ASSESSMENT OF THREE SCHISTOSOMIASIS ENDEMIC AREAS USING KATO-KATZ TECHNIQUE AND ELISA ANTIGEN AND ANTIBODY TESTS

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Abstract. This study aimed to determine the prevalence of schistosomiasis in selected schistosomiasis-endemic provinces. Areas identified as endemic and near elimination level for schistosomiasis were purposively selected. School-based collection of stool and blood samples was conducted and samples were examined using Kato-Katz technique and ELISA Ag and Ab tests, respectively. Results showed zero prevalence in Davao City, 0.5% in Davao del Sur, and 3.6% in Compostela Valley using Kato-Katz technique. Higher prevalences were observed for Davao City, Davao del Sur, and Compostela Valley with 5.0% and 34.4%; 3.0% and 19.2%; and 14.4%, and 56.5% using ELISA Ag and Ab tests, respectively. Results of the study showed that the use of Kato-Katz technique in highly endemic areas is still helpful in diagnosis of infected individuals. In low endemic areas, surveillance of schistosomiasis using ELISA Ab test may provide a better evaluation of the transmission status of the infection at population level necessary in the policy formulation for appropriate surveillance and implementation of control measures. ELISA Ag test, on the other hand, may provide more accurate diagnosis of the infection in low transmission areas necessary in the treatment of the infection that could contribute to the control of transmission of the infection in the community. Further studies are needed to support the use of these diagnostic techniques in a stratification scheme to be utilized by the National Schistosomiasis Control and Elimination Program in light of the other strategies being implemented at the community level.

Keywords: schistosomiasis, *Schistosoma japonicum*, algorithm, antigen test, antibody test, Kato-Katz technique, ELISA, surveillance

INTRODUCTION

Schistosomiasis, caused by *Schistosoma japonicum*, is endemic in 12 out of the 16 administrative regions of the Philippines, covering 28 provinces, 15 cities, and 190 municipalities (DOH, 2009). A systematic review and meta-analysis of disability-related effects of schistosomiasis has indicated that the disability weight of schistosomiasis (2% to 15%) is greater than the previous estimate of 0.5% (King *et al*, 2005). Schistosomiasis can bring about morbidities that include anemia,

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growth stunting, undernutrition, predisposition to and exacerbation of co-infections, cognitive underdevelopment, decreased work capacity, and chronic pain (King and Dangerfield-Cha, 2008). The Department of Health (DOH) currently undertakes surveillance in active and passive case finding in endemic areas through parasitological examination using the Kato-Katz technique. Kato-Katz technique is the gold standard for the detection of schistosome infections in humans and currently used as the diagnostic method for disease surveillance. It has a sensitivity that ranges from 51.1% to 84.1% when compared with six repeated examinations (Lin *et al*, 2008a), but likely misses light intensity infections in areas with low transmission (Doenhoff *et al*, 2004, Bergquist *et al*, 2009, Stothard *et al*, 2009; Rollinson *et al*, 2013).

In the Philippines, areas with prevalence greater than or equal to 1% are classified as schistosomiasis endemic areas, while areas with prevalence less than 1% are classified as areas at near elimination level (DOH, 2009). In areas where elimination of schistosomiasis is the target, case detection through Kato-Katz technique may pose a major challenge because it may lack the necessary sensitivity to accurately determine the prevalence of schistosomiasis (Utzinger *et al*, 2005).

This study aimed to assess selected schistosomiasis-endemic provinces in collaboration with the Disease Prevention and Control Bureau (DPCB)-Infectious Disease Office. Specifically, this study aimed to determine the status of surveillance and control of the disease in the human population in three areas identified by the Schistosomiasis Control and Elimination Program (SCEP); and to determine the prevalence of schistosomiasis in the three areas; and to determine applicable algorithms for surveillance, mass diagnosis, and treatment in low transmission areas.

MATERIALS AND METHODS

Study site and population

Three areas were purposively selected, two areas at near elimination level for schistosomiasis, namely Davao City and Davao del Sur, and one endemic area, Compostela Valley. Two districts or municipalities were selected from each province in coordination with the concerned local government units (LGU) and regional office of DOH. The districts and municipalities included were: Calinan District and Tugbok District for Davao City, Digos and Hagonoy for Davao del Sur, and Nabunturan and Maragusan for Compostela Valley.

Study participants included public elementary school children aged 9-to-15 years old from two selected barangay schools from each selected municipality. Study participants were residents of the selected schistosomiasis endemic municipalities and have not received any treatment with praziquantel within the last six months of the study period in March and June 2015.

