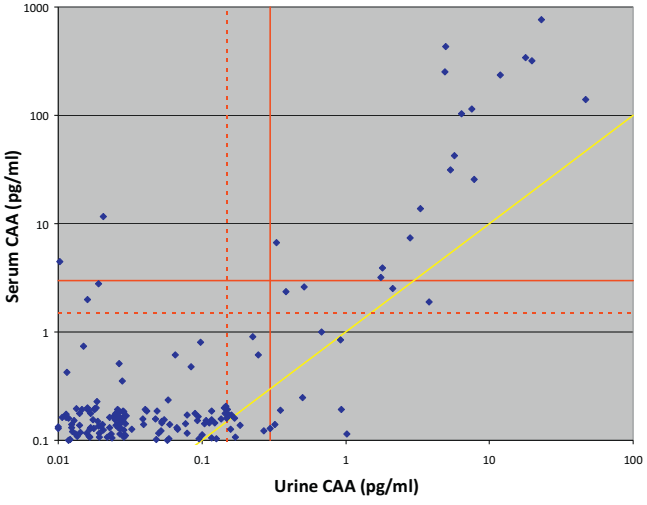

Fig. 2. Scattergram of serum and urine CAA concentrations as determined by the UCP-LF CAA assay. The solid red lines indicate high-specificity cut-off levels, while the dotted red lines indicate lower specificity levels. Samples that have concentrations in the in-between region would be classified as 'indecisive'. The solid yellow diagonal line represents where CAA concentrations in serum and urine would be equal, indicating that most serum CAA concentrations are higher than urine CAA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

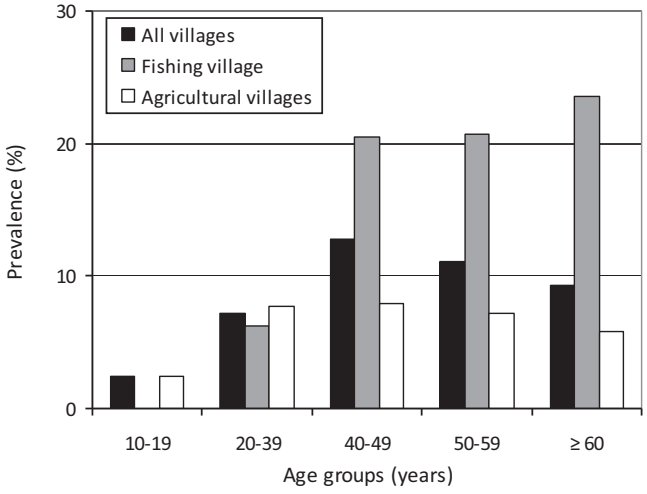

Fig. 3. Distribution of urine CAA positive scores with age, stratified by endemic settings. Three egg-positives were in the 40–49 age-group, one in the 50–59 age-group and two in the 60–69 age-group.

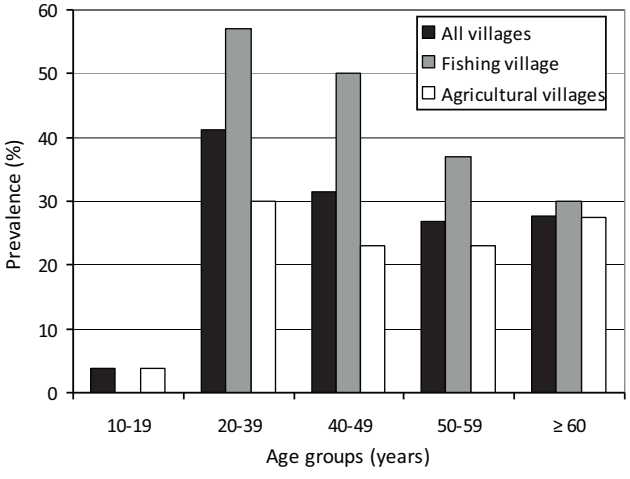

Fig. 4. Distribution of serum IHA positive scores with age in the different endemic settings.

