EVALUATION OF FECAL AND SEROLOGICAL TESTS FOR THE DIAGNOSIS OF SCHISTOSOMIASIS IN SELECTED NEAR-ELIMINATION AND ENDEMIC AREAS IN THE PHILIPPINES

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Abstract. Schistosomiasis caused by *Schistosoma japonicum* is endemic in the Philippines. The Kato-Katz Technique (KKT) is the most commonly used technique for schistosomiasis surveillance, but may have inadequate sensitivity for surveillance. Our study aimed to determine the best schistosomiasis surveillance test(s) for near-elimination and endemic areas in the Philippines. The study population was randomly selected school children aged 9-15 years. The study locations were the provinces of Bohol and Zamboanga del Norte (ZDN) for the near-elimination areas and Agusan del Sur (ADS) for the endemic area. A total of 1,112 study participants were included in the study. Each participant provided a stool and a blood sample to test for schistosomiasis. Each stool sample was examined using the KKT and Formalin Ether Concentration Technique (FECT). Each blood sample was examined using the Circumoval Precipitin Test (COPT), and the Enzyme-Linked Immunosorbent Assay (ELISA) antibody (Ab) and antigen (Ag) tests. We calculated the prevalence of schistosomiasis using each test. We also calculated the sensitivity and specificity of each test in the near-elimination and endemic areas using a combination of the KKT, FECT and ELISA Ag tests as a reference standard. The results showed a zero prevalence in the studied near-elimination areas and a 17.6% prevalence in the studied endemic areas using the KKT; a 0.1% prevalence in the studied near-elimination areas and a 2.5% prevalence in the studied endemic areas using FECT; an 11.0% prevalence in the studied near-elimination areas and a 27.2% prevalence in the endemic areas using the COPT, a 16.8% in the studied near-elimination areas and a 58.5% prevalence in the endemic areas using the ELISA Ab test, and an 8.6% prevalence in the studied near-elimination areas and a 30.5% prevalence in the endemic areas using the ELISA Ag test. The sensitivities were 0.0%, 1.6%, 24.6%, 36.9%, and 98.5% and the specificities were 100.0%, 100.0%, 90.3%, 85.1%, and 100.0% for the KKT, FECT, COPT, ELISA Ab,

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and ELISA Ag tests, respectively in near-elimination areas. The sensitivities were 43.2%, 6.1%, 40.5%, 84.5%, and 75.0% and the specificities were 100.0%, 100.0%, 81.9%, 59.3%, and 100.0% for the KKT, FECT, COPT, ELISA Ab, and ELISA Ag tests, respectively, in the endemic areas. Our results showed higher prevalences of schistosomiasis using the serological than the fecal tests. They also showed generally higher sensitivities for the serological tests than the fecal tests. In areas with lower prevalences, serological testing may be more appropriate for population screening for schistosomiasis in the Philippines.

Keywords: *Schistosoma japonicum*, schistosomiasis, Kato-Katz, FECT, COPT, ELISA, antigen detection test, antibody detection test

INTRODUCTION

Schistosomiasis caused by *Schistosoma japonicum* is endemic in 12 of the 16 administrative regions of the Philippines (Department of Health, 2010). King *et al* (2005) conducted a systematic review and meta-analysis of disability due to schistosomiasis and reported the disability weight of schistosomiasis (2-15%) was greater than previously estimated (0.5%). Schistosomiasis can cause anemia, stunt growth, undernutrition, predisposition to and exacerbation of co-infections, cognitive underdevelopment, decreased work capacity and chronic pain (King *et al*, 2005).

Schistosomiasis can be diagnosed on stool examination, such as with the Kato-Katz Technique (KKT) or the Formalin Ether Concentration Technique (FECT); it can also be diagnosed by examining the serum for schistosome-specific antibodies, such as with the Circumoval Precipitin Test (COPT) or Enzyme-Linked Immunosorbent Assay (ELISA) (Doenhoff *et al*, 2004). The Philippine Department of Health (DOH) currently conducts active and passive surveillance for schistosomiasis using government health facilities (DOH, 2010). The DOH monitors schistosomiasis endemic areas by conducting stool examinations using the KKT. A single stool exam using the KKT has a sensitivity of 51.1-84.1% compared to six stool exams in the same person using the KKT (Lin *et al*, 2008).