Parasitological examination

School-based collection of stool and blood samples was conducted in coordination with the Department of Education (DepEd). Stool cups with appropriate collection instructions were distributed to the students by the trained school nurses and/or teachers. Stool samples collected were brought to the field laboratory by trained DepEd nurses/barangay health workers for immediate processing and analysis using the Kato-Katz technique. All Kato-Katz slides were properly labeled with assigned codes. Two aliquots from each sample were prepared and initially examined by trained field microscopists

from DOH and LGU under the supervision of the research team. The slides were properly stored for quality control purposes. Schistosomiasis and other intestinal parasites found during examination were recorded in the Results Form provided to each microscopist.

Serological examination

Trained field staff from the research team and DOH performed blood extraction through venipuncture. Approximately 4 ml of blood was collected from each participant for both ELISA Ab and Ag detection tests. Blood samples were placed in red top tube labeled with their corresponding assigned codes. The blood samples were stored in a cold chain for transport. These samples were processed and analyzed by trained personnel in the University of the Philippines Manila-National Institutes of Health (UPM-NIH) Central laboratory.

An ELISA test was performed using kits (Inno Vision International, Hong Kong). *Schistosoma japonicum* IgG ELISA kit and *Schistosoma japonicum* soluble egg antigen (SEA) ELISA kit were used to detect antibody and antigen, respectively. The assays were carried out following the manufacturer's protocol. Absorbance was measured using iMark™ Microplate Reader (Biorad, Hercules, CA) at 450 nm following the recommended absorbance cut-off values. Results were recorded as either positive or negative for each assay.

Quality control

The accuracy and reliability of parasitological assessment was ensured through quality control measures. This involved proper collection of specimens, use of freshly prepared reagents, appropriate laboratory techniques, meticulous examination of processed specimens, and accurate reporting of findings. Ten percent of all Kato-Katz slides were re-examined by a reference microscopist without the knowledge of initial results in order to ensure accuracy of microscopic readings (Montresor *et al*, 1998). Positive and negative control sera included in the ELISA kits were used for quality control for ELISA Ab test and ELISA Ag test.

Data processing and analysis

Results were recorded on pre-tested forms and encoded using Epi Info version 3.5.4. Double encoding was done to ensure accuracy of data. Data collected were used to derive the following parameters: 1) prevalence and intensity of schistosomiasis in the areas at near elimination level and the endemic area using Kato-Katz technique, ELISA Ab, and ELISA Ag tests; and 2) prevalence of moderate to heavy intensity infections using Kato-Katz technique.

Ethical considerations

The study protocol, informed consent and assent forms, as well as patient information sheet were reviewed and approved by the University of the Philippines Manila-Research Ethics Board (UPMREB-2013-NIH-P2-047). Confidentiality was maintained through replacement of participant identifiers with codes. Only authorized personnel from the research team were allowed access to the results to guarantee the anonymity of study participants and the confidentiality of information gathered. Accomplished informed consent and assent forms were collected. All study participants found positive for schistosomiasis and other parasitic infections were referred to the local health units for appropriate management following DOH guidelines. Feedback of the results of the study and recommendations were provided to the concerned authorities at the end of the study.

Table 1
Prevalence of schistosomiasis among school-age children in selected study sites using Kato-Katz technique (March 2015, June 2015).

Study sites	Stools examined (*n*)	Positive for *S. japonicum*	
		Kato-Katz technique *n* (%)	Moderate to heavy intensity infection by Kato-Katz technique *n* (%)
Areas at near elimination level			
Davao City			
Calinan District	172	0 (0.0)	0 (0.0)
Riverside	80	0 (0.0)	0 (0.0)
Dacudao	92	0 (0.0)	0 (0.0)
Tugbok District	191	0 (0.0)	0 (0.0)
Los Amigos	90	0 (0.0)	0 (0.0)
Tugobok Proper	101	0 (0.0)	0 (0.0)
Subtotal	363	0 (0.0)	0 (0.0)
Davao del Sur			
Digos	207	0 (0.0)	0 (0.0)
Colorado	102	0 (0.0)	0 (0.0)
Igpit	105	0 (0.0)	0 (0.0)
Hagonoy	226	0 (0.8)	0 (0.8)
Kibuaya	117	2 (1.7)	2 (1.7)
Sinayawan	109	0 (0.0)	0 (0.0)
Subtotal	433	2 (0.5)	2 (0.5)
Endemic area			
Compostela Valley			
Nabunturan	260	8 (3.1)	2 (0.8)
Magsaysay	129	2 (1.6)	1 (0.8)
Basak	131	6 (4.6)	1 (0.8)
Maragusan	241	10 (4.2)	2 (0.8)
Tigbao	125	6 (4.8)	0 (0.0)
Mapawa	116	4 (3.5)	1 (0.9)
Subtotal	501	18 (3.6)	3 (0.6)
Total	1,297	20 (1.4)	5 (0.3)

