RESEARCH NOTE

FIELD EVALUATION OF A DIPSTICK DYE IMMUNOASSAY IN LARGE-SCALE SCREENING OF HUMAN *SCHISTOSOMA JAPONICUM* INFECTIONS IN CHINA

Rujin Xu, Yueqin Chen, Fang He, Yenan Shen and Chunyan Qian

First People's Hospital of Linping District, Linping Campus, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang Province, China

Abstract. Schistosomiasis japonica remains a major public health concern in China. Diagnosis is central to control and elimination of schistosomiasis in China. As parasitological techniques are laboratory-intensive, time-consuming and insensitive in low-endemicity regions, compounded with poor compliance, immunodiagnostics with high sensitivity and specificity provide an alternative tool. Here we examined the efficacy of a dipstick dye immunoassay (DDIA) in a large-scale screening of human *Schistosoma japonicum* infection among adult residents (n = 404) of a village located a schistosomiasis endemic region near Poyang Lake, Jiujiang, Jiangxi Province in comparison with an enzyme-linked immunosorbent assay (ELISA), an indirect hemagglutination assay (IHA) and a miracidium hatching test, the gold standard. A 13.4% prevalence of *S. japonicum* infection was detected using the gold standard test, with a diagnostic sensitivity, specificity, positive predictive value and negative predictive value of 94.4, 67.7, 31.1, and 98.8%, respectively for DDIA; 87.0, 65.1, 27.8, and 97.0%, respectively for ELISA and 75.9, 72.9, 30.1, and 95.1%, respectively for IHA. Assays of serum samples of healthy subjects (n = 418) who resided in non-endemic regions and had never traveled in schistosomiasis-endemic areas revealed a diagnostic specificity of DDIA, ELISA and IHA of 98.1, 97.4 and 98.8%, respectively. In conclusion, DDIA proved to be a simple and rapid immunodiagnostic method with high sensitivity and specificity as evaluated in a large-scale screening of human *S. japonicum* infection in an endemic region of China.

Keywords: *Schistosoma japonicum*, diagnostic efficacy, dipstick dye immunoassay, schistosomiasis japonica screening, China

Correspondence: Yueqin Chen, First People's Hospital of Linping District, Linping Campus, The Second Affiliated Hospital of Zhejiang University School of Medicine, No. 369 Yingbin Road, Linping District, Hangzhou City, Zhejiang Province 310006, PR China
Tel: +86-571-8936-9917 E-mail: yhxcn2007@aliyun.com

INTRODUCTION

Diagnosis is central to schistosomiasis control (Lv *et al*, 2022). Currently, diagnosis of schistosomiasis japonica mainly employs parasitological and immunodiagnostic techniques (Zhang *et al*, 2016b). Parasitological examinations using Kato-Katz technique and/or miracidium hatching test remain the gold standard for definitive diagnosis of human *Schistosoma japonicum* infection (Zhou *et al*, 2007). However, parasitological techniques are time-consuming, with low compliance (Zhou *et al*, 2007) and, importantly, have a high percent missed diagnosis in regions with low schistosomiasis japonica endemicity (Zhou *et al*, 2011b). Thus, immunodiagnostic assays have been accepted as a simple and sensitive tool for screening target populations in schistosomiasis-endemic areas (Zhu, 2005).

Zhu *et al* (2002) developed a rapid, simple and inexpensive dipstick dye immunoassay (DDIA) targeting anti-*S. japonicum* specific antibodies, which has proven to be highly sensitive and specific for detection of schistosomiasis japonica (Xu *et al*, 2011a; Xu *et al*, 2011b; Zhou *et al*, 2011a). However, there has been no large-scale field evaluation of DDIA efficacy in the diagnosis of schistosomiasis japonica. Hence, this study evaluated DDIA method in comparison with an enzyme-linked immunosorbent assay (ELISA), an indirect hemagglutination assay (IHA) and a miracidium hatching test, the gold standard, in a large-scale screening of human *S. japonicum* infection in an endemic focus of China. Our findings may provide field evidence for the use of DDIA for large-scale serological screening of *S. japonicum*-infected individuals, which provides insights into the option of a diagnostic tool for schistosmiasis japonica screening during the stage moving towards elimination of schistosomiasis.

