Communication

Competency and challenges in malaria microscopy in China

Jianhai Yin, He Yan, Mei Li, Yao Ruan, Xueqiang Zhang, Liying Wang, Cunli Cao, Zhigui Xia, Shuisen Zhou*

National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, WHO Collaborating Centre for Tropical Diseases, National Center for International Research on Tropical Diseases, Ministry of Sciences and Technology, Key Laboratory of Parasite and Vector Biology, Ministry of Health, Shanghai, China.

Summary	Precise diagnosis is a key measure for malaria control and elimination, and malaria microscopy is still the gold standard method recommended by the World Health Organization (WHO) for malaria diagnosis. Analysis of the competency in malaria microscopy in China will benefit to identify the challenges in this skill and provide some suggestions for improvement in order to reach the requirement of WHO procedures for certification of malaria elimination, and finally contribute to malaria elimination by 2020 in China. According to a series of external assessment activities about malaria microscopy, malaria microscopists from both the national and provincial level but not the levels below provincial level performed quite well in *Plasmodium spp* identification, but their competency in differentiation of *P. ovale* and *P. vivax* and parasite counting by microscopy were not good enough at all levels. Therefore, it is necessary to strengthen the competency in species identification and parasite counting especially at the lower levels in the first line through training and practice as well as regular quality assurance with enough policy support.

Keywords: Malaria microscopy, species identification, parasite counting, quality assurance

1. Introduction

The annual incidence rate of indigenous malaria in China is decreasing, but malaria is still a very significant health problem, especially the importation of malaria has been an important challenge against malaria elimination in this country (*1,2*). Precise and prompt laboratory diagnosis and appropriate treatment is a key strategy to control and eliminate malaria. There are many limitations such as low sensitivity within the detection limit, poor specificity due to morphological changes that are enhanced by staining and similarities between several parasites, and operator dependence because even highly qualified microscopists can make an incorrect or incomplete assessment of the *Plasmodium spp*. A variety of diagnostic methods are used for *Plasmodium* parasites identification and speciation. However, malaria microscopy on Giemsa-stained thick and thin blood smears with species identification and parasite counting is still the gold standard method recommended by the World Health Organization (WHO) for malaria diagnosis, clinical trials efficacy evaluation and epidemiological surveys. Moreover, qualified microscopy competency is a major indicator of WHO procedures for certification of malaria elimination (*3*).

However, it is difficult to maintain competency in malaria microscopy with the rapid decline of indigenous malaria cases, especially in first line clinics, due to its complexity and time-consuming and inconsistency of results compared with other diagnostic assays such as rapid diagnostic tests (RDT) and polymerase chain reactions (PCR) (*4-6*). It is also potentially due to the lack of fiscal and personnel investments in malaria microscopy (*7*).

Therefore, the China malaria diagnosis reference laboratory network (*8*) based on centres for disease control and prevention or institutes of parasitic diseases at different levels covering all 24 historical malaria-endemic provinces has been set up. It performs

*Address correspondence to:
Dr. Shuisen Zhou, National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, WHO Collaborating Centre for Tropical Diseases, National Center for International Research on Tropical Diseases, Ministry of Sciences and Technology, Key Laboratory of Parasite and Vector Biology, Ministry of Health, Shanghai 200025, China.
E-mail: shuisenzhou@126.com

Table 1. Results of malaria microscopy in EQAs, 2013-2015

Scoring	Identification of species						Quantification of parasite counts					
	2013-1	2013-2	2014-1	2014-2	2015-1	2015-2	2013-1	2013-2	2014-1	2014-2	2015-1	2015-2
Win	39	43	42	42	42	42	11	9	15	16	16	14
Lose	6	2	3	3	3	0	4	9	0	2	2	4
Total	45	45	45	45	45	42	15	18	15	18	18	18

EQA, External Quality Assessment.

quality assurance to guarantee and maintain the performance of diagnostic assays including malaria microscopy in clinics and in the field. Meanwhile, China participates in different external assessment activities about malaria microscopy organized by WHO. In addition, competency in malaria microscopy at different administrative levels was expected to improve through systematic training before technique competitions for malaria parasite detection (*9*). Above all, a comprehensive analysis of the competency in malaria microscopy in China can help us identify the challenges in this skill and provide some suggestions for improvement, and contribute to the achievement of malaria elimination by 2020 in China.

