COMPARISON OF THE MIRACIDIUM HATCHING TEST AND MODIFIED KATO-KATZ METHOD FOR DETECTING *SCHISTOSOMA JAPONICUM* IN LOW PREVALENCE AREAS OF CHINA

Hong-qing Zhu, Jing Xu, Rong Zhu, Chun-li Cao, Zi-ping Bao, Qing Yu, Li-juan Zhang, Xiao-lin Xu, Zheng Feng and Jia-gang Guo

National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, WHO Collaborating Center for Malaria, Schistosomiasis and Filariasis; The Key Laboratory of Parasite and Vector Biology, National Health and Family Planning Commission, People's Republic of China

Abstract. The diagnosis of *Schistosoma japonicum* (Sj) infection in low prevalence areas of the People's Republic of China is challenging due to the sensitivity of the detection methods, leading to an underestimation of the disease burden. We compared the sensitivities of the miracidium hatching test (MHT) with the modified Kato-Katz method (KK) and the combination of the two methods (KK-MHT) to detect Sj infection in low prevalence areas of China. The stool samples of 3,853 residents from 8 villages with a light to moderate prevalence (0-23%) of Sj infection were examined by KK, MHT and KK-MHT. The findings were inconsistent. The KK-MHT conbination gave more positives than either the KK or MHT alone. Using the KK-MHT, we determined the missed rates with the KK (mR_K) and MHT (mR_H) to be 30.1% and 10.2%, respectively. At light prevalence sites (infection rate ≤10%) the mR_K was 60.6%, significantly higher than the mR_K of 22.3% found at moderate prevalence sites (10-23%). However, the mR_H at the light and moderate prevalence sites were 11.54% and 9.90%, respectively (p>0.05). The combination KK-MHT had the best sensitivity in low Sj prevalence areas in China and the KK method alone was the least sensitive. Using KK alone as a screening method will result in an underestimation of Sj infection disease burden.

Keyword: schistosomiasis japonica, fecal examination, Kato-Katz method, miracidium hatching test, PR China

INTRODUCTION

In the mid 1950s, at the beginning of the national schistosomiasis control program, schistosomiasis was rampant in 12 provinces and 426 counties (cities) in China (Zheng, 2003). However, remarkable progress has been made over the past 50 years in controlling schistosomiasis. It still remains endemic in 7 provinces (Hunan, Hubei, Jiangxi, Anhui, Jiangsu, Sichuan and Yunnan) and 189 counties / cities in China (Hao *et al*, 2010).

Since the 1980s, using mass chemo-

Correspondence: Hongqing Zhu
Tel: (86) 21 54650863; Fax: (86) 21 54650863
E-mail: zhu.hq@163.com
Zheng Feng
Tel: (86) 21 54653007; Fax: (86) 21 54653512
E-mail: zfeng09@gmail.com

therapy with praziquantel has dramatically reduced the prevalence and the morbidity of schistosomiasis in China (Zhu and Guo, 2009). The higher sensitivity and simple performance of serological tests make them more acceptable to health professionals and patients in endemic areas and popularized in use as an integrated part of the national schistosomiasis control program to screen individuals for infection in endemic areas (Wu, 2005). However, the main problem with this approach is the low specificity of the test because of persisting antibodies due to previous infection and cross-reactions with other helminth infections (Wu *et al*, 1985; Guan and Shi, 1996; Feng *et al*, 1998). Therefore, parasitological diagnostic techniques are the methods of choice to definitively diagnose *Schistasoma japonicum* (Sj) infection in China (Wu, 2005; Chen *et al*, 2007; Xu *et al*, 2007).

Techniques currently used in China to detect Sj eggs in the stool are the modified Kato-Katz method (KK) and the miracidium hatching test (MHT). Although the MHT is highly specific, there are some problems with this method: it is influenced by temperature, quality of the hatching water and examiner experience. After treatment with praziquantel, the MHT may be affected by damage to the eggs by praziquantel (Xiao *et al*, 1980; Min *et al*, 1987). The MHT may miss some egg positives leading to an underestimation of prevalence (Zhu *et al*, 2006). The KK method is recommended by the World Health Organization (WHO) for the routine diagnosis of schistosomiasis (WHO Expert Committee, 1985; Yao *et al*, 1992; Zhang *et al*, 1999) especially in heavily endemic areas, because of its accessibility, simplicity and minimal cost. The KK method has been used to diagnose schistosomiasis in China since the early 1980s (Huang *et al*, 2007). As control programs have improved, the prevalence and intensity of schistosomiasis has decreased (Zhou *et al*, 2005). Consequently, the sensitivity of these parasitological techniques is inadequate for low prevalence areas. The sensitivity of the KK method decreases in low prevalence settings where the expected number of eggs is small (Wu *et al*, 2002; Jin *et al*, 2005).

