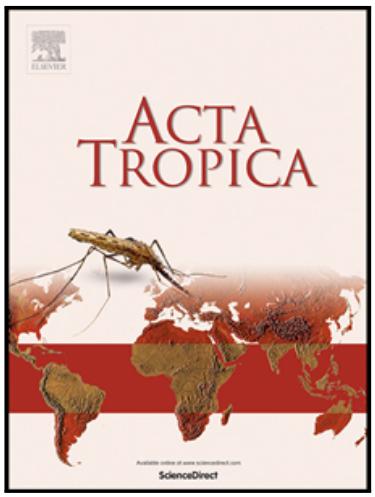

Diagnostic performance of two specific Schistosoma japonicum immunological tests for screening Schistosoma haematobium in school children in Zambia

Li-Juan Zhang , Victor Mwanakasale , Jing Xu , Le-Ping Sun , Xiao-Mei Yin , Jian-Feng Zhang , Ming-Chuang Hu , Wu-Min Si , Xiao-Nong Zhou

PII: S0001-706X(18)31658-9
DOI: https://doi.org/10.1016/j.actatropica.2019.105285
Reference: ACTROP 105285

To appear in: *Acta Tropica*

Received date: 26 December 2018
Revised date: 22 November 2019
Accepted date: 27 November 2019

Please cite this article as: Li-Juan Zhang , Victor Mwanakasale , Jing Xu , Le-Ping Sun , Xiao-Mei Yin , Jian-Feng Zhang , Ming-Chuang Hu , Wu-Min Si , Xiao-Nong Zhou , Diagnostic performance of two specific Schistosoma japonicum immunological tests for screening Schistosoma haematobium in school children in Zambia, *Acta Tropica* (2019), doi: https://doi.org/10.1016/j.actatropica.2019.105285

Diagnostic performance of two specific *Schistosoma japonicum* immunological tests for screening *Schistosoma haematobium* in school children in Zambia

Li-Juan Zhang[1],Victor Mwanakasale[2], Jing Xu[1*], Le-Ping Sun[3], Xiao-Mei Yin[4], Jian-Feng Zhang[3], Ming-Chuang Hu[4], Wu-Min Si[4], Xiao-Nong Zhou[1*]

1 National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, People's Republic of China, WHO Collaborating Center for Tropical Diseases, Shanghai, People's Republic of China, National Center for International Research on Tropical Diseases, Shanghai, People's Republic of China, Key Laboratory of Parasite and Vector Biology, Ministry of Health, Shanghai, People's Republic of China.

2 School of Medicine, Copperbelt University, KITWE, ZAMBIA.

3 Institute for schistosomiasis control, Wuxi, Jiangsu, P. R. China.

4 Institute for schistosomiasis control, Hefei, Anhui, P. R. China.

*Corresponding author: xujing@nipd.chinacdc.cn & zhouxn1@chinacdc.cn

Abstract

Dipstick Dye Immunoassay (DDIA) and Indirect Haemagglutination Assay(IHA), are two commercially available kits which have been widely used for screening *Schistosoma japonicum* in P.R. China. Whether they can be used for screening of *Schistosoma haematobium* are not clear. In order to evaluate the diagnostic efficiency of DDIA and IHA for screening *Schistosoma haematobium*, serum samples were collected from pupils in endemic areas in Zambia, Southern Africa, and tested by DDIA and IHA by single-blind manner. Meanwhile, the pupils were microscopically examined by infection with *Schistosoma* and soil-transmitted helminths, visually observed for parasite eggs. Of the enrolled 148 pupils, 61% tested positive for *S. haematobium* infection, while 31% and 36% of pupils were infected with hookworm and *Ascaris* respectively. Regarding the parasitological tests as reference standard, for the diagnosis of *S. haematobium* infection, IHA performed higher sensitivity (74%, 95% CI: 65%-83%) than that of DDIA (60%, 95%CI: 49%-70%). The sensitivities of IHA and DDIA are significant higher in 10-14 years old students than those of 7-9 years old group. The specificity of DDIA and IHA were 61% (95%CI: 49%-74%) and 72% (95%CI: 60%-84%), respectively. The co-infection with STHs decreased the specificity of DDIA but had no impact on that of IHA. Our study indicated that IHA has more potential as an alternative diagnostic tool for identifying schistosomiasis haematobium but need further improvement.

Keywords: Schistosomiasis haematobium; Urine filtration; Diagnosis; Immunological assay

1. Introduction

Schistosomiasis is an infectious disease caused by the parasites of the genus *Schistosoma*. It affects over 200 million people worldwide(WHO, 2017) and account for an estimated 1.9 million disability-adjusted life years(DALYS) annually. *S. haematobium, S. mansoni and S. japonicum* are the major species causing the majority of schistosomiasis in humans(Colley et al., 2014). *S. haematobium* and *S. mansoni* both occur in Africa and the Middle East, whereas only *S mansoni* is present in the Americas. *S japonicum* is localized to Asia, primarily the Philippines and China. Recent reports showed that more than 90% of documented cases of schistosomiasis in non-endemic countries were infected in Africa with history of water exposure (Clerinx and Van Gompel, 2011; Helleberg M, 2009; Salvana and CH, 2008). The main symptoms of *S. haematobium* infection are hematuria and dysuria due to a chronic inflammation of the bladder and urethra. And it can lead to complications such as anemia, chronic cystitis, cancer of the bladder, and genital lesions. In addition, for women *S. haematobium* infection can lead to genital diseases, infertility, abortion, etc(Santos et al., 2014; van der Werf et al., 2003; Zida et al., 2016). Timely diagnosis and treatment can reduce the burden of schistosomiasis haematobium.