2. Competency in malaria microscopy

First of all, three rounds of External Competency Assessment (ECA) of malaria microscopists were held in China organized by WHO by 2015. Microscopists (35 person-times) from the malaria endemic provinces including Anhui, Yunnan, Henan, Hubei, Hainan, Jiangsu, Sichuan, Shanghai, Guangxi, Fujian and National Institute of Parasitic Diseases (NIPD) in the year 2010; Yunnan, Anhui, Jiangsu, Henan, Hainan, Shanghai, Shandong, Guizhou, Guangxi and NIPD in the year 2012; and Yunnan, Jiangsu, Sichuan, Henan, Guangxi, Shanghai, Fujian, Guizhou, Shandong, Anhui, Tengchong of Yunnan and NIPD in the year 2015, were assessed through a scheduled examination of *Plasmodium spp* identification and parasite counting respectively. Overall, their performance was generally good ($\chi^2 = 2.520$, $p = 0.112$). In detail, there was one microscopist ranked in Level 1, seven Level 2 and four Level 3 in 2010; five Level 1, four Level 2 and two Level 3 in 2012; and eight Level 1, two Level 2 and two Level 4 in 2015. And five microscopists were assessed repeatedly, and two of them raised their competency from level 2 to level 1 and the other three remained the same (two remained level 1, one remained level 2).

Moreover, two rounds of External Quality Assessment (EQA) Programme for malaria microscopy (*10*) in NIPD each year were also organized by WHO during 2013 and 2015, 15 slides were included per round, totally covering 34 slides of *P. falciparum*, 23 slides of *P. vivax*, 3 slides each of P. malariae and *P. knowlesi*, 2 slides of *P. ovale* and 25 negative slides.

As a result, all the *P. falciparum*-positive slides and negative slides were identified correctly, while 3 slides of *P. vivax*-positve were misdiagnosed as *P. ovale*, one slide of *P. malariae*-positive was misdiagnosed as *P. knowlesi*, one *P. knowlesi*-positive was misdiagnosed as *P. ovale*, one *P. knowlesi*-positive and one *P. ovale*-positive as *P. vivax* (Table 1). As a result, no significance was found in the identification of species ($\chi^2 = 2.217$, $p = 0.330$), while parasite counting was statistically different among these three years ($\chi^2 = 13.665$, $p = 0.001$): 2014 was better than 2013 ($\chi^2 = 8.627$, $p = 0.003$), no difference in 2015 *vs.* 2013 ($\chi^2 = 3.391$, $p = 0.066$) and 2015 *vs.* 2014 ($\chi^2 = 0.996$, $p = 0.318$).

Since then, a national competency assessment programme concerning *Plasmodium spp* identification only has been organized by national laboratory (*8*). Briefly, nineteen microscopists from 19 provincial malaria diagnosis laboratories took part in the activity. Only two participants correctly identified all 20 slides, seven failed in one slide, six incorrectly identified two slides, two individuals failed in three slides, and one each incorrectly identified four and six slides, respectively. Particularly, twelve participants identified the *P. vivax*-positive slide as *P. ovale*. There existed a *P. ovale* positive slide that was the most difficult one for participants with nine failing to identify it with most of them (6/9) incorrectly identifying it as *P. vivax*.

Last but not least, malaria microscopy including blood slide preparation and *Plasmodium spp* identification and parasite counting is a very important part of National technique competition for diagnosis of parasitic diseases carried out in China, four representatives working for disease control and prevention at different levels were selected as contestants (age < 45 and at least two contestants from county-level) per province every year with no duplicates in the entries between years. Although a quite good performance was found in every year, the competency in malaria microscopy was not good enough (Table 2). There was significance of performance in blood film preparation (F = 17.3, $p < 0.01$) and malaria microscopy on the whole (F = 4.5, $p < 0.01$) from 2011 to 2015 (Table 2). Moreover, malaria parasite identification of *P. falciparum* (F = 14.2, $p < 0.01$) and *P. vivax* (F = 3.4, $p < 0.01$) were also different among these five competitions (Table 2). Additionally, *P. ovale*-positive slides were added into the competition from the

Table 2. Comparison of malaria microscopy in the national competitions, 2011-2015

Content	2011	2012	2013	2014	2015	*F* value	*p* value
Blood film preparation							
(mean ± SD)	86.9 ± 10.4	87.3 ± 9.2	90.8 ± 9.7	94.4 ± 4.0	91.0 ± 6.2	17.3	< 0.01
Malaria microscopy							
(mean ± SD)	44.3 ± 22.0	53.4 ± 25.4	56.8 ± 24.7	52.9 ± 23.1	54.1 ± 26.1	4.5	< 0.01
Parasite identification							
(detection rate, %)							
P. falciparum	55.3	67.8	62.1	64.5	61.1	14.2	< 0.01
P. vivax	73.9	62.6	68.8	79.0	65.3	3.4	< 0.01

year 2014, and there was no difference in performance between 2014 (36.3%) and 2015 (47.6%).

3. Challenges and prospects

Various assessment activities and competitions not only implement the quality assurance for malaria microscopy, but also are good models to be used as training courses to improve the competency of microscopists.