In the present study, we used the combination of KK and the MHT (KK-MHT) to assess the sensitivity of KK and MHT individually in low schistosomiasis prevalence areas in China, which was expressed by the missed infection rate (false negative rate).

MATERIALS AND METHODS

Study subjects and sample collection

The study was carried out in schistosomiasis endemic areas with low and medium prevalences: Hubei, Jiangsu and Jiangxi provinces in the lake region, and Sichuan and Yunnan provinces in the mountainous region of China. Study sites were classified by prevalence rates: 1%, 1%, 5%, and 10% based on the previous records. Two villages were selected for each prevalence level. All the residents in each of the 8 villages were enrolled in the examination. A total of 9,853 villagers of both genders aged 6-65 years were included in the study.

Finger-prick blood samples were collected from each individual for Sj screening using ELISA kit (Manual of Schistosomiasis Control and Prevention, China, 2000) (Kangbaide Biotechnology, Shenzhen, China). Three stool samples were obtained from each examinee with a positive ELISA screen (n=2,824) on consecutive days and obtained from those

Table 1
Method used to calculate the false negative rate and sensitivity of evaluated techniques.

Examination method		Miracidium hatching		Total
		Positive	Negative	
Kato-Katz	Positive	A	B	A+B
	Negative	C	D	C+D
	Total	A+C	B+D	A+B+C+D

with water contact exposure (1,029).

With the MHT, the stool samples were screened through a nylon tissue bag to concentrate the eggs. Hatching took place in a well-lit room at 25±3ºC and then the samples were checked for swimming miracidia at 4, 8 and 12 hours (Bureau of Disease Control, 2000). Three to 5 positive control samples were tested simultaneosly.

Fecal samples were examined for Sj eggs using the modified KK thick smear method (WHO Expert Committee, 1985; Bureau of Disease Control, 2000).The slides were read and all positives were confirmed by two examiners.

A training session was conducted for stool examiners prior to the study to improve standardization.

Questionnaire

A questionnaire was filled out by the subject to obtain demographic information and relevant history: name, address, gender, age, education, water contact and recent treatment for schistosomiasis.

Data analysis

The questionnaire and examination results were double entered into a database. All data were processed using SPSS, version 13.0. The chi-square test was used to determine significant differences in rates among the different groups. Statistical significance was set at $p \leq 0.05$.

To assess the sensitivity of the MHT and KK, we used the infection rate detected by the KK-MHT combination as the standard. The false negative rates, expressed as the missed rate with KK (mR_K) and missed rate with the MHT (mR_H), were calculated as shown in Table 1.

The sensitivity of the KK-MHT = (A+B+C)/ (A+B+C+D) x100%.

The missed rates for the KK (mR_K) and MHT (mR_H) methods:

mR_K=C/(A+B+C) x100%

mR_H=B/(A+B+C) x100%

Ethical considerations

The study protocol was approved by the Review Board of the National Institute of Parasitic Diseases Control and Prevention, Chinese Center for Disease Control and Prevention. Verbal informed consent was obtained from all adult participants or guardians before inclusion in the study. All procedures performed were in accordance with the national control program. The personal information was kept confidential.

RESULTS

Sensitivities of the tested methods

A total of 9,853 subjects from 8 villages with a schistosomiasis prevalence of

Table 2
Comparision of stool examination by modified Kato-Katz method and miracidium hatching test.

No. of villages	Prevalence range (%)	No. of samples	KK-MHT		KK			MHT		
			No. positive	Positive rate (%)	No. positive	Positive rate (%)	mR_K (%)	No. positive	Positive rate (%)	mR_H (%)
5	≤10	1,704	104	6.1	41	2.4	60.6	92	5.4	11.5
3	10-23	2,149	404	18.8	314	14.6	22.3	364	16.9	9.9
Total		3,853	508	13.2	355	9.2	30.1	456	11.8	10.2

KK, Kato-Katz method; MHT, miracidium hatching test; mR_K, missed rate with Kato-Katz method; mR_H, missed rate with miracidum hatching test.