MATERIALS AND METHODS

Recruitment of subjects

From March to June, 2011, residents (n = 404), 15-70 years of age, were randomly sampled in a village located near Poyang Lake, Jiujiang, Jiangxi Province (Fig 1) where *S. japonicum* is highly endemic (Tseng *et al*, 2014). Blood and stool samples were collected from subjects for subsequent detection *S. japonicum*

Fig 1 - Map of PR China showing location of the study area for large-screening of schistosomiasis japonica using a dipstick dye immunoassay (March - June, 2011)

infection using DDIA, ELISA, IHA, and miracidium hatching test (MHT). Negative controls (n = 418) were serum samples from healthy individuals, 18-70 years of age, who resided in non-endemic regions and had never travelled in schistosomiasis-endemic regions (kindly provided by the Hangzhou Municipal Blood Center, Hangzhou, PR China).

The study protocols were approved by the Ethics Review Committee of First People's Hospital of Linping District (permission no. YHYY-2010102). Prior written informed consent was obtained from all participants. Prior written consent was waived for blood samples from Hangzhou Municipal Blood Center as names, gender and addresses of blood donors were redacted.

MHT procedure

MHT was performed as described previously (Zhu *et al*, 2014). Briefly, approximately 30 g of stool samples were sieved through a nylon tissue bag to collect parasite eggs, which were suspended in dechlorinated water, incubated at 25-28 °C and miracidium hatching observed at 4, 8 and 12 hours. Miracidia hatching from ova were visualized microscopically and the presence of swimming miracidia was defined as an infection.

DDIA, ELISA and IHA procedures

DDIA was conducted using a commercial kit (Wuxi Saide Biotechnology Development Corp, Wuxi, PR China). In brief, a 50-µl aliquot of a blue dye-labeled soluble egg antigen (SEA) solution was added into a well of a polyvinyl chloride plate, followed by a 10-µl aliquot of serum sample and the solution was gently mixed prior to insertion of the dipstick for 5-10 minutes until the solution was completely absorbed. Presence of both test and control bands on the dipstick is defined as a positive result, and the presence of only control band as negative.

ELISA and IHA tests were conducted using a commercial kit (Shenzhen Combined Biotech Co Ltd, Shenzhen, PR China and Anji Medical Technology Co Ltd, Hefei, PR China, respectively). A 2.1-fold higher A450 nm of test serum than that of negative control serum is considered a positive ELISA test and a serum antibody titer of ≥1:10 a positive IHA test (Xu *et al*, 2011b).

Data analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated by the equations below using MHT as the gold standard for diagnosis of *S. japonicum* human infection.

$$\text{Sensitivity} = \frac{\text{(Number of true positives)}}{\text{(Number of true positives+number of false negatives)}}$$

$$\text{Specificity} = \frac{\text{(Number of true negatives)}}{\text{(Number of true negatives+number of false positives)}}$$

$$\text{PPV} = \frac{\text{(Number of true positives)}}{\text{(Numer of true positives+number of false positives)}}$$

$$\text{NPV} = \frac{\text{(Number of true negatives)}}{\text{(Number of true negatives+number of false negatives)}}$$

RESULTS

Among subjects (n = 404) randomly sampled from a village located in a highly endemic schistosomiasis region near Poyang Lake, Jiujiang, Jiangxi Province, frequencies of *S. japonicum* positive subjects using DDIA, ELISA and IHA were ~2.8 folds higher than that of the MHT gold standard (Table 1). Sensitivity, specificity, PPV, and NPV was 94.4, 67.7, 31.1, and 98.8%, respectively for DDIA; 87.0, 65.1, 27.8 and 97.0%, respectively for ELISA and 75.9, 72.9, 30.1 and 95.1%, respectively for IHA.

Among stool-negative samples, there 34 samples that were positive for all three immunodiagnostic kits (data not shown). If these subjects together with those with positive stools (n = 54) were considered as being schistosomiasis cases, then percent immunodiagnostic positives ranged 85-97%. Of stool negative subjects, 180 (57%) had a history of contact with *S. japonicum*-infested water and 43% without such history (Table 2). Among the former group, DDIA and ELISA positives were nearly equal (~40%) and slightly higher than that of IHA (31%), and among the latter group, ELISA positives were higher (10%) than those of DDIA and IHA (4% each assay type).