RESULTS

A total of 1,297 study participants were included in the study, from whom stool samples and blood samples were collected and examined. A total of 796 participants (61.4%) were from the areas at near elimination level. Of this total, 363 (28.0%) were from Davao City and 433 (33.4%) were from Davao del Sur. A total of 501 (38.6%) study participants were from the endemic area, Compostela Valley.

Table 1 shows the prevalence of schistosomiasis using Kato-Katz technique. Compostela Valley had the highest preva-

Table 2
Prevalence of schistosomiasis among school-age children in selected study sites using ELISA Ag and ELISA Ab (March 2015, June 2015).

Study sites	Positive for *S. japonicum*	
	ELISA Ag *n* (%)	ELISA Ab *n* (%)
Areas at near elimination level		
Davao City		
Calinan District	4 (2.3)	42 (24.4)
Riverside	0 (0.0)	0 (0.0)
Dacudao	4 (4.4)	42 (42.3)
Tugbok District	14 (7.3)	83 (43.5)
Los Amigos	3 (3.3)	73 (81.1)
Tugobok Proper	11 (10.9)	10 (9.9)
Subtotal	18 (5.0)	125 (34.4)
Davao del Sur		
Digos	6 (2.9)	50 (24.1)
Colorado	2 (2.0)	20 (19.6)
Igpit	4 (3.8)	30 (28.6)
Hagonoy	7 (3.0)	33 (14.6)
Kibuaya	5 (4.2)	30 (25.6)
Sinayawan	2 (1.7)	3 (2.8)
Subtotal	13 (3.0)	83 (19.2)
Endemic area		
Compostela Valley		
Nabunturan	50 (19.2)	142 (54.6)
Basak	11 (8.5)	69 (53.5)
Magsaysay	39 (29.8)	73 (55.7)
Maragusan	22 (9.1)	141 (58.5)
Tigbao	8 (6.4)	89 (71.2)
Mapawa	14 (12.1)	52 (44.8)
Subtotal	72 (14.4)	283 (56.5)
Total	103 (7.9)	491 (37.9)

lence of schistosomiasis among the study sites at 3.6% and a prevalence of moderate to heavy intensity infection of 0.6%. The highest prevalence of schistosomiasis was found in the Municipality of Maragusan at 4.2%. A prevalence of 0.5% was found in Davao del Sur. No case of schistosomiasis was seen in Davao City using Kato-Katz technique.

Table 2 summarizes the results of ELISA Ag and Ab tests. Compostela Valley had the highest seroprevalence for *S. japonicum* using ELISA Ab test at 56.5%. In the ELISA Ab test, the seroprevalence of Davao City and Davao del Sur were 34.4% and 19.2%, respectively. In the ELISA Ag test, the highest seroprevalence of 14.4% was observed in Compostela Valley followed by Davao City at 5.0% and Davao del Sur at 3.0%.

DISCUSSION

Kato-Katz technique is the most commonly used method for diagnosing *S. japonicum* in field surveys because it is relatively simple to perform, quantitative, and relatively inexpensive. However, its sensitivity may be decreased following widespread chemotherapy, which results in generally low worm burden (Doenhoff *et al*, 2004). Lin *et al* (2008a) showed that the sensitivity of this method varies from 40% to 100%, while a recent study conducted by Belizario *et al* (2013) showed a sensitivity of 16.0% and a specificity of 96.2%. Various studies have shown that increased number of smears per sample increases the sensitivity of the test (Yu *et al*, 1998; da Frota *et al*, 2011; Carneiro *et al*, 2012; Endris *et al*, 2013); however, this would be a limiting procedure in the conduct of fieldwork due to increased requirements in terms of human resources, time, and cost.