In the Philippines, the DOH classifies areas with a schistosomiasis prevalence ≥1% of the population as being a schistosomiasis endemic area and areas with prevalence <1% as being near-elimination areas (DOH, 2009). In near-elimination areas, the most commonly used screening method (the KKT) may lack the sensitivity to determine the actual prevalence of schistosomiasis (Utzinger *et al*, 2005).

Our study aimed to determine the best diagnostic test(s) to conduct active surveillance for *S. japonicum* in near-elimination and endemic areas in the Philippines. We aimed to determine the prevalences of schistosomiasis using the KKT, the FECT, the COPT, the ELISA antibody (Ab) detection test, and the ELISA antigen (Ag) detection test at the selected study sites. We compared the sensitivity and specificity of the different studied diagnostic techniques for *S. japonicum* in near-elimination and endemic areas.

MATERIALS AND METHODS

Study site and population

We selected three provinces for our study. Two provinces were near-elimi-

nation areas (Bohol and Zamboanga del Norte) and one province was endemic for schistosomiasis (Agusan del Sur). Two municipalities were selected from each province in coordination with the concerned regional offices of the DOH. The municipalities were: Trinidad and Talibon in Bohol, Pinan and Polanco in Zamboanga del Norte and Bunawan and Prosperidad in Agusan del Sur. The latest sentinel surveillance data from the Provincial Health Offices for the selected municipalities reported no cases of schistosomiasis in Trinidad or Talibon in Bohol during 2013 and no cases of schistosomiasis in Polanco, and a prevalence of 0.45% was reported in Pinan, Zamboanga del Norte in 2013. The prevalences of schistosomiasis were reported to be 12.9% in Bunawan and 9.1% in Prosperidad, in Agusan del Sur.

In this study, we selected children in grades 5-6 as suggested by Montresor *et al* (1998). Two hundred school children were targeted from each municipality; the number needed is based on a paper by Montresor *et al* (1998) who stated that a sample size of 200-250 is adequate in each ecologically homogeneous area to evaluate the prevalence of infection. All school children from grades 5-6 who gave written informed consent and their guardian did as well were included in the study. The schools were chosen based on the recommendation of the respective local government units for the study sites. Study participants were residents of the study areas who had not received praziquantel during the previous six months. The study was conducted during February and March, 2014.

Fecal examination

Blood and stool samples were obtained at the study schools with approval of the Department of Education (DepEd). The stool samples were then brought to the field laboratory where they were examined using the KKT and FECT. Parasites were diagnosed using the World Health Organization bench aids for the diagnosis of intestinal parasites (WHO, 2012).

Serological examination

Approximately 4 ml venous blood was obtained from each participant and kept cold until examined for schistosomiasis using the COPT and ELISA Ab and Ag detection tests at the University of the Philippines, Manila – National Institutes of Health (UPM-NIH) Central laboratory.

For the COPT, 2 drops of serum was added to lyophilized *S. japonicum* eggs on a glass slide, covered with a cover slip and sealed with paraffin wax. The slide was then incubated at 37°C for 24-48 hours and then examined for the presence of segmented precipitates, indicating a positive result (Garcia *et al*, 1981).

The ELISA tests were performed using kits by Inno Vision International (Hong Kong). *Schistosoma japonicum* IgG ELISA kits and *Schistosoma japonicum* Ag ELISA kits were used following the manufacturer's instructions. Absorbance was measured using an iMark™ Microplate Reader (Bio-Rad, Hercules, CA) at 450 nm. Results were recorded as positive or negative for each assay.

Quality control

Ten percent of the Kato-Katz slides were re-examined by a reference microscopist blinded to the first examiner's results (Montresor *et al*, 1998). FECT positive samples were confirmed by a reference microscopist in the field. Positive and negative control sera were used for quality control with the COPT, ELISA Ab test and ELISA Ag test.

Data processing and analysis

Recorded results were entered into Microsoft Excel (2007; Microsoft, Redmond, WA). The data were entered in duplicate. The following were calculated: 1) prevalences and intensities of schistosomiasis in the near-elimination and endemic areas using the KKT, FECT, COPT, ELISA Ab and ELISA Ag tests; 2) the prevalences of moderate to heavy intensity infections using the KKT; 3) sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each test in the near-elimination and endemic areas.