0-23% were screened with ELISA for schistosomiasis. Of these, 2,824 (28.66%) were positive. Stool samples were obtained from these subjects (n=2,824) and from others at high risk by history of water contact (n=1,029). The stool samples were tested with the MHT and KK methods. The egg positivity rate for all subjects (n=3,853) using the KK-MHT combination was 13.2%. The positivity rate with the KK method alone was 9.2%, significantly lower ($\chi^2 = 30.546$, $p<0.001$) than the combined method. The positivity rate with the MHT method was 10.2%, which was not significantly different from the combined method (Table 2).

The mR_K (30.1%) was significantly higher than the mR_H (10.2%) ($\chi^2 = 62.339$, $p<0.001$). The KK and MHT methods were less sensitive than the KK-MHR combination (Fig 1) in areas where the prevalence of schistosomiasis is <23%.

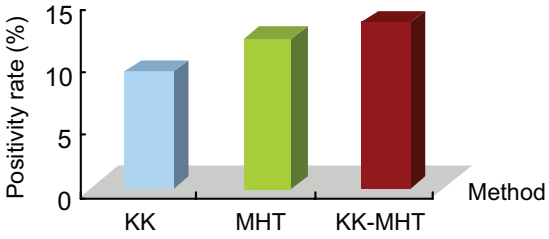
Sensitivities of examined methods by prevalence rates

The study villages were divided into two groups: low prevalence group (prevalence ≤10%), higher prevalence group (prevalence >10% but ≤23%). The mR_K was high (60.6%) in the lower prevalence areas (village/province: Shiyuan/Hubei; Huanglin/Sichuan, Majiayi/Yunnan, Shuijin/Yunnan, Yanjiang/Jiangsu) and 22.3% in the higher prevalence areas (village/province: Shunhe/Sichuan, Dingshan/Jiangxi, Pohu/Jiangxi) compared to the KK-MHT. The mR_K were significantly different from eachother by prevalence area ($\chi^2 = 30.546$, $p < 0.001$). The mR_H was 11.5% in the lower prevalence area and 9.9% in the higher prevalence area, but this difference was not significant ($\chi^2 = 0.164$, p=0.685) (Fig 2).

DISCUSSION

In recent years, the program to control schistosomiasis in China using environmental modification for snail control and chemotherapy has dramatically reduced the prevalence of infection (Wu *et al*, 2002; Wu, 2005; Wang *et al*, 2005; Wang *et al*, 2009a,b; Chen *et al*, 2011; Zhong *et al*, 2011).

Although serological techniques are convenient, the gold standard for diagnosing *S. japonica* infection is stool parasitological examination (Wu, 2005; Chen *et al*, 2007). The most commonly used stool



KK, Kato-Katz method; MHT, miracidium hatching test.

Fig 1–Sensitivities of examined methods.

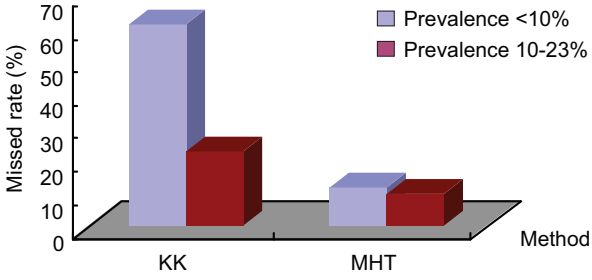


Fig 2–The missing rate by KK and MHT compared with KK-MHT.

parasitological examination methods are the KK and MHT. The KK method has suboptimal sensitivity (Jin *et al*, 2005; Zhu *et al*, 2006) leading to underestimation of the prevalence of schistosomiasis particularly in areas with a lower prevalence. The KK method has been found to have lower sensitivity in a patients with a light infection (1-100 epg) compared to moderately (101-400 epg) and heavily infected individuals (> 400 epg) in a study from a village with a relatively light to moderate prevalence (14-25%) (Yu *et al*, 1998).

The present study found the KK method was significantly less sensitive than the combined KK-MHT method in low to moderate schistosomiasis prevalence areas. The lower sensitivity in low prevalence areas is possibly due to lower intensities of infections in those areas and only small amount of feces for the examination. The KK method is less sensitive when the number of eggs in the stool is low (Jin *et al*, 2005; Zhu *et al*, 2006). The KK method evaluates about 40 mg of stool, while the MHT evaluates 30 g of stool. This gave a missed rate of about 10% in both lower and medium prevalence areas and the difference was not significant.

Based on the findings of this study, we suggest the KK-MHT combination method gives a better estimation of the prevalence of schistosomiasis in light to moderately prevalent areas in China.

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