ELISA has been regarded as the method being most likely to meet stringent requirements for field use in terms of reliability and sensitivity (Zhou *et al*, 2011). According to the study of Zhou *et al* (2011) the sensitivity of this method varies from 65.5% to 100%, while most reported specificities of this method are less than 60%. ELISA test can be done through antigen detection or antibody detection. ELISA Ag detection indicates existence of active infection. Its ability to detect active infection reduces the rate of false positive results compared to antibody detection test. Results from various studies suggest that egg antigen detection provide greater diagnostic sensitivity and specificity than worm antigen detection (McLaren *et al*, 1978; Mott and Dixon, 1982; Grenfell *et al*, 2012). In a study conducted by Belizario *et al* (2013), ELISA Ag was shown to have a sensitivity of 43.8% and a specificity of 86.0%. On the other hand, ELISA Ab was shown to have a sensitivity of 81.3% and a specificity of 71.9%. ELISA Ab detection is unable to differentiate between active and past infections (Lin *et al*, 2008b), but was shown to have a significantly higher sensitivity compared with COPT, which is currently the only serological test being used in the Philippines (Belizario *et al*, 2013).

In this study, there were cases wherein individuals tested positive for ELISA Ag but negative for ELISA Ab. This could be a result of the early stages of infection being poorly immunogenic (Doenhoff *et al*, 2004) or a result of late seroconversion which causes delay in the development of detectable antibodies in the blood (Depietropaolo *et al*, 2005). IgM production usually occurs at the fourth week of the infection, while IgG production usually occurs at the fifth week of the infection (Boxall *et al*, 2006; Lorenzo *et al*, 2015). It has been shown in another SEA – ELISA test that serum IgG levels are significantly higher in chronic than acute *S. japonicum* infection, while IgM levels are higher in the acute infection (Hu *et al*, 1999; Weerakoon *et al*, 2015). The kits used in this study are specific for IgG antibodies, hence the affected individual may still be at the acute phase of infection where the antibody produced is still the IgM with no detectable increase in the IgG antibody levels (Rosenstock *et al*, 2000).

Results of this study showed that the areas declared at near elimination level, Davao City and Davao del Sur, had a prevalence of less than 1% using Kato-Katz technique. Compostela Valley, which was classified as an endemic area, had prevalence greater than 1% using Kato-Katz technique. These results are in line with the DOH classification standard

wherein areas with prevalence less than 1% are classified at near elimination level, and areas with prevalence greater than or equal to 1% are classified as schistosomiasis endemic area (DOH, 2009).

Prevalence levels using ELISA Ag and Ab tests were higher in all three areas compared with the prevalence obtained using Kato-Katz technique. Several studies corroborate with our findings where higher seroprevalence levels were observed compared with the prevalence levels obtained by parasitological examination (Dias *et al*, 1989; Carneiro *et al*, 2012; Cai *et al*, 2014). It was shown in a study by Belizario *et al* (2013) that seroprevalence levels of schistosomiasis using ELISA Ag and Ab tests are approximately three to six times higher than the prevalence levels obtained using Kato-Katz technique.

Mass treatment using praziquantel was conducted in the municipality of Nabunturan three days prior to the conduct of the fieldwork in Compostela Valley. The drug acts within an hour of ingestion by paralyzing the worms (Gryseels *et al*, 2006). Mass treatment in Nabunturan could have affected the prevalence reported in this study using Kato-Katz technique given that the drug primarily controls schistosome egg production by destroying the adult worms (Cioli *et al* 2000; Gryseels *et al*, 2006; Weerakoon *et al*, 2015). However, this does not affect the results of serological tests. Antibodies for Schistosoma remain in the individual for more than one year and up to two years after treatment with praziquantel (Zhou *et al*, 2007; Lin *et al*, 2008b). Prevalence levels of schistosomiasis in Davao City and Davao del Sur found in this study using ELISA Ab test do not reflect the supposed prevalence levels of areas classified as near elimination. Prevalence of less than 1% for five consecutive years is required to be classified at near elimination level (DOH, 2009). Relatively high prevalence by ELISA Ab test suggests possible continuing transmission or persistence of infection in these areas at least in the last two years.

In the early stages of a control program where morbidity is still high, the use of Kato-Katz technique proves to be useful. In areas where there is low level of endemicity, the use of Kato-Katz technique alone might not be enough due to its low sensitivity in diagnosing infections especially in individuals with low egg burden. In the case of Compostela Valley, which is classified as endemic area for schistosomiasis, the use of Kato-Katz technique remains indispensible in the surveillance of the infection and formulation of appropriate control measures such as mass treatment. However, in Davao City and Davao del Sur, which are classified as areas at near elimination level, the use of Kato-Katz technique alone for surveillance may lead to underreporting of cases.