4. Discussion

In the current study it was found that triplicate Kato–Katz thick smears were only 13% sensitive against the highly sensitive UCP-LF CAA assay for *S. japonicum* diagnosis, thereby providing further evidence to the already increasingly obvious conception that the widely used Kato–Katz technique for helminth diagnosis, including *S. japonicum* and *S. mansoni*, is insensitive (Knopp et al., 2009; Lin et al., 2008; Siqueira et al., 2011; Utzinger et al., 2011). This issue is particularly pertinent with respect to detecting schistosome infection in preschool-aged children (Coulibaly et al., 2013; Stothard et al., 2011; Verani et al., 2011) and in well-controlled areas leading to considerable understating of the real situation (Zhou et al., 2011). With more sensitive diagnostics, better strategic decisions will be made (Collins et al., 2012).

The observed prevalence in the current study area in P.R. China was raised significantly according to the diagnostic approach; from 1.4% by Kato–Katz to 9.7% by the urine UCP-LF CAA assay; a six-fold increase. Meanwhile, serum antibodies indicated a prevalence of 27.1%. If the two different transmission settings – the fishing and the agricultural villages – were analysed separately, the increase in prevalence of active infections was even more remarkable, rendering the villages back from infection control into morbidity control and from transmission interruption into infection control (Zhou et al., 2011). With respect to the control approach, the IHA assay is considered as highly sensitive, but – although schistosome-specific – it will not be able to discriminate between active and past infections. Together with the rapid time to result, ease of use and batch testing, it is therefore used largely as a screening tool in the P.R. China, with the active infection cases being confirmed by multiple Kato–Katz thick smears. However, to our surprise, of the five egg positive cases that were also tested by IHA, only two contained antibodies, while similarly, nearly 50% of the CAA positives were antibody negative, the assay having only a positive predictive value of 18% (Table 3). This implies that half of the active infections were actually missed using the IHA as a first-line screening tool, thereby allowing these individuals to go untreated and continue to contribute to transmission. Regarding this underestimation of the true infection status, it must be emphasised that in fact the percentage CAA positivity is on the low side as probably a considerable portion of the indecisive cases will in fact also be positive.

Serum CAA concentrations were found to be significantly higher than urine levels, as has usually been observed in previous studies that still employed the CAA–ELISA (Polman et al., 1995; van Dam et al., 1996a). Inversely, more positive cases were found testing CAA presence in urine as compared to what was found in serum (sensitivity *versus* 'gold' standard of 93% *versus* 77%; see Table 3), which can be attributed to the lower LOD as well as the higher sample volume used in the urine assay. In combination with a higher compliance using urine rather than serum, this will make the urine UCP-LF CAA assay the more favoured candidate for further implementation. Triplicate Kato–Katz from a single stool sample in the present low-endemicity setting only appeared to be 13% sensitive *versus* our combined 'gold' standard.

In the current study, the cut-off threshold that was chosen according to the protocol outlined by Corstjens et al. (2014) turned out to be rather high. It is conceivable that a relatively large percentage in the 'indecisive' group would also be positive, but to determine this issue with high fidelity, repeated testing or higher sample volumes will be needed. For serum, higher volumes are essentially not acceptable for large studies, but for urine this would be possible, although it will require significantly more elaborate procedures as well as higher reagent and device costs. An option to further investigate in practice is the concept of pooled samples, which might reduce labour and costs by 50–80%, depending on the prevalence (Cringoli et al., 2013; Mekonnen et al., 2013). In case a rapid estimation of the overall worm burden in a population would be needed, the individual infection status is not required and the pooling approach will be a very cost-efficient method to determine e.g. the effect of mass treatment.

Categorising the urine CAA and specific antibody positivity by age, the highest prevalence rates of active infections found by the UCP-LF CAA assay were in older people (aged 40 years and above), and this finding was particularly pronounced in the fishing village. The IHA prevalence rates overall were found to be stable at ages 20 years and higher, while in the fishing village they appeared to decrease at higher ages. The latter is a common phenomenon in schistosomiasis antibody serology and could well be an effect of immune down-regulation in chronic schistosome infections (van Lieshout et al., 1995). Divergent from the decreasing trend with age of antibody positives active infections as measured by CAA tend to increase with age. If serum CAA results were added to the age-prevalence pattern as well, all age groups from 20 years onwards show a similar prevalence for the fishermen (data not shown). Using the ultra–sensitive UCP-LF CAA for further research could assist elucidating these mechanisms.