Reference standard used was the combination of the KKT, FECT, and ELISA Ag test. The combination of these 3 tests was used to increase the sensitivity of the reference standard. The COPT and ELISA Ab test were not included in the reference standard due to their low specificities, giving high false positive rates (Garcia *et al*, 1981; Xu *et al*, 2011). True positive infection status was then defined as a sample from an individual positive for *S. japonicum* in any one of the three diagnostic tests mentioned (Glinz *et al*, 2010; Van Dam *et al*, 2015). Following this approach, the specificities of the studied tests were combined and assumed to be the reference standard of 100% based on previous studies (Bogoch *et al*, 2006; Steinmann *et al*, 2008; Utzinger *et al*, 2008; Knopp *et al*, 2009; Midzi *et al*, 2009; Glinz *et al*, 2010; Van Dam *et al*, 2015).

Logistic regression was used to determine whether the differences in prevalences of schistosomiasis using the ELISA Ag and Ab tests were significantly different between near- elimination and endemic areas, between those aged ≤12 years and those aged >12 years and different by gender. Associations were assessed between study sites and age groups, and between study sites and gender. We also calculated the odds ratios of associations between study sites and age groups and between study sites and gender.

Ethical considerations

The study protocol, consent forms, and patient information sheets were reviewed and approved by the University of the Philippines, Manila - Research Ethics Board (UPMREB-2013-NIH-P2-047). Consent forms were distributed to the targeted study participants and their parents/guardians and only those who gave informed consent were included in the study. All study participants with a positive test for schistosomiasis or other parasitic infections were referred to local health units to obtain appropriate treatment following DOH guidelines.

RESULTS

A total of 1,112 study subjects aged 9-15 years were included in the study, of whom 748 participants (67.2%) were from a near-elimination area and 364 (32.8%) were from an endemic area. Table 1 shows the ages and genders of participants by study site.

Table 2 shows the prevalences of schistosomiasis by stool examination technique. The prevalence of schistosomiasis in endemic areas using KKT was 17.6%, with 4.7% having a moderate to heavy intensity infection. No cases of schistosomiasis were seen using the KKT in near-elimination areas. The prevalence of schistosomiasis in the endemic areas using the FECT was 2.5%. One case of schistosomiasis was identified using the FECT in a near-elimination area.

Table 3 summarizes the results of the serological examinations. The near- elimi-

Table 1
Ages and genders of study participants by study site.

Ages in years by study site	Gender		Total
	Female	Male	
Age groups in near-elimination areas			
<10	11	4	15
10-12	155	110	265
>12	222	246	468
Subtotal	388	360	748 (67.2%)
Age groups in endemic areas			
Age not recorded	0	1	1
<10	13	7	20
10-12	76	62	138
>12	108	97	205
Subtotal	197	167	364 (32.8%)
Total	585	527	1,112

Table 2
Prevalences of schistosomiasis using the Kato-Katz Technique (KKT) and the Formalin Ether Concentration Technique (FECT) among study subjects at study sites.

Study sites	No. of stool specimens examined	Stool specimen positive for *S. japonicum*		
		KKT	Those with moderate to heavy infections by KKT	FECT
		No. (%)	No. (%)	No. (%)
Near-elimination Areas	748	0 (0.0)	0 (0.0)	1 (0.1)
Endemic Areas	364	64 (17.6)	17 (4.7)	9 (2.5)
Total	1,112	64 (5.8)	17 (1.5)	10 (0.9)

nation areas had seroprevalences of 11.0%, 16.8%, and 8.6% using the COPT, ELISA Ab, and ELISA Ag tests, respectively. The endemic areas had seroprevalences of 27.2%, 58.5%, and 30.5% using the COPT, ELISA Ab, and ELISA Ag tests, respectively.

Tables 4 and 5 show the results of the logistic regression analysis for ELISA Ag and Ab tests. With the ELISA Ag test, study subjects living in endemic areas had a 4.69 times greater odds of having schistosomiasis than subjects living in near-elimination areas (Table 4). Age was retained as a variable in the logistic regression model to adjust for its effect on estimates. Males were not significantly more likely to have schistosomiasis than females with the ELISA Ag test. There was also no significant associations between study site and age and between study site and gender with the ELISA Ag test.

Table 3
Seroprevalences of schistosomiasis using the Circumoval Precipitin Test, Enzyme-Linked Immunosorbent Assay (ELISA) Antigen (Ag), and ELISA Antibody (Ab) tests among study subjects.