The diagnosis and quantification of the *S. haematobium* infection can be determined through microscopic examination for the eggs and egg counting by urine filtration. However, this is often unreliable due to the day-to-day variations in egg excretion, and lack of sensitivity to detect light infections(Gray et al., 2011). Haematuria is one of the most striking indicators of urinary schistosomiasis, but its sensitivity is quite low in populations having lower intensity infection through continued control programs conducted in African countries(King and Bertsch, 2013). In populations with low worm burdens, antibody-based assays such as enzyme-linked immunosorbent assay are proved to be effective tools for screening, but have limited use in the field due to the requirement of extra equipment and no available commercial kits (Olveda et al., 2014).

P.R. China has a long history for discovering and implementing immunoassays against *S. japonicum*. The current well-developed and commercially available immunoassays such as indirect hemagglutination assay (IHA) and dipstick dye immunoassay (DDIA) have been integrated into national control programs and clinical use in hospitals in P.R. China, performing higher sensitivities and specificities(Xu et al., 2005). Whether these commercial kits in China could be used for screening of imported schistosomiasis haematobium in China or in African continent remains uncertain. In this study, we evaluated the efficacy of DDIA and IHA against *S. Japonicum* for the screening of *S. haematobium*, using serum samples collected from school-aged children from endemic areas in Zambia.

2. Materials and Methods

2.1 Ethical statement

The study was approved by the Tropical Diseases Research Centre (TDRC) ethics committee in Ndola, Zambia. Parents/guardians of the children were informed about

the study procedures, the benefits and risks as well as their voluntary participation. Written informed consents were obtained from parents/guardians of the children before being enrolled into the study. Parents/guardians of the study children were at liberty to withdraw them from the study at any time. Confidentiality of details of the study children were observed throughout the study period. Participants who were found to be parasitological positives were given free praziquantel and/or albendazole treatment using the dose pole according to the instruction of WHO(Moser et al., 2017; Palha De Sousa et al., 2014).

2.2 Sample size and study population

Before conducting the field survey, we estimated that DDIA and IHA would perform both sensitivity and specificity of 80% for diagnosis of *S. haematobium* infection(Zhu et al., 2005). Other parameters were set as follows: 5% level of significance ($\alpha = 0.05$), 95% confidence interval and 5% margin of error ($D = 0.05$). Thus at least 62 positive and negative specimens respectively were needed to evaluate the efficacies of the kits. Urine, stool and blood samples were collected from pupils in Kenani primary school, in Nchelenge District, Luapula province of Zambia, and Kawama Primary school in Luanshya District, Copperbelt province of Zambia, which were estimated to have with a high prevalence and a relatively low prevalence of *S.haematobi*um respectively(Kalinda et al., 2018) (Figure 1). Demographic information of each enrolled child was collected including name, gender, place of birth, how long enrolled at the school and history of taking antischistosomal drugs etc.

< Figure 1 near here>

2.3 Urine specimen collection and urine filtration

Two Urine specimens were collected from each child for microscopic examination on two different days in one week. At least 10 ml of urine from each child was collected between 10:00 a.m. and 14:00 p.m. Mixed 10 ml of urine was drawn by a syringe and filtered by a filter holder with a millipore membrane. The filter was then placed onto a microscope glass-slide and examined for *S. haematobium* eggs by microscopy (KE, 1983). The infection intensity was recorded as the number of eggs/10 ml of urine and classified as light infection (less than 50 eggs/10 ml of urine) or heavy infection (more than 50 eggs/10 ml of urine)(WHO, 2013a).

2.4 Stool sample collection and examination

Each pupil was asked to provide two feces on two days in one week. Stool specimen was processed using the Kato-Katz thick smear method with three slides to determine the *S. mansoni* and soil transmitted helminths(STHs) infection. Three slides from a single stool specimen were prepared using a standard template (41.7mg per smear). All slides were read after their initial preparation by two qualified technicians. The number of eggs in each slide were counted and recorded. Infection intensity of patients was expressed as the arithmetic mean of eggs per gram of feces (EPG)(Ross et al., 2007).

2.5 Blood handling and immunological tests

2ml of venous blood were collected from each pupil and then centrifuged to separate sera to clean Eppendorf tubes. Serum specimens were tested by well-trained technicians, without knowing the results of urine filtration, stool examination and another immunological test. DDIA (Lot. 1405231) was supplied by Saide Bio LTD, Jiangsu, P.R. China and the IHA kit (20131216) was provided by Anji Bio LTD, Anhui, P. R. China.