Underreporting of cases may result to underestimation of the burden of infection and may not likely reflect the actual status of infection in the areas, resulting to inappropriate control measures. Combination of diagnostic technique with poor sensitivity and areas with low infection levels may lead to inaccurate picture of disease burden which could be a threat to the success of a control and/or elimination program (Utzinger *et al*, 2005; Bergquist *et al*, 2009; Rollinson *et al*, 2013). It is imperative to know the true infection status of a community so that appropriate prevention and control strategies can be implemented (Rollinson *et al*, 2013).

The low prevalence of infection using Kato-Katz technique in the areas declared at near elimination level shows

a significant progress in the control of schistosomiasis. Shift of the program goal from control to elimination would mean a need to improve diagnosis of the infection using more sensitive tests and to develop algorithms for monitoring and surveillance of the infection. Utzinger *et al* (2011) noted that development and implementation of optimal methodologies for diagnosis is crucial in all aspects of schistosomiasis control as programs shift their emphasis from control to elimination of schistosomiasis.

The use of a more sensitive test plays a major role in the elimination of schistosomiasis in the Philippines. Persistent levels of schistosomiasis in low transmission areas should be addressed accordingly as this may cause considerable morbidity and contribute to the continuous transmission of the infection (Zhou, 2014). The high sensitivity of ELISA, as shown in various studies (Zhou *et al*, 2011; Grenfell *et al*, 2012; Belizario *et al*, 2013), and its ability to detect cases in areas where no case is being detected using Kato-Katz technique demonstrate its effectiveness and utility in the diagnosis and surveillance of schistosomiasis in low transmission areas.

The repeated stool collection and examination necessary to increase the sensitivity of Kato-Katz technique, which proves to be more labor intensive and costly (Yu *et al*, 1998; Hubbard *et al*, 2003; Zhu *et al*, 2005; Lin *et al*, 2008a; Xu *et al*, 2011), merits the efficiency of ELISA in low transmission areas as it does not require multiple collection of samples that will eventually increase the cost and manpower to perform the test. The use of ELISA in the surveillance of schistosomiasis would play a major role in the control and elimination of schistosomiasis, particularly in providing a more accurate status of the infection necessary in the formulation of appropriate control and elimination measures.

The use of Kato-Katz technique in the diagnosis of schistosomiasis may result in cases being missed and may lead to individuals left undiagnosed and untreated. These same individuals may remain infected and be responsible for the continued transmission of the infection. Continued transmission and persistence of the infection may affect the quality of life of these individuals and cause significant health burden. Similarly, the use of Kato-Katz technique in the surveillance of the infection may cause misclassification of low intensity infection areas (de Vlas and Gryseels, 1992; Yu *et al*, 2007; Lin *et al*, 2008a; Utzinger *et al*, 2011) and may lead to implementation of inappropriate control measures. Results of the study point to the need to use more sensitive tests in the surveillance of schistosomiasis in areas at near elimination level.

In areas where infection prevalence and intensity remain high, direct methods such as Kato-Katz technique show reasonable accuracy (Utzinger *et al*, 2011). Surveillance of the infection in highly endemic areas using Kato-Katz technique is still recommended. However, in low endemic areas, ELISA Ab test may have greater utility in epidemiologic surveys (Lin *et al*, 2008b). Fig 1 shows the proposed algorithm for the surveillance of schistosomiasis in endemic areas. The use of ELISA Ab test in low endemic areas in the surveillance of schistosomiasis may provide a better evaluation of the transmission status of the infection at population level (Lin *et al*, 2008b).

In the clinical setting, Kato-Katz technique remains to be the recommended diagnostic tool in areas with high endemicity while ELISA Ag test is the recommended diagnostic tool in areas with low

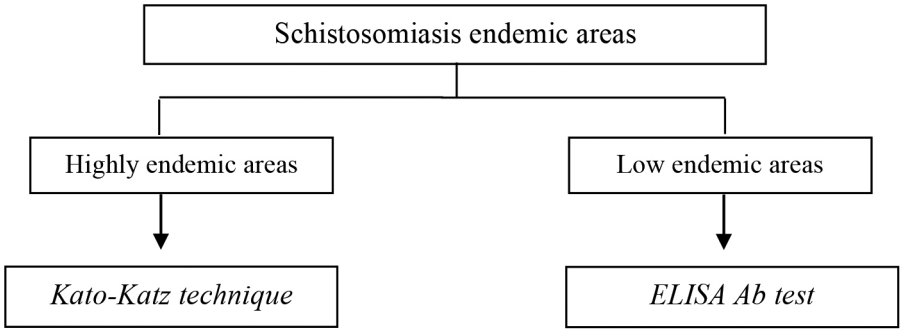

Fig 1–Proposed algorithm for the surveillance of schistosomiasis in endemic areas.