Although the competency for *Plasmodium spp* identification was quite good at both national and provincial levels according to the results of WHO ECA, EQA Programme and National Competency Assessment Programme referred to above, it was still difficult to differentiate *P. ovale* from *P. vivax*. This may be attributed to the morphologic similarity between them (*11*) or because of only sporadic ovale malaria cases in China (*12,13*). However, ovale malaria cases have increased in China due to travellers returning from endemic areas (*1,2*). In addition, it was also not good enough in parasite counting at these two levels, but malaria parasite density is critical for patient management especially when parasite resistance to available therapy is increasing and particularly in clinical trials and drug efficacy studies.

Meanwhile, the competency in *Plasmodium spp* identification and parasite counting by microscopy at levels below the provincial level was lower nationally. To some extent, differences in competency between endemic and non-endemic provinces had an impact on the overall level (*9,14*). It also may be attributed to less opportunity to detect malaria cases with the reduction in local cases, or because several much simpler and rapid diagnostic assays were used instead of malaria microscopy (*4*). While microscopists at county level are the main health workers for malaria control and prevention as the first line, their deficiency in malaria diagnosis will be a big challenge for malaria elimination.

Therefore, certified microscopists should receive more training courses as well as be invited as teachers in training malaria microscopy as a first line with strong policy supports, but it must pay much more attention to the identification of rare malaria parasites in China. Moreover, other diagnostic tools such as nucleic acid amplification with high sensitivity and specificity should be considered in routine malaria diagnosis, especially because of its advantages in detecting asymptomatic infections. In addition, quality assurance of malaria diagnosis must be carried out regularly.

References

1. Feng J, Xiao H, Xia Z, Zhang L, Xiao N. Analysis of malaria epidemiological characteristics in the People's Republic of China, 2004-2013. Am J Trop Med Hyg. 2015; 93:293-299.
2. Yin JH, Yang MN, Zhou SS, Wang Y, Feng J, Xia ZG. Changing malaria transmission and implications in China towards National Malaria Elimination Programme between 2010 and 2012. PloS One. 2013; 8:e74228.
3. World Health Organization. WHO procedures for certification of malaria elimination. Wkly Epidemiol Rec. 2014; 89:321-325.
4. Hawkes M, Kain KC. Advances in malaria diagnosis. Expert Rev Anti Infect Ther. 2007; 5:485-495.
5. Proux S, Suwanarusk R, Barends M, Zwang J, Price RN, Leimanis M, Kiricharoen L, Laochan N, Russell B, Nosten F, Snounou G. Considerations on the use of nucleic acid-based amplification for malaria parasite detection. Malaria J. 2011; 10:323.
6. Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, do Rosario VE, Thaithong S, Brown KN. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol Biochem Parasitol. 1993; 61:315-320.
7. Johnston SP, Pieniazek NJ, Xayavong MV, Slemenda SB, Wilkins PP, da Silva AJ. PCR as a confirmatory technique for laboratory diagnosis of malaria. J Clin Microbiol. 2006; 44:1087-1089.
8. Yin JH, Yan H, Huang F, Li M, Xiao HH, Zhou SS, Xia ZG. Establishing a China malaria diagnosis reference laboratory network for malaria elimination. Malaria J. 2015; 14:40.
9. Fu Q, Li SZ, Wang Q, Zhang L, Liu W, Zheng X, Zhang SS, Xia ZG, Zhou SS, Chen Z, Wang LY, Zhou XN. Report of analysis of National Technique Competition

for Diagnosis of Parasitic Diseases in 2011--II Analysis of capabilities of *Plasmodium* detection. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi. 2012; 24:274-278. (in Chinese)

10. Ashraf S, Kao A, Hugo C, Christophel EM, Fatunmbi B, Luchavez J, Lilley K, Bell D. Developing standards for malaria microscopy: External competency assessment for malaria microscopists in the Asia-Pacific. Malaria J. 2012; 11:352.

11. Collins WE, Jeffery GM. *Plasmodium ovale*: Parasite and disease. Clin Microbiol Rev. 2005; 18:570-581.

12. Yao LN, Zhang LL, Ruan W, Chen HL, Lu QY, Yang TT. Species identification in 5 imported cases previously diagnosed as Vivax malaria by parasitological and nested PCR techniques. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2013; 31:221-223, 234. (in Chinese)

13. Zhou RM, Zhang HW, Deng Y, Qian D, Liu Y, Chen WQ, Yan QY, Su YP, Zhao XD, Xu BL. Laboratory detection on two cases with imported *Plasmodium ovale* infection. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2013; 31:127-130. (in Chinese)

14. Zhang SS, Xia ZG, Yin JH, Yan H, Zhou SS, Li SZ, Zheng X, Huang F, Li M, Chen HT, Wang Q, Zhang L, Liu W, Xiao N, Zhou XN. Analysis report of the national technique competition for diagnosis of parasitic diseases in 2012: I. Capability analysis of *Plasmodium* detection. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2013; 31:131-134. (in Chinese)