For DDIA, a drop of 50µl blue colloidal dye-labelled soluble egg antigens (SEA) solution from the buffer bottle was added into a polyvinyl chloride (PVC) well and 20 µl of serum specimen was added. The solution in the well was mixed lightly for about one minute. A dipstick was then inserted into the well. The result was read after the solution was absorbed completely. The appearance of two blue bands on the dipstick indicated a positive reaction while the appearance of a single blue band in the control position was regarded as a negative reaction(Zhu et al., 2005).

For IHA, 100µl of buffer was placed into the first well of the transverse line, whereas 25µl was added into wells 2 and 3. 25µl of serum was added to the first well and thoroughly mixed. 25µl of mixture was transferred to the second well and mixed, and then25µl of the mixture in the second well moved into the third well. 75µl and 25µl of mixture in well 1 and 3 were discarded. Thus, the concentrations of serum in the first, second and third wells were 1:5, 1:10 and 1:20, respectively. Positive and negative control sera samples provided by the company were tested simultaneously on each plate. 25µl sensitized red blood cell was placed into each well. Observations were made by the naked eye after 30 min at room temperature. The titer in the test sera was recorded as one dilution before that which yielded a clear, sharp dark spot similar to that in the negative control wells. Titers of 10 indicated a positive result(Fei et al., 2016; Fenwick et al., 2009; Jie et al., 2016).

2.6 Data management and statistical analysis

Data were double entered into Microsoft Excel 2007 and analyzed using SPSS V 20.0. Only data from subjects who gave consent and from whom both urine and blood samples were collected were used for analyses. Using urine filtration and microscopic examination as a gold standard, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for DDIA and IHA were calculated. 95% confidence intervals (CI) were also calculated for all diagnostic scores. The Chi square was used to compare the difference of parameters between strata. We judged a *P* value less than 0.05 to be significant.

3. Results

3.1 Demographic data

A total of 148 school children were enrolled in this field trial and 147 children were examined and analyzed. Among the147 students, 68 were from the Kenani Primary

School in Nchelenge District, while 79 were from the Kawama Primary School in Luanshya District. The studied population was composed of 47% (69/147) girls and 53% (78/147) boys, with the median age of 9 years old (in the range of 7-14 years). Total 46 pupils had been treated with praziquantel one or two years before the study, 39 of them being from the Kenani Primary school.

3.2 Results of parasitological examination

A total of 147 pupils had their urine samples examined for presence of *S. haematobium* ova by microscopy. Of these pupils, 130 provided two urine samples while 17 only provided one urine sample. *S. haematobium* eggs were found in 90 pupils, 66 from Kenani primary school and 24 from Kawama primary school, with a total positive rate of 61% (90/147, 95%CI:53%-69%). Among the 90 infected children, 52 presented with light infection intensity and 38 with heavy infection intensity. A higher frequency of high infection intensity was found in the older children (10-14 years old) group than the younger children (7-9 years old) (χ^2=4.014, P<0.05). No significant difference in infection intensity was found in gender strata and between the two primary schools (Table 1).

< Table 1 near here>

Among 145 pupils received stool examination, 145 pupils had ancylostomiasis and 52 students got infection of *Ascaris*. In addition, eggs of *S. mansoni* were found in feces of two children from Kenani primary school，who were also infected with *S. haematobium* and hookworm. Integrated with the results of urine and stool examinations, 37% (55/147) of the children were infected with at least two species of parasites, while 50% (74/147) of pupils were infected with one helminth.

3.3 Performance of IHA and DDIA

A total of 146 children were included for assessing the performance of IHA and DDIA, who both received immunological examinations and urine filtration (Figure 2). The positive rates determined by DDIA and IHA were 51% (75/146, 95%CI: 43%-59%) and 56% (82/146, 95%CI:48%-64%)..

< Figure 2 near hear>

Compared with the results of urine filtration, DDIA and IHA performed with a sensitivity of 60% (53/89, 95%CI: 49%-70%) and 74% (66/89, 95%CI: 65%-83%) for the whole population, respectively (Table 2). DDIA and IHA all presented higher sensitivity in the older children than younger pupils by Chi-square analysis ($\chi^2_{\text{for DDIA}}$=5.17, *P*=0.02, $\chi^2_{\text{for IHA}}$=16.01, *P*=0.00). The sensitivity of IHA in children with heavy infection intensity was 86% (32/37, 95%CI: 75%-98%), significantly higher than 65% (34/52, 95%CI: 52%-78%) in those with light infection intensity (χ^2=5.02，*p*=0.03)(Figure 3). The sensitivity of DDIA and IHA did not differ in STHs infection strata.

< Table 2 near here>
< Figure 3 near here>

DDIA and IHA showed specificity of 61% (35/57, 95%CI: 49%-74%) and 72%(41/57, 95%CI: 60%-84%) respectively. In stratified analysis, the specificity of DDIA and IHA did not differ in strata of age, gender. Only DDIA gave a specificity of 77% (20/26) in children without STHs infection, significantly higher than that of 47% (14/30) in those with STHs infection (χ^2=5.35, *P*=0.02) (Figure 3).