Study sites	No. of blood samples examined	Positive for *S. japonicum* by test type		
		COPT No. (%)	ELISA Ab No. (%)	ELISA Ag No. (%)
Near-elimination areas	748	82 (11.0)	126 (16.8)	64 (8.6)
Endemic areas	364	99 (27.2)	213 (58.5)	111 (30.5)
Total	1,112	181 (16.3)	339 (30.5)	175 (15.7)

Table 4
Logistic regression analysis results for Enzyme-Linked Immunosorbent Assay Antigen test.

Variables	Odds ratio (Standard error)	95% Confidence interval	*p*-value
Age group (>12 yrs old)	0.99 (0.18)	0.71, 1.40	0.974
Sex (M)	1.34 (0.23)	0.95, 1.88	0.091
Endemic areas	4.69 (0.81)	3.34, 6.59	<0.001
Constant	0.09 (0.01)	0.07, 0.12	<0.001

Table 5
Logistic regression analysis results for Enzyme-Linked Immunosorbent Assay Antibody test.

Variables	Odds ratio (Standard error)	95% Confidence interval	*p*-value
Age group	1.47 (0.23)	1.09, 1.99	0.012
Sex (M)	1.90 (0.28)	1.42, 2.54	<0.001
Endemic areas	6.96 (1.00)	5.25, 9.24	<0.001
Constant	0.20 (0.02)	0.17, 0.25	<0.001

With the ELISA Ab test, subjects living in endemic areas had a 6.96 times greater odds of having schistosomiasis than subjects living in near-elimination areas (Table 5). There was no significant association between study site and age group and between study site and gender with the ELISA Ab test. Study subjects aged >12 years had a 1.5 times greater odds of having schistosomiasis than study subjects aged ≤12 years. Male study subjects had a 1.9 times greater odds of having schistosomiasis than female study subjects with the ELISA Ab test.

Table 6
Sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) for the selected diagnostic techniques in near-elimination areas using the combination of the Kato-Katz Technique (KKT), Formalin Ether Concentration Technique (FECT), and Enzyme-Linked Immunosorbent Assay (ELISA) Antigen Test as the reference standard.

Diagnostic test	Sensitivities (%)	Specificities (%)	Positive predictive values (%)	Negative predictive values (%)
KKT	0.0	100.0	0.0	91.3
FECT	1.6	100.0	100.0	91.4
Circumoval precipitin test	24.6	90.3	19.5	92.6
ELISA antibody	36.9	85.1	19.1	93.4
ELISA antigen	98.5	100.0	100.0	99.9

Table 7
Sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) for the selected diagnostic techniques in endemic areas using the combination of the Kato- Katz Technique (KKT), Formalin Ether Concentration Technique (FECT), and Enzyme-Linked Immunosorbent Assay (ELISA) Antigen Test as the reference standard.

Diagnostic test	Sensitivities (%)	Specificities (%)	Positive predictive values (%)	Negative predictive values (%)
KKT	43.2	100.0	100.0	72.0
FECT	6.1	100.0	100.0	60.9
Circumoval precipitin test	40.5	81.9	60.6	66.8
ELISA antibody	84.5	59.3	58.7	84.8
ELISA antigen	75.0	100.0	100.0	85.4

Table 6 shows the sensitivities, specificities, PPVs, and NPVs for each specified test for near-elimination areas using the KKT, FECT and ELISA Ag tests as the reference standard. The sensitivities were 0.0%, 1.6%, 24.6%, 36.9%, and 98.5% for the KKT, FECT, COPT, ELISA Ab, and ELISA Ag tests, respectively, for the near-elimination areas. The specificities were 100.0%, 100.0%, 90.3%, 85.1%, and 100.0% for the KKT, FECT, COPT, ELISA Ab, and ELISA Ag tests, respectively, for the near-elimination areas. The PPVs were 0.0%, 100.0%, 19.5%, 19.1%, and 100.0% for the KKT, FECT, COPT, ELISA Ab, and ELISA Ag tests, respectively, for the near-elimination areas. The NPVs were 91.3%, 91.4%, 92.6%, 93.4%, and 99.9%

for the KKT, FECT, COPT, ELISA Ab, and ELISA Ag tests, respectively, for the near-elimination areas.