The commercially available POC–CCA urine cassette test could potentially replace the Kato–Katz test with respect to rapid identification of areas at-risk for *S. mansoni* (Bergquist, 2013). Benefits include substantial savings in time for collection and processing of specimens (urine instead of stool), as well as direct savings in terms of the costs of testing and treatment delivery (Colley et al., 2013; Knopp et al., 2013). However, in low-endemicity areas that were the subject of the current study, the POC–CCA did not show sufficient sensitivity to be useful (data not shown). Nonetheless, in an accompanying paper, where the same assay was evaluated in highly endemic settings in the Philippines for *S. japonicum* and in Cambodia for *Schistosoma mekongi*, the POC–CCA was shown to be helpful as an alternative or supplement to the Kato–Katz technique (van Dam et al., 2015).

The main arguments for the high specificity of the UCP-LF CAA assay used in this study are antigen uniqueness (Bergwerff et al., 1994), detection by highly sensitive and specific monoclonal antibodies (careful selection of hybridoma for optimal performance) (Deelder et al., 1989) and extraction of CAA by TCA sample pretreatment. Polman et al. (2000) have reported high sensitivity and a near perfect specificity for the CAA, irrespective of target population and pretreatment method, supporting the finding by Bergwerff et al. (1994). However, there was a lower specificity with CCA explained by cross-reactivity with a common epitope with granulocytes requiring increased cut-off levels (van Dam et al., 1996b). TCA extraction is the pretreatment of choice as it not only seems to influence specificity, but also effectively dissociates potential immune complexes (De Jonge et al., 1987), thereby increasing sensitivity, at least theoretically. Fast clearance after effective treatment (van't Wout et al., 1992), and increase of CAA levels over the course of an experimental infection (van Dam et al., 1996a) provide additional leverage to the arguments put forth here. Using sets of negative control samples, and the application of a high-specificity cut-off threshold furthermore substantiated the very high specificity of the UCP-LF CAA assay (Corstjens et al., 2014; van Dam et al., 2013).

A rapid and cost-effective diagnostic test capable of detecting as few as one or two schistosome worm pairs is urgently needed for the elimination stage of schistosomiasis (Knopp et al., 2013). There are significant differences between traditional diagnostic methods, such as stool microscopy and serology on the one hand, PCR, LAMP and detection of circulating antigens, on the other hand. While serology is an indirect technique, the other approaches are direct and therefore applicable, depending on sensitivity, for indicating the status of infection or treatment outcomes. Both PCR and LAMP can be seen as improvements on the stool examination approach as they target worm products excreted by the host faecal route, while

the circulating antigens are either detected in serum or urine. From operational and compliance points of view, collection of urine is a preferable option, as it is less invasive than collection of blood (or stool) samples. Different diagnostic approaches are not always comparable because of variations in epidemiological settings. One explanation for the inconsistencies between these diagnostic tests relates to the current lack of a definitive standard reference test to detect all *Schistosoma* infections in real-life settings. The introduction and wide use of the Kato–Katz technique standardised the situation for highly endemic areas, but as we move to the other extreme in large parts of the endemic world, a more sensitive standard is needed (Utzinger et al., 2011). The important question is "What are the best diagnostic methods?" Addressing this question will inform policy decision at different stages of schistosomiasis control (from morbidity to infection control, transmission control, transmission interruption, and ultimately elimination) (Bergquist et al., 2009).

Finally, the UCP-LF CAA assay as an ultra-sensitive and straightforward method has been employed for the first time to urine samples from *S. japonicum*-infected individuals from a low prevalence area in P.R. China. As this test can be performed on urine samples, compliance will be less of an issue than standard Kato–Katz applied on stool or serological approaches. These characteristics, in combination with the significantly higher prevalence of active schistosome infections shown in the current study, make the UCP-LF CAA assay uniquely useful for the Chinese national schistosomiasis control/elimination programme, having implications with respect to case-finding, treatment strategies and verification of local elimination. To this end, initial steps have been made to upscale the production of the test with the required thorough quality control assessment and stability tests. The control programme is currently entering the elimination phase where an intensification and enlargement of the existing diagnostic arsenal is one of the essential requirements for success.

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