The PPV and NPV for DDIA was 71% (53/75, 95%CI: 60%-81%) and 49% (35/71, 95%CI: 38%-61%), while the overall PPV and NPV of IHA was 80% (66/82, 95%CI: 72%-89%) and 64% (41/64, 95%CI: 52%-76%), respectively. The results showed that IHA performed better screening efficacy to find the patients still shedding parasites among the sero-positives.

< Figure 4 near hear>

4. Discussion

Different from other *Schistosoma species* that causes intestinal schistosomiasis, *S. haematobium* causes urogenital schistosomiasis. It is endemic in 53 countries in Africa and Arabian Peninsula where people are living in poverty. It is estimated that 112 million people are infected with *S. haematobium* in sub-Saharan Africa (Steinmann et al., 2006; WHO, 2013b). Meanwhile, the reports on travelers or immigrants acquired infection of *S. haematobium* from African continents become more and many of them were underwent misdiagnosis during the process of seeking treatment(Hua et al., 2013; Wang et al., 2013).

The parasitological examination of urine remains the gold standard for the diagnosis of active schistosomiasis haematobium. With the implementation of interventions against schistosomiasis haematobium in endemic areas, the worm load on population will get lower and eggs often escape detection even in concentrated urine samples(Clerinx and Van Gompel, 2011). Antibody-based immunoassay is the method of choice for sensitive detection of schistosome exposure, because the eggs are not yet produced in the early stage of infection(Bergquist et al., 2009). Two commercially available diagnostic tests-the urine-circulating cathodic antigen(CCA) strip and the soluble egg antigen enzyme-linked immunosorbent assay(SEA-ELISA), had been evaluated for detection of *S. haematobium* infection in 150 schoolchildren from Zanzibar, the sensitivity of the urine-CCA strip was 9% and the SEA-ELISA showed a 89% sensitivity, a 70% specificity, a 57% positive predictive value and a 90% negative predictive value(Stothard et al., 2009). The result of the SEA-ELISA was better. However, the operation of the SEA-ELISA is a little complex which limited its application in the field settings.

Immunological tests for schistosomiasis diagnosis had been well developed in The People's Republic of China. IHA and DDIA, are two kits which have been integrated into national control programmes and wildly used in field settings due to their advantages such as high sensitivity, easy use and rapidity(Jie et al., 2016; Zhang et al., 2016).To explore the feasibility of IHA and DDIA for screening of S. haematobium , We evaluated the diagnostic efficacy of IHA and DDIA using human sera collected from endemic areas in Zambia.

In comparison with urine filtration, the sensitivity of IHA and DDIA was 74% and 60% respectively, both increased with age. The sensitivity of IHA also increased with the infection intensity, although no difference existed in strata of gender. The tendency of DDIA performing lower sensitivity than that of IHA for detecting *S. haematobium* is consistent with our former studies when these kits were used to diagnose schistosomiasis japonica(Xu et al., 2007; Xu et al., 2005; Xu et al., 2011b). Meanwhile, 40% and 26% cases with egg positives were misdiagnosed as antibody negatives by DDIA and IHA. The phenomena could be attributed to the following reasons: (1) Some kinds of antibody against *S. haematobium* in serum are species-specific and couldn't be recognized by and/or combined with the SEA of *S. japonicum*; (2) The antibodies excess in serum in those cases with high infection intensity caused prozone phenomenon (3) Kits are insensitive to some cases with low antibody level and serology tests turn positive only about 6 to 12 weeks after exposure.

The specificity of IHA and DDIA was moderate with a value of 72% and 61% , respectively. This finding is also similar to our former studies when diagnosing schistosomiasis japonica in field settings(Xu et al., 2011a; Xu et al., 2011b). Analysis showed that the co-infection with STHs decreased the specificity of DDIA but had no impact on that of IHA, which were inconsistent with our former studies (Xu et al., 2005; Xu et al., 2011b). Possible reasons causing the false positives could be due to the accumulation of antibodies against *Schistosoma haematobium* as a part of children who had received treatment before this study might had the exposure history. Further studies should be conducted in non-endemic areas of schistosomiasis haematobium.

In summary, our study showed that IHA or DDIA performing the moderate sensitivity and specificity f and cannot simply used or screening schistosomiasis haematobium. However, IHA and DDIA reagents have been widely used to screen schistosomiasis japonicum in China, and both reagents have been registered through China Food and Drug Administration (CFDA). Improvement of diagnostic efficacy of Chinese products could be made through adjusting the concentration or ratio of kit components based on further laboratory or field survey. IHA has more potential as an alternative diagnostic tool for identifying schistosomiasis haematobium after improvement.

Funding

This manuscript was supported by the National Important Scientific & Technology Project (grant no. 2016ZX10004222-004, 2018ZX10101002-002). We acknowledge partial financial support by the National Natural Science Foundation of China (grant no. 81301454).

Authors' contribution

JX, VM and XNZ contributed to the design of the study. JX, VM, LPS, XMY, FB, NS, NKC, JFZ, MCH, WMS and LJZ implemented the fieldwork and data collection. JX and LJZ analysed the data. LJZ wrote the manuscript. All authors contributed to the writing of the manuscript and approved the submitted version of the manuscript.