Table 7 shows the sensitivities, specificities, PPVs, and NPVs for each specified test for endemic areas using the KKT, FECT and ELISA Ag tests as the reference standard. The sensitivities were 43.2%, 6.1%, 40.5%, 84.5%, and 75.0% for the KKT, FECT, COPT, ELISA Ab, and ELISA Ag tests, respectively, in endemic areas. The specificities were 100.0%, 100.0%, 81.9%, 59.3%, and 100.0% for the KKT, FECT, COPT, ELISA Ab, and ELISA Ag tests, respectively, in endemic areas. The PPVs were 100.0%, 100.0%, 60.6%, 58.7%, and 100.0% for the KKT, FECT, COPT, ELISA Ab, and ELISA Ag tests, respectively, in endemic areas. The NPVs were 72.0%, 60.9%, 66.8%, 84.8%, and 85.4% for the KKT, FECT, COPT, ELISA Ab, and ELISA Ag tests, respectively, in endemic areas.

DISCUSSION

In our study, we found higher prevalence rates of schistosomiasis using serological tests than fecal tests in both near-elimination and endemic areas, similar to a previous study from Brazil that reported a 16% prevalence rate with the KKT and at 47.2% prevalence rate with the ELISA Ab test for schistosomiasis (Carneiro *et al*, 2012). In a study from China, Cai *et al* (2014) reported a prevalence of 12.7% using the ELISA Ab test, 4.7% using the ELISA Ag test and 1.5% using the KKT among 1,864 samples from an endemic area.

Comparing serological tests, we found higher prevalence rates with antibody tests (COPT and ELISA Ab) than the antigen test (ELISA Ag) in both near-elimination and endemic areas. Comparing antibody tests, the ELISA Ab test gave a higher prevalence rate than the COPT in both near-elimination and endemic areas, similar to a study from Brazil that reported higher rates of schistosomiasis using the ELISA IgG test (11.6%) than the COPT (5.4%) (Espirito-Santo *et al*, 2015). However, antibody tests have higher false positive rates than antigen tests since they cannot differentiate between current and previous infections.

In our study in near-elimination areas, the ELISA Ag test had the highest sensitivity at 98.5%, with a specificity of 100%. In our study in endemic areas, the ELISA Ab test had the highest sensitivity at 84.5%, but the lowest specificity at 59.3%. Ab detection tests generally have low specificities due to not differentiating between active and previos infection and cross-reactivity with other parasites. The especially low specificity of antibody tests in endemic areas (Doenhoff *et al*, 2004) make it unfit for screening; the ELISA Ag test would be more appropriate with a sensitivity of 75% and a specificity of 100%.

The KKT is the current gold standard to diagnose schistosomiasis, having a specificity of 100% but had a sensitivity of 0% in near-elimination areas in our study. This indicates the KKT is inappropriate as a screening tool for near-elimination areas in the studied areas. In endemic areas in our study, the KKT had a sensitivity of 43.2% and a specificity of 100%. Despite the lower sensitivity of the KKT compared with the ELISA Ag and Ab tests, the prevalence rates seen in our study are the same as those of the DOGH, since they used the KKT for their screening. This also suggests that if the prevalence of schistosomiasis is >1%, the KKT is an appropriate screening test for the studied areas.

Better screening tests are needed to inform schistosomiasis elimination pro-

grams in the Philippines. The ELISA Ag test suggests itself due to its reliability and sensitivity (Zhou *et al*, 2011), especially for near-elimination areas. In endemic areas, the KKT is still useful.

In summary, in our study, the KKT was still adequate for schistosomiasis screening in endemic areas but the ELISA Ag test should be added to this technique in near- elimination areas in the study location. This can better inform schistosomiasis control programs in the study areas.

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REFERENCES

Bogoch II, Raso G, N'Goran EK, Marti HP, Utzinger J. Differences in microscopic diagnosis of helminths and intestinal protozoa among diagnostic centres. *Eur J Clin Microbiol Infect Dis* 2006; 25: 344-7.

Cai YC, Xu JF, Steinmann P, *et al*. Field comparison of circulating antibody assays *versus* circulating antigen assays for the detection of schistosomiasis japonica in endemic areas of China. *Parasites Vectors* 2014; 7: 138.