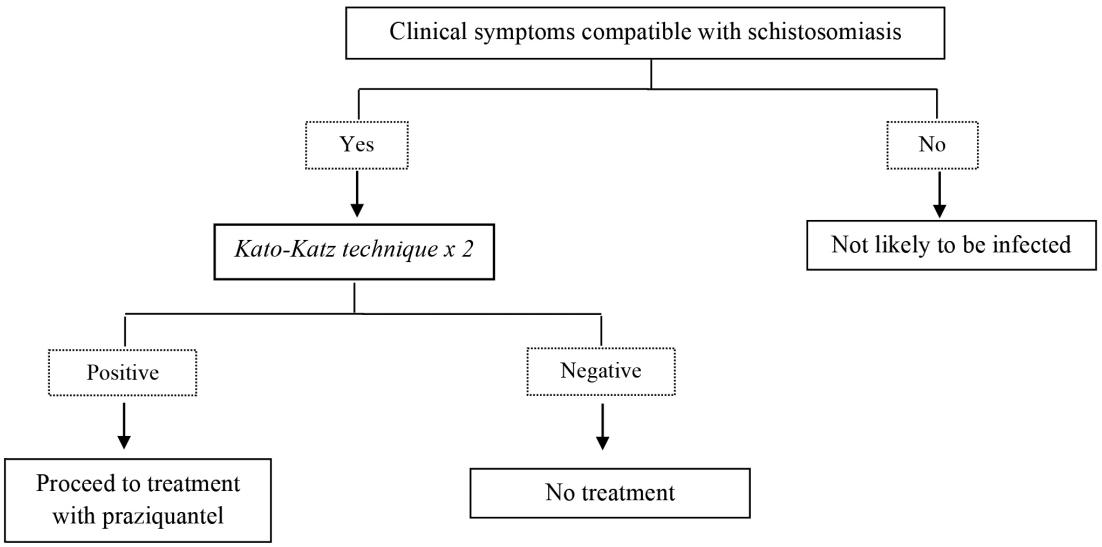

Fig 2–Proposed algorithm for the clinical diagnosis of schistosomiasis in highly endemic areas.

endemicity. Fig 2 shows the proposed algorithm for the clinical diagnosis of schistosomiasis in highly endemic areas. Alejandria *et al* (2010) recommends examination of two triplet Kato-Katz smears from two stool specimens prepared from the outer layer of the first stool defecated. The same study mentioned that more than two stool specimens may increase sensitivity, but can be time-consuming and may pose follow-up and compliance difficulties. Fig 3 shows the proposed algorithm for the clinical diagnosis of schistosomiasis in low endemic areas. Given the high sensitivity of ELISA Ag test in the detection of active infections relative to Kato-Katz technique, its use in low endemic areas may provide more accurate diagnosis of the infection necessary in the treatment of the infection that could contribute to the control of transmission of the infection in the community.

Results of this study indicates that the use of Kato-Katz technique in highly

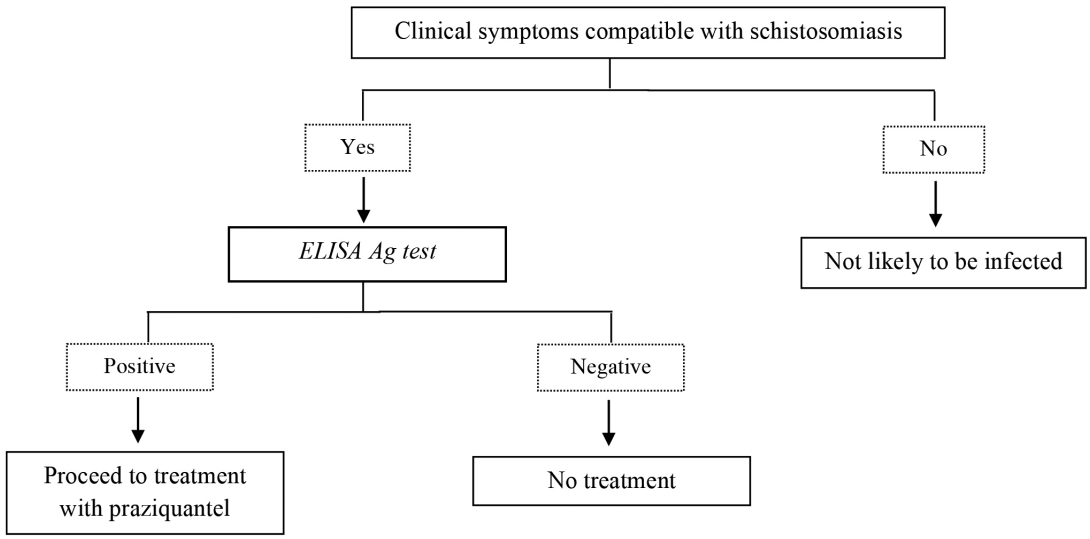

Fig 3–Proposed algorithm for the clinical diagnosis of schistosomiasis in low endemic areas.