Conflict of interest statement

The authors declare that they have no competing interests

Acknowledgements

We wish to thank the Vice-Chancellor of the Copperbelt University, Kitwe, Zambia, Professor Naison ngoma, for having approved the collaboration between the National Institute of Parasitic Diseases, China CDC, and the Copperbelt University, School of Medicine. We are also grateful to the Dean of the Copperbelt University, School of Medicine, Professor Kasonde Bowa, for having facilitated the travel of the Chinese scientists to Zambia and having supported the execution of the field work for the study. We also thank the Permanent Secretary of the Ministry of Health for having approved the execution of this study in Zambia. We also thank the education authorities in Luapula and Copperbelt provinces for the support rendered to the conduction of the study in the two schools.

References

Bergquist, R., Johansen, M.V., Utzinger, J., 2009. Diagnostic dilemmas in helminthology: what tools to use and when? Trends in parasitology 25, 151-156.

Bevilacqua, N., Pane, S., Vairo, F., Nicastri, E., Paglia, M.G., Ame, S.M., Schepisi, M.S., Kitua, A., Mangi, S., Racalbuto, V., Meschi, S., Ippolito, G., 2012. Accuracy of indirect haemagglutination and western blot assays for the detection of anti-Schistosoma antibodies in non-severe febrile patients in two Tanzanian hospitals. Scandinavian journal of infectious diseases 44, 453-458.

Clerinx, J., Van Gompel, A., 2011. Schistosomiasis in travellers and migrants. Travel medicine and infectious disease 9, 6-24.

Colley, D.G., Bustinduy, A.L., Secor, W.E., King, C.H., 2014. Human schistosomiasis. Lancet 383, 2253-2264.

Fei, H., Zhao-Jun, L., Yi-Feng, L., Min, Y., Shu-Ying, X., Yue-Min, L., Jian-Ying, L., Zu-Lu, G., Yong, P., Jin-Ming, W., Dan-Dan, L., 2016. [Study on cut-off value of IHA method for schistosomiasis diagnosis in different endemic areas]. Zhongguo xue xi chong bing fang zhi za zhi = Chinese journal of schistosomiasis control 28, 644-647.

Fenwick, A., Webster, J.P., Bosque-Oliva, E., Blair, L., Fleming, F.M., Zhang, Y., Garba, A., Stothard, J.R., Gabrielli, A.F., Clements, A.C., Kabatereine, N.B., Toure, S., Dembele, R., Nyandindi, U., Mwansa, J.,

Koukounari, A., 2009. The Schistosomiasis Control Initiative (SCI): rationale, development and implementation from 2002-2008. Parasitology 136, 1719-1730.

Gray, D.J., Ross, A.G., Li, Y.S., McManus, D.P., 2011. Diagnosis and management of schistosomiasis. BMJ 342, d2651.

Helleberg M, T.S., 2009. Schistosomiasis in Danish travellers and immigrants. Uqeskr Laeger 171, 7.

Hua, H.Y., Wang, W., Cao, G.Q., Tang, F., Liang, Y.S., 2013. Improving the management of imported schistosomiasis haematobia in China: lessons from a case with multiple misdiagnoses. Parasites & vectors 6, 260.

Jian-Feng, Z., Li-Yong, W., Jing, X., You-Sheng, L., Xiao-Lan, Y., Guang-Hui, R., Tie-Wu, J., Wei, W., Xiao-Nong, Z., 2019. [Current status and transmission risks of oversea imported schistosomiasis in China]. Zhongguo xue xi chong bing fang zhi za zhi = Chinese journal of schistosomiasis control 31, 26-32.

Jie, Z., Wei, G., Fu-Rong, W., Yao, R., Sheng-Ming, L., Jing, X., Tie-Wu, J., Xiao-Nong, Z., 2016. [Study on value of IHA in diagnosis of schistosomiasis japonica]. Zhongguo xue xi chong bing fang zhi za zhi = Chinese journal of schistosomiasis control 28, 375-380.

Kalinda, C., Chimbari, M.J., Mukaratirwa, S., 2018. Schistosomiasis in Zambia: a systematic review of past and present experiences. Infectious diseases of poverty 7, 41.

KE, M., 1983. A reusable polyamide filter for diagnosis of S. haematobium infection by urine filtration. Bull Soc Pathol Exot Filiales 76, 4.

King, C.H., Bertsch, D., 2013. Meta-analysis of urine heme dipstick diagnosis of Schistosoma haematobium infection, including low-prevalence and previously-treated populations. PLoS neglected tropical diseases 7, e2431.

Liang, S., Abe, E.M., Zhou, X.N., 2018. Integrating ecological approaches to interrupt schistosomiasis transmission: opportunities and challenges. Infectious diseases of poverty 7, 124.

Moser, W., Schindler, C., Keiser, J., 2017. Efficacy of recommended drugs against soil transmitted helminths: systematic review and network meta-analysis. BMJ 358, j4307.