Carneiro TR, Pinheiro MCC, de Oliveira SM, Hanemann AL, Queiroz JAN, Bezerra FSM. Increased detection of schistosomiasis with Kato-Katz and SWAP-IgG-ELISA in a northeastern Brazil low-intensity transmission area. *Rev Soc Brasileira Med Trop* 2012; 45: 510-13.

Department of Health (DOH). Administrative Order 2009-0013: Declaring the month of july every year as the mass treatment and awareness month for schistosomiasis in the established endemic areas in the Philippines. Manila: DOH, 2009.

Department of Health (DOH). Clinical practice guidelines for the diagnosis, treatment and prevention of *Schistosoma japonicum* infections in the Philippines. Manila: DOH, 2010.

Doenhoff M, Chiodini P, Hamilton J. Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies. *Trends Parasitol* 2004; 35-9.

Espírito-Santo MCC, Alvarado-Mora MV, Pinto PLS, *et al*. Comparative study of the accuracy of different techniques for the laboratory diagnosis of Schistosomiasis mansoni in areas of low endemicity in Barra Mansa City, Rio de Janeiro State, Brazil. *BioMed Res Int* 2015; 2015: 135689.

Garcia EG, Tapales FP, Valdez CA, Mitchell GF, Tiu WU. Attempts to standardize the circumoval precipitin test (COPT) for schistosomiasis japonica. *Southeast Asian J Trop Med Public Health* 1981; 12: 384-95.

Glinz D, Silue KD, Knopp S, *et al*. Comparing diagnostic accuracy of Kato–Katz, Koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. *PLOS Negl Trop Dis* 2010; 4: e754.

Kanamura HY, Dias LCS, Glasser CM, *et al*. Detection of IgM antibodies to *Schistosoma mansoni* gut-associated antigens for the study of the dynamics of schistosomiasis transmission in an endemic area with low worm burden. *Rev Inst Med Trop São Paulo* 1998; 40: 225-31.

King CH, Dickman K, Tisch DJ. Reassessment

of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* 2005; 365 (9740): 1561-9.

Knopp S, Rinaldi L, Khamis IS, *et al*. A single FLOTAC is more sensitive than triplicate Kato-Katz for the diagnosis of low-intensity soil-transmitted helminth infections. *Trans R Soc Trop Med Hyg* 2009; 103: 347-54.

Lin DD, Liu JX, Liu YM, *et al*. Routine Kato-Katz technique underestimates the prevalence of *Schistosoma japonicum*: a case study in an endemic area of the People's Republic of China. *Parasitol Int* 2008; 57: 281-6.

Midzi N, Butterworth AE, Mduluza T, Munyati S, Deelder AM, van Dam GJ. Use of circulating cathodic antigen strips for the diagnosis of urinary schistosomiasis. *Trans R Soc Trop Med Hyg* 2009; 103: 45-51.

Montresor A, Crompton DWT, Hall A, Bundy DAP, Savioli L. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. *Trans R Soc Trop Med Hyg* 1998; 92: 470-1.

Steinmann P, Du ZW, Wang LB, *et al*. Extensive multiparasitism in a village of Yunnan province, People's Republic of China, revealed by a suite of diagnostic methods. *Am J Trop Med Hyg* 2008; 78: 760-9.

Utzinger J, Zhou X, Chen M, Bergquist R. Conquering schistosomiasis in China. *Acta Trop* 2005; 96: 69-96.

Utzinger J, Rinaldi L, Lohourignon LK, *et al.* FLOTAC: a new sensitive technique for the diagnosis of hookworm infections in humans. *Trans R Soc Trop Med Hyg* 2008; 102: 84-90.

Van Dam GJ, Xu J, Bergquist R, *et al*. An ultrasensitive assay targeting the circulating anodic antigen for the diagnosis of *Schistosoma japonicum* in a low-endemic area, People's Republic of China. *Acta Trop* 2015; 141: 190-7.

World Health Organization (WHO). Bench aids for the diagnosis of intestinal parasites. Geneva: WHO, 2012.

Xu J, Peeling R, Chen JX, *et al*. Evaluation of immunoassays for the diagnosis of *Schistosoma japonicum* infection using archived sera. *PLOS Negl Trop Dis* 2011; 5: e949.

Zhou YB, Zheng HM, Jiang QW. A diagnostic challenge for schistosomiasis japonica in China: consequences on praziquantel-based morbidity control. *Parasit Vectors* 2011; 4: 194.