endemic areas is still helpful in diagnosis of infected individuals, but its use in areas classified as near elimination level may lead to underestimation of the actual prevalence of the infection. The use of more sensitive diagnostic tests is warranted in the surveillance of schistosomiasis in low endemic areas in order to get a better estimate of the prevalence and status of transmission of the infection necessary in policy formulation for appropriate surveillance and implementation of control measures. ELISA Ab test show great utility in the surveillance of schistosomiasis in low endemic areas and may provide better evaluation of the transmission status at population level. ELISA Ag test, on the other hand, may provide more accurate diagnosis of the infection in areas with low endemicity. Stratification of areas using more sensitive tools as well as implementation of appropriate control measures according to level of endemicity are in order. Further studies, however, are needed to support the use of these diagnostic techniques in a stratification scheme to be utilized by the National Schistosomiasis Control and Elimination Program in light of other strategies being implemented at the community level.

The persistence of *Schistosoma* infection could be due to the continued existence of the parasite's life cycle in the community. Breaking this cycle is critical in eliminating *Schistosoma* infection in highly endemic and near elimination areas. Targeting the human aspect of the parasite's life cycle is necessary together with providing preventive chemotherapy through mass drug administration of praziquantel to ensure morbidity control of *Schistosoma* infection. However, this focus does not address other factors that contribute to the continuing cycle in the transmission of infection in these areas. Reduction of open defecation through health promotion and health education, improvement of sanitation, snail control, and monitoring of animal reservoir hosts through parasitic surveillance should likewise be addressed.

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REFERENCES

Alejandria M, Salvana EM, Belizario VY, *et al*. Clinical practice guidelines for the diagnosis, treatment, and prevention of *Schistosoma japonicum* infections in the Philippines: 2010 Update. Manila: Department of Health, 2010.

Belizario V, Destura R, Gabunada RR, Petronio-Santos JA, Amarillo ML, de Veyra C. Assessment of schistosomiasis endemic areas through parasitological and serological techniques. Final report submitted to Department of Health Philppines; 2013.

Belizario V, Destura R, Gabunada RR, Petronio-Santos JA, Amarillo ML, de Veyra C. Assessment of schistosomiasis endemic areas through parasitological and serological techniques. Final report submitted to Department of Health Philippines, 2013.

Bergquist R, Johansen MV, Utzinger J. Diagnostic dilemmas in helminthology: what tools to use and when? *Trends Parasitol* 2009; 25: 151-6.

Boxall E, Herborn A, Kochethu G, *et al*. Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. *Transfus Med* 2006; 16: 79-83.

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 Bras Med Trop* 2012; 45: 510-3.

Cioli D. Praziquantel: is there real resistance and are there alternatives? *Curr Opin Infect Dis* 2000; 13: 659-63.

da Frota SM, Carneiro TR, Queiroz JA, Alencar LM, Heukelbach J. Combination of Kato-Katz faecal examinations and ELISA to improve accuracy of diagnosis of intestinal schistosomiasis in a low-endemic setting in Brazil. *Acta Trop* 2011; 120: 138-41.

de Vlas SJ, Gryseels B. Underestimation of *Schistosoma mansoni* prevalences. *Parasitol Today* 1992; 8: 274-7.

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.

Depietropaolo D, Powers J, Gill J, Foy A. Diagnosis of Lyme disease. *Am Fam Physician* 2005; 72: 297-304.

Dias LCS, Kawazoe U, Glasser CM, *et al*. Schistosomiasis mansoni in the municipality of Pedro de Toledo (São Paulo, Brazil) where the *Biomphalaria tenagophila* is the snail host: I. Prevalence in human population. *Rev Inst Med Trop Sao Paulo* 1989; 31: 110-8.

Doenhoff M, Chiodini P, Hamilton J. Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies. *Trends Parasitol* 2004; 20: 35-9.