Olveda, D.U., Olveda, R.M., McManus, D.P., Cai, P., Chau, T.N., Lam, A.K., Li, Y., Harn, D.A., Vinluan, M.L., Ross, A.G., 2014. The chronic enteropathogenic disease schistosomiasis. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases 28, 193-203.

Palha De Sousa, C.A., Brigham, T., Chasekwa, B., Mbuya, M.N., Tielsch, J.M., Humphrey, J.H., Prendergast, A.J., 2014. Dosing of praziquantel by height in sub-Saharan African adults. The American journal of tropical medicine and hygiene 90, 634-637.

Ross, A.G., Vickers, D., Olds, G.R., Shah, S.M., McManus, D.P., 2007. Katayama syndrome. The Lancet. Infectious diseases 7, 218-224.

Salvana, E., CH, K., 2008. schistosomiasis in travelers and immigrants. Current Infectious Disease Reports 10, 8.

Santos, J., Gouveia, M.J., Vale, N., Delgado Mde, L., Goncalves, A., da Silva, J.M., Oliveira, C., Xavier, P., Gomes, P., Santos, L.L., Lopes, C., Barros, A., Rinaldi, G., Brindley, P.J., da Costa, J.M., Sousa, M., Botelho, M.C., 2014. Urinary estrogen metabolites and self-reported infertility in women infected with Schistosoma haematobium. PloS one 9, e96774.

Steinmann, P., Keiser, J., Bos, R., Tanner, M., Utzinger, J., 2006. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. The Lancet. Infectious diseases 6, 411-425.

Stothard, J.R., Sousa-Figueiredo, J.C., Standley, C., Van Dam, G.J., Knopp, S., Utzinger, J., Ameri, H., Khamis, A.N., Khamis, I.S., Deelder, A.M., Mohammed, K.A., Rollinson, D., 2009. An evaluation of urine-CCA strip test and fingerprick blood SEA-ELISA for detection of urinary schistosomiasis in schoolchildren in Zanzibar. Acta tropica 111, 64-70.

van der Werf, M.J., de Vlas, S.J., Brooker, S., Looman, C.W., Nagelkerke, N.J., Habbema, J.D., Engels, D., 2003. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. Acta tropica 86, 125-139.

Wang, W., Liang, Y.S., Hong, Q.B., Dai, J.R., 2013. African schistosomiasis in mainland China: risk of transmission and countermeasures to tackle the risk. Parasites & vectors 6, 249.

Weerakoon, K.G., Gobert, G.N., Cai, P., McManus, D.P., 2015. Advances in the Diagnosis of Human Schistosomiasis. Clinical microbiology reviews 28, 939-967.

WHO, 2013a. Schistosomiasis progress report 2001-2011 and strategic plan 2012-2020, in: Organization, W.H. (Ed.), Geneva.

WHO, 2013b. Schistosomiasis: progress report 2001-2011 and strategic plan 2012-2020. World Health Organization, Geneva.

WHO, 2017. Schistosomiasis and soil-transmitted helminthiases: number of people treated in 2016. Releve epidemiologique hebdomadaire 92, 749-760.

Xu, J., Chen, N.G., Feng, T., Wang, E.M., Wu, X.H., Chen, H.G., Wang, T.P., Zhou, X.N., Zheng, J., 2007. [Effectiveness of routinely used assays for the diagnosis of schistosomiasis japonica in the field]. Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi = Chinese journal of parasitology & parasitic diseases 25, 175-179.

Xu, J., Feng, T., Guo, J.G., Zheng, H., Wang, Q., Wu, X.H., Zhou, X.N., 2005. Comprehensive evaluation of several diagnosis agents of schistosomiasis japonica in China. Chin J Schisto Contr 17, 116-119.

Xu, J., Feng, T., Lin, D.D., Wang, Q.Z., Tang, L., Wu, X.H., Guo, J.G., Peeling, R.W., Zhou, X.N., 2011a. Performance of a dipstick dye immunoassay for rapid screening of Schistosoma japonicum infection in areas of low endemicity. Parasites & vectors 4, 87.

Xu, J., Peeling, R.W., Chen, J.X., Wu, X.H., Wu, Z.D., Wang, S.P., Feng, T., Chen, S.H., Li, H., Guo, J.G., Zhou, X.N., 2011b. Evaluation of Immunoassays for the Diagnosis of Schistosoma japonicum Infection Using Archived Sera. PLoS neglected tropical diseases 5, e949.

Xu Yun, Gong Zhi-Hong, Ning An, Hui-qun, X., 2017. Clinical Analysisof Imported Schistosomiasis Cases. Contemporary Medicine 23, 4.

Yi, P., Yuan, L.P., Wang, Z.H., He, Y.K., Jing, Q.S., Zhou, J., Wang, H.B., Li, S.M., Franziska, B., 2011. [Retrospective survey of 184 patients infected with Schistosoma haematobium from African countries]. Zhongguo xue xi chong bing fang zhi za zhi = Chinese journal of schistosomiasis control 23, 441-442.