From assays of 418 serum samples collected from healthy subjects who resided in non-*S. japonicum* endemic regions and had never traveled in schistosomiasis-endemic regions,

Table 1

Detection of *Schistosoma japonicum* human infection using dipstick dye immunoassay (DDIA), enzyme linked immunosorbent assay (ELISA), indirect hemagglutination assay (IHA), and miracidium hatching test (MHT) among residents (n = 404), 15-70 years of age, randomly sampled in a village located in a schistosomiasis-endemic region near Poyang Lake, Jiujiang, Jiangxi province (March - June, 2011)

MHT	DDIA		ELISA		IHA	
	Number of positives	Number of negatives	Number of positives	Number of negatives	Number of positives	Number of negatives
Number of positives	51	3	47	7	41	13
Number of negatives	113	237	122	228	95	255

Table 2

History of residents (n = 404), 15-70 years of age, randomly sampled in a village located in a schistosomiasis-endemic region near Poyang Lake, Jiujiang, Jiangxi province (March - June, 2011) and *Schistosoma japonicum* infection using dipstick dye immunoassay (DDIA), enzyme linked immunosorbent assay (ELISA) and indirect hemagglutination assay (IHA)

Subject	Number (n)	DDIA		ELISA		IHA	
		Number of positives	Positive rate (%)	Number of positives	Positive rate (%)	Number of positives	Positive rate (%)
Schistosomiasis case (stool positive as well as (DDIA + ELISA + IHA) positive but stool negative)	88	85	97	81	92	75	85
Stool negative with a history of contact with *Schistosoma japonicum*-infested water	180	74	41	75	42	55	31
Stool negative with no history of contact with *S. japonicum*-infested water	136	5	4	13	10	6	4

diagnostic specificity was 98.1, 97.4 and 98.8% for DDIA, ELISA and IHA, respectively.

DISCUSSION

Although parasitological tools are the currently gold standards for diagnosis of schistosomiasis japonica, these techniques suffer from a problem of missing diagnosis (Zhou *et al*, 2007), 60.6 and 11.5% for Kato-Katz technique and MHT respectively in endemic regions of PR China where there is <10 % prevalence of human *S. japonicum* infection (Zhu *et al*, 2014). Following implementation of an integrated schistosomiasis control strategy in the country (Wang *et al*, 2017), former endemic regions are currently characterized as having low endemicity (Liu *et al*, 2016; Shi *et al*, 2016; Zhang *et al*, 2016a).

In our study we employed MHT as the gold standard for detection of human *S. japonicum* infection because it has a significantly lower missing diagnosis rate compared to Kato-Katz technique (Zhu *et al*, 2014). To compensate for missing (false negative) MHT results, in our study samples that were stool-negative but positive for all three immunodiagnostic assays (DDIA, ELISA and IHA) are defined as *S. japonicum*-infected cases.

Our study shows DDIA had the highest sensitivity for detecting *S. japonicum* human infection, followed by ELISA then IHA. According to China national criterion, a seropositive individual with a history of contact with *S. japonicum*-infested water is defined a case of schistosomiasis (MOH, 2006). The study site is historically hyper-endemic for *S. japonicum*, where 1-2 rounds of mass drug administration with praziquantel are carried out annually, resulting in a marked decline in prevalence and intensity of *S. japonicum* infection (Yu *et al*, 2013). As a result, we detected low *S. japonicum* infection (13.4 %) using MHT. Therefore, among subjects who were stool-negative but seropositive, some may be infected and some may have been cured of schistosomiasis but still retained circulating serum anti-schistosome antibodies (Zhu *et al*, 2008). In a large-scale screening program, seropositives are regarded as schistosomiasis-suspected cases, and whether praziquantel is to be administered depends on clinical signs, symptoms and duration since the last treatment.

The advantages of DDIA over ELIZA and IHA are that it does not require any specialized instrument, such as microplate reader or microscope for determination of results, which can be observed by eye within 10 minutes (Zhu *et al*, 2002), while ELISA requires at least 4 hours and IHA at least 30 min to obtain a readout (Zhou *et al*, 2007). In addition, DDIA was reported to be effective for detecting human *S. mansoni* and *S. haematobium* infections (Zhu *et al*,

2006; Hua *et al*, 2013). Currently, China is transferring its successful experience on schistosomiasis control to assist in Africa schistosomiasis elimination program (Xu *et al*, 2016a; Xu *et al*, 2016b). DDIA should provide a new tool for surveillance of African schistosomiasis and help facilitate the progress towards a successful elimination (Savioli *et al*, 2017). However, further studies to evaluate DDIA efficacy in the diagnosis of African schistosomiasis in a large-scale setting are warranted.

In conclusion, the study confirms the dipstick dye immunoassay is a simple, rapid, and highly sensitive and specific immunodiagnostic tool feasible for large-scale screening of human *S. japonicum* infections in endemic regions of China and elsewhere.

ACKNOWLEDGEMENT

The study was supported by the Research and Development Project for Novel Technology and Products in Zhejiang Provincial Health Commission (grant no. 2021PY018).