Endris M, Tekeste Z, Lemma W, Kassu A. Comparison of the Kato-Katz, Wet Mount, and Formol-Ether concentration diagnostic techniques for intestinal helminth infections in Ethiopia. *ISRN Parasitol* 2013;

ID 180439.

Grenfell RFQ, Silva-Moraes V, Taboada D, de Mattos ACA, de Castro AKS, Coelho PMZ. Immunodiagnostic methods: what is their role in areas of low endemicity? *Sci World J* 2012; ID 593947.

Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. *Lancet* 2006; 368(9541): 1106-18.

Hu M, Kirinoki M, Yokoi H, Kawai S, Chigusa Y, Matsuda H. Human antibody isotype responses to *Schistosoma japonicum* egg antigen. *Southeast Asian J Trop Med Public Health* 1999; 30: 24-8.

Hubbard A, Liang S, Maszle D, Qiu D, Gu X, Spear RC. Estimating the distribution of worm burden and egg excretion of *Schistosoma japonicum* by risk group in Sichuan province, China. *Parasitology* 2003; 125: 221-31.

King CH, Dangerfield-Cha M. The unacknowledged impact of chronic schistosomiasis. *Chron Illn* 2008; 4: 65-79.

King CH, Dickman K, Tisch DJ. Reassessment of the cost of chronic helminthic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* 2005; 365: 1561-9.

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* 2008a; 57: 281-6.

Lin DD, Xu JM, Zhang YY, *et al*. Evaluation of IgG ELISA for the diagnosis of *Schistosoma japonicum* in a high prevalence, low intensity endemic area of China. *Acta Trop* 2008b; 107: 128-33.

Lorenzo AA, de Guzman TS, Sia Su GL. Detection of IgM and IgG antibodies against hepatitis E virus in donated blood bags from a national voluntary blood bank in Metro Manila, Philippines. *Asia Pacific J Trop Dis* 2015; 5: 604-5.

McLaren M, Draper CC, Roberts JM. Studies on the enzyme linked immunosorbent assay (ELISA) test for *Schistosoma mansoni* infections. *Ann Trop Med Parasitol* 1978; 72: 243-53.

Mott KE, Dixon H. Collaborative study on antigens for immunodiagnosis of schistosomiasis. *Bull World Health Organ* 1982; 60; 729-53.

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.

Rollinson D, Knopp S, Levitz S, *et al*. Time to set the agenda for schistosomiasis elimination. *Acta Trop* 2013; 128:423-40.

Rosenstock S, Jorgensen T, Andersen L, Bonnevie O. Seroconversion and seroreversion in IgG antibodies to *Helicobacter pylori*: a serology based prospective cohort study. *J Epidemiol Community Health* 2000; 54: 444-50.

Stothard JR, Sousa-Figueiredo JC, Standley C, *et al*. An evaluation of urine-CCA strip test and finger prick blood SEA-ELISA for detection of urinary schistosomiasis in schoolchildren in Zanzibar. *Acta Trop* 2009; 111: 64-70.

Utzinger J, N'Goran E, Caffrey C, Keiser J. From innovation to application: social-ecological context, diagnostics, drugs, and integrated control of schistosomiasis. *Acta Trop* 2011; 120: 121-37.

Utzinger J, Zhou XN, Chen MG, Bergquist R. Conquering schistosomiasis in China: the long march. *Acta Trop* 2005; 96: 69-96.

Weerakoon KGAD, Gobert GN, Cai P, McManus DP. Advances in the diagnosis of human schistosomiasis. *Clin Microbiol Rev* 2015; 28: 939-67.

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* 2011; 5: e949.

Yu JM, de Vlas SJ, Yuan HC, Gryseels B. Variations in fecal *Schistosoma japonicum* egg counts. *Am J Trop Med Hyg* 1998; 59: 370-5.

Yu JM, de Vlas SJ, Jiang QW, Gryseels B. Comparison of the Kato-Katz technique, hatching test and indirect hemagglutination assay (IHA) for the diagnosis of *Schistosoma japonicum* infection in China. *Parasitol Int* 2007; 56: 45-9.

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: consquences on praziquantel-based morbidity control. *Parasit Vectors* 2011; 4: 194.

Zhou XN. Value of a novel diagnostics of rSP13-ELISA toward schistosomiasis elimination in China. *Sci China Life Sci* 2014; 57: 647-8.

Zhu HQ, Cao CL, Gao FH, Guo JG, Bao ZP. Evaluation of effectiveness of modified Kato-Katz technique for diagnosis of schistosomiasis japonica. *Chin J Schistosom Control* 2005; 17: 273-7.