Zhang, J.F., Xu, J., Bergquist, R., Yu, L.L., Yan, X.L., Zhu, H.Q., Wen, L.Y., 2016. Development and Application of Diagnostics in the National Schistosomiasis Control Programme in The People's Republic of China. Advances in parasitology 92, 409-434.

Zhu, Y.C., Socheat, D., Bounlu, K., Liang, Y.S., Sinuon, M., Insisiengmay, S., He, W., Xu, M., Shi, W.Z., Bergquist, R., 2005. Application of dipstick dye immunoassay (DDIA) kit for the diagnosis of schistosomiasis mekongi. Acta tropica 96, 137-141.

Zida, A., Briegel, J., Kabre, I., Sawadogo, M.P., Sangare, I., Bamba, S., Yacouba, A., Ouedraogo, A., Yonli, D., Drabo, F., Traore, L.K., Ouedraogo-Traore, R., Guiguemde, R.T., Wacker, J., 2016. Epidemiological and clinical aspects of urogenital schistosomiasis in women, in Burkina Faso, West Africa. Infectious diseases of poverty 5, 81.

Figure Legends

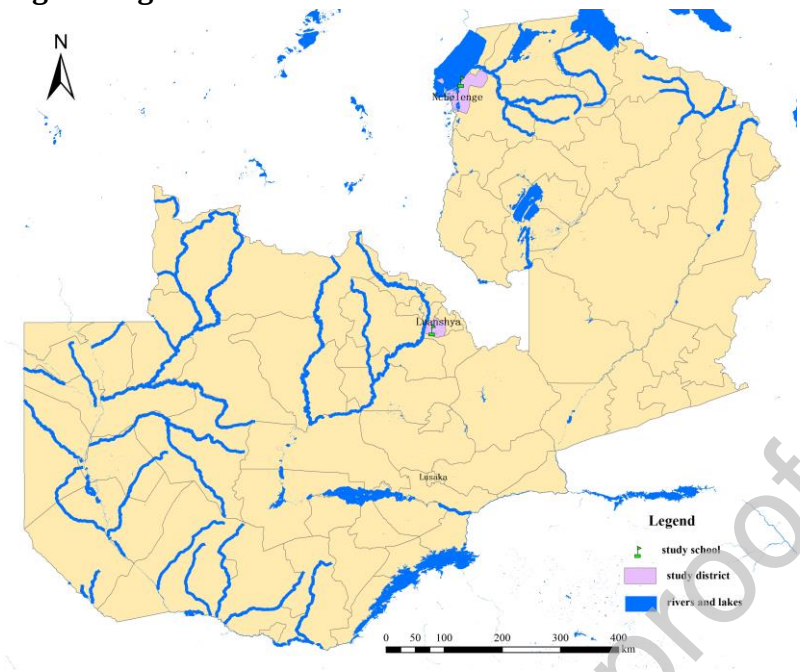

Figure 1 Location of pupils' bio-samples collected

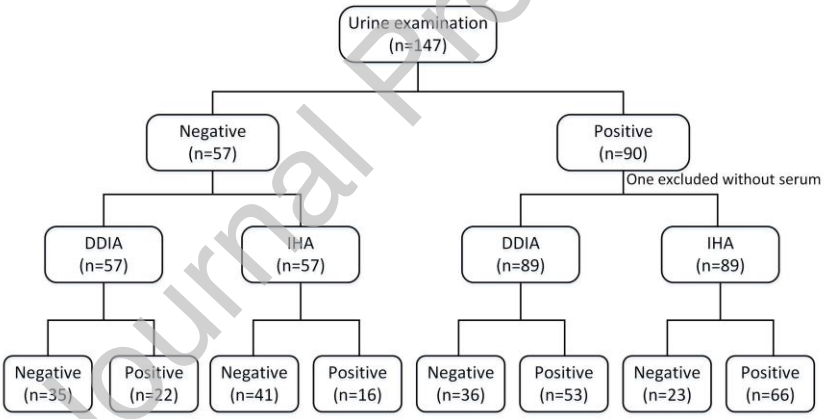

Figure 2 Comparison of results with urinary filtration and immunological tests