CONFLICTS OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

REFERENCES

Hua HY, Wang W, Cao GQ, Tang F, Liang YS. Improving the management of imported schistosomiasis haematobia in China: lessons from a case with multiple misdiagnoses. *Parasit Vectors* 2013; 6: 260

Liu Y, Zhou YB, Li RZ, *et al*. Epidemiological features and effectiveness of schistosomiasis control programme in mountainous and hilly region of the People's Republic of China. *Adv Parasitol* 2016; 92: 73-95.

Lv C, Deng W, Wang L, Qin Z, Zhou X, Xu J. Molecular techniques as alternatives of diagnostic tools in China as schistosomiasis moving towards elimination. *Pathogens* 2022; 11: 287.

Ministry of Health (MOH). Diagnostic criteria for schistosomiasis. Beijing: People's Medical Publishing House Co Ltd; 2006. p 1-10. [in Chinese]

Savioli L, Albonico M, Colley DG, *et al*. Building a global schistosomiasis alliance: an opportunity to join forces to fight inequality and rural poverty. *Infect Dis Poverty* 2017; 6: 65.

Shi L, Li W, Wu F, Zhang JF, Yang K, Zhou XN. Epidemiological features and control progress of schistosomiasis in waterway-network region in the People's Republic of China. *Adv Parasitol* 2016; 92: 97-116.

Tseng KH, Liang S, Ibaraki M, Lee H, Shum CK. Study of the variation of schistosomiasis risk in Lake Poyang in the People's Republic of China using multiple space-borne sensors for the monitoring and modelling. *Geospat Health* 2014; 8: 353-64.

Wang X, Wang W, Wang P. Long-term effectiveness of the integrated schistosomiasis control strategy with emphasis on infectious source control in China: a 10-year evaluation from 2005 to 2014. *Parasitol Res* 2017; 116: 521-8.

Xu J, Bergquist R, Qian YJ, *et al*. China-Africa and China-Asia collaboration on schistosomiasis control: A SWOT analysis. *Adv Parasitol* 2016a; 92: 435-66.

Xu J, Feng T, Lin DD, *et al*. Performance of a dipstick dye immunoassay for rapid screening of *Schistosoma japonicum* infection in areas of low endemicity. *Parasit Vectors* 2011a; 4: 87.

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

Xu J, Yu Q, Tchuenté LA, *et al*. Enhancing collaboration between China and African countries for schistosomiasis control. *Lancet Infect Dis* 2016b; 16: 376-83.

Yu Q, Zhao GM, Hong XL, Lutz EA, Guo JG. Impact and cost-effectiveness of a comprehensive schistosomiasis japonica control program in the Poyang Lake region of China. *Int J Environ Res Public Health* 2013; 10: 6409-21.

Zhang SQ, Sun CS, Wang M, Lin DD, Zhou XN, Wang TP. Epidemiological features and effectiveness of schistosomiasis control programme in lake and marshland region in the People's Republic of China. *Adv Parasitol* 2016a; 92: 39-71.

Zhang JF, Xu J, Bergquist R, *et al*. Development and application of diagnostics in the national schistosomiasis control programme in the People's Republic of China. *Adv Parasitol* 2016b; 92: 409-34.

Zhou XN, Xu J, Chen HG, *et al*. Tools to support policy decisions related to treatment strategies and surveillance of schistosomiasis japonica towards elimination. *PLoS Negl Trop Dis* 2011a; 5: e1408.

Zhou YB, Yang MX, Wang QZ, *et al*. Field comparison of immunodiagnostic and parasitological techniques for the detection of schistosomiasis japonica in the People's Republic of China. *Am J Trop Med Hyg* 2007; 76: 1138-43.

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

Zhu HQ, Xu J, Zhu R, *et al*. Comparison of the miracidium hatching test and modified Kato-Katz method for detecting *Schistosoma japonicum* in low prevalence areas of China. *Southeast Asian J Trop Med Public Health* 2014; 45: 20-5.

Zhu Y, He W, Liang Y, *et al*. Development of a rapid, simple dipstick dye immunoassay for schistosomiasis diagnosis. *J Immunol Methods* 2002; 266: 1-5.

Zhu YC. Immunodiagnosis and its role in schistosomiasis control in China: a review. *Acta Trop* 2005; 96: 130-6.

Zhu YC, Hassen S, He W, Cao GQ. Preliminary study on detection of schistosomiasis mansoni with dipstick dye immunoassay kit. *Chin J Schisto Control* 2006; 18: 419-21. [in Chinese]

Zhu YC, Wu GL, Guan XH. Immunology of *Schistosoma* Infection. Shanghai: Shanghai Scientific and Technological Literature Press; 2008. p. 101-25. [in Chinese]