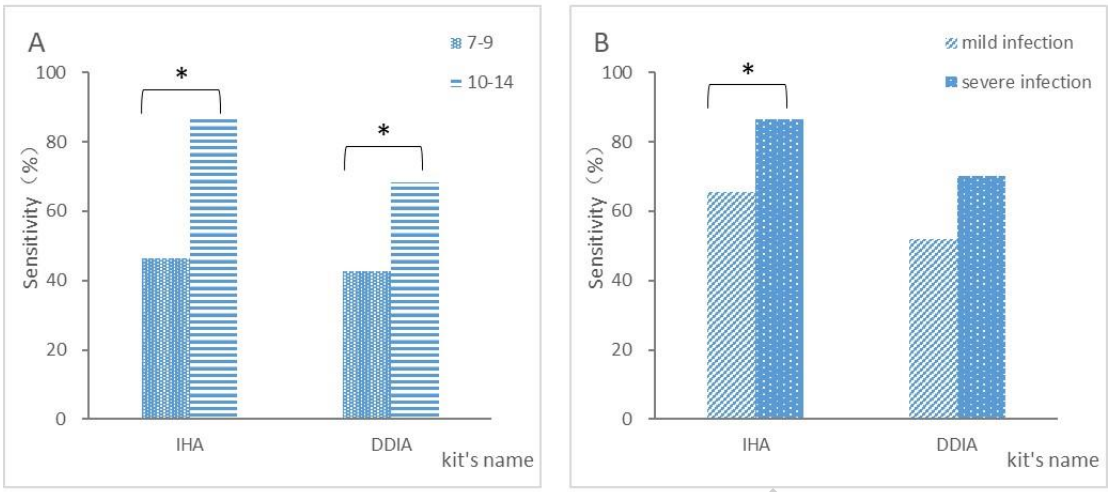

Figure 3 Sensitivity of IHA and DDIA A, by age and B, by infection intensity (*means that the difference was statistically significant)

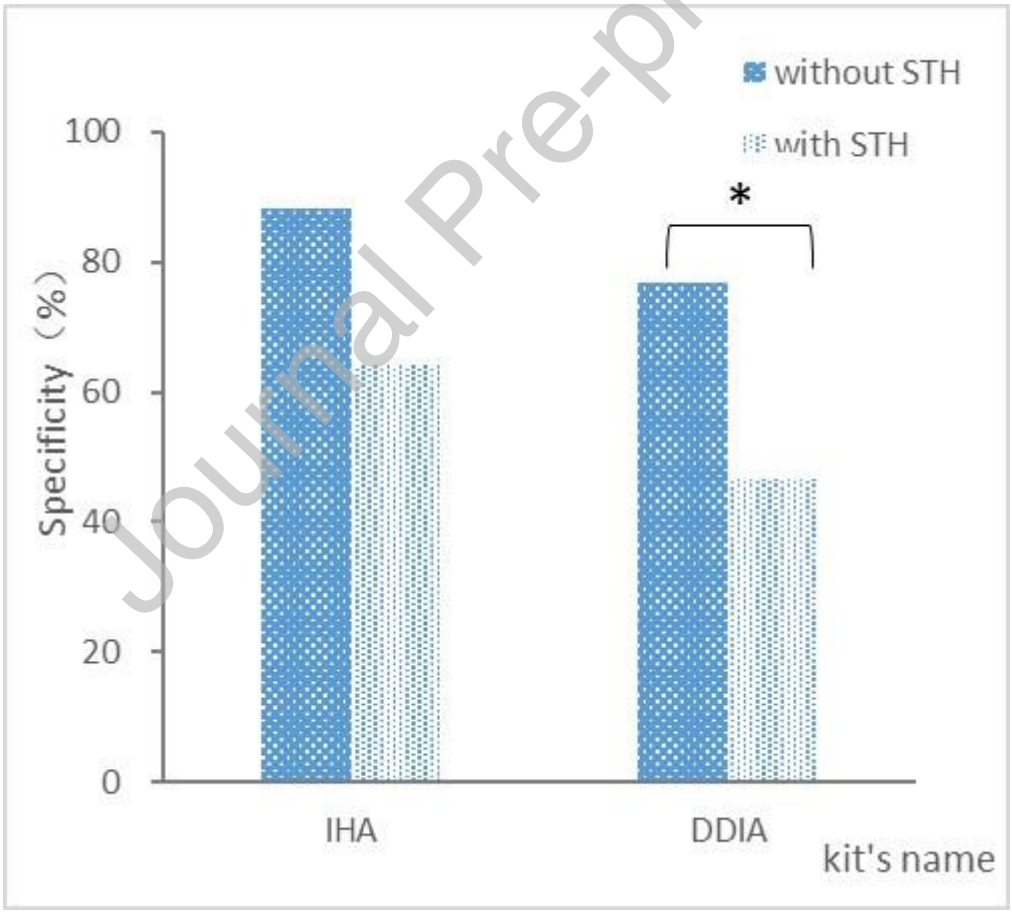

Figure 4 Specificity of IHA and DDIA by STHs infection status (*means that the difference was statistically significant)

Table 1 Demographic characteristics and results of urine filtration

Variables	Group	No. examined	Infection intensity		Positive rates
			Mild	Heavy	[95%CI]
Sex	girl	69	17	19	52%(40%-64%)
	boy	78	35	19	69%((59%-79%)
age	7-9	78	22	8	37%(26%-48%)
	10-14	69	30	30	87%(79%-95%)
school	Kenani School	68	35	31	97%(93%-100%)
	Kawama School	79	17	7	30%(20%-41%)

Table 2 Performance characteristics of DDIA and IHA compared with urine filtration

Indictors assessed	DDIA	IHA
No. true positives	53	66
No. false positives	22	16
No. true negatives	35	41
No. false negatives	36	23
Sensitivity [95%CI]	60%(49%, 70%)	74%(65%, 83%)
Specificity [95%CI]	61%(49%, 74%)	72%(60%, 84%)
PPV [95%CI]	71%(60%, 81%)	80%(72%, 89%)
NPV [95%CI]	49%(38%, 61%)	64(52%, 76%)