Tropical Medicine and Health Vol. 42 No.4, 2014, 149–153
doi: 10.2149/tmh.2014-07
Copyright© 2014 by The Japanese Society of Tropical Medicine

Original Paper

Performance of Rapid Diagnostic Tests for *Plasmodium ovale* Malaria in Japanese Travellers

Ryutaro Tanizaki[1]*, Yasuyuki Kato[1], Moritoshi Iwagami[2], Satoshi Kutsuna[1], Mugen Ujiie[1], Nozomi Takeshita[1], Kayoko Hayakawa[1], Shuzo Kanagawa[1], Shigeyuki Kano[2] and Norio Ohmagari[1]

Received 2 April, 2014 Accepted 21 August, 2014 Published online 3 September, 2014

Abstract: Background: Rapid diagnostic tests (RDTs) are used widely in the diagnosis of malaria. Although the effectiveness of RDTs for malaria has been described in many previous studies, the low performance of RDT particularly for *Plasmodium ovale* malaria in traveller has rarely been reported.
Methods: This was a retrospective cohort study conducted on Japanese travellers diagnosed with malaria at the National Center for Global Health and Medicine between January 2004 and June 2013. The diagnosis of malaria was confirmed by microscopic examination, RDT, and polymerase chain reaction in all patients. The RDTs used in our study were Binax NOW Malaria (Binax Inc., Scarborough, Maine, USA) (BN) and SD Malaria Antigen Pf/Pan (Standard Diagnostics Inc., Korea) (SDMA). We compared the sensitivity of the RDTs to *P. ovale* malaria and *Plasmodium vivax* malaria.
Results: A total of 153 cases of malaria were observed, 113 of which were found among Japanese travellers. Nine patients with *P. ovale* malaria and 17 patients with *P. vivax* malaria undergoing RDTs were evaluated. The overall sensitivity of RDTs for *P. ovale* malaria and *P. vivax* malaria was 22.2% and 94.1%, respectively ($P < 0.001$). The sensitivity of SDMA for *P. ovale* malaria and *P. vivax* malaria was 50% and 100%, respectively. The sensitivity of BN for *P. vivax* malaria was 90.0%, but it was ineffective in detecting the cases of *P. ovale* malaria.
Conclusions: The sensitivity of RDTs was not high enough to diagnose *P. ovale* malaria in our study. In order not to overlook *P. ovale* malaria, therefore, microscopic examination is indispensable.
Key words: *Plasmodium ovale*, malaria, rapid diagnostic test, Japanese, traveller

Introduction

Malaria is a serious and sometimes fatal disease. Early and accurate detection of malaria infection is important to reduce its associated morbidity and mortality [1]. The gold standard for the detection of malaria infection is microscopic examination, and the most reliable method is genetic examination by polymerase chain reaction (PCR) or deoxyribonucleic acid (DNA) sequencing [2]. Rapid diagnostic tests (RDTs) for malaria are widely used as a substitute for microscopic examination because of their good accessibility. RDTs detect different target antigens such as histidine rich protein 2 (HRP2) [3–5] or *Plasmodium falciparum*-specific lactate dehydrogenase (PfLDH) [5–7] for *Plasmodium falciparum*, *Plasmodium vivax*-specific lactate dehydrogenase (Pv-pLDH) [8], and aldolase [3, 4, 6] or plasmodium lactate dehydrogenase (pLDH) [5, 7, 9, 10] for *P. vivax*, *Plasmodium ovale* and *Plasmodium malariae*.

Although the usefulness of RDTs for *P. falciparum* is well recognized [11, 12], RDTs are often ineffective in detecting other malaria species. Moreover, very few reports have described the performance of RDT in travellers particularly regarding *P. ovale* malaria. For returned travellers in non-endemic settings, it is sometimes difficult to distinguish *P. vivax* malaria from *P. ovale* malaria without PCR because the clinical and microscopic features are very similar. We investigated the performance of RDTs for the detection of *P. ovale* malaria in Japanese travellers by comparing their performance with that of RDTs for *P. vivax* malaria.

[1] Disease Control and Prevention Center, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, JAPAN
[2] Department of Tropical Medicine and Malaria, Research Institute, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, JAPAN
*Corresponding author:
Tel: +81-3-3202-7181
Fax: +81-3-3207-1038
E-mail: k_taro023@yahoo.co.jp

Methods

This study was a retrospective cohort study on Japanese travellers diagnosed with malaria at the National Center for Global Health and Medicine (NCGM) between January 2004 and June 2013. Malaria cases were diagnosed by microscopic examination using initial blood samples at the NCGM Research Institute. Parasite concentration (parasitemia) was calculated by counting the number of parasites per 200,000 red blood cells in a thin blood smear. The RDTs used in our study were Binax NOW Malaria (Binax Inc., Scarborough, Maine, USA) (BN), which detects histidine-rich protein for the identification of falciparum malaria and plasmodium aldolase for the identification of non-falciparum malaria, and SD Malaria Antigen Pf/Pan (Standard Diagnostics Inc., Korea) (SDMA), which detects plasmodium lactic acid dehydrogenase (pLDH) for the identification of all four human malaria parasites. BN could not be obtained in our institute between July 2008 and May 2010, which made it necessary to use SDMA during the period instead of BN. RDTs were performed once for each sample. Concerning the line intensity reading, a test line strong enough to read was deemed positive. Microscopic examination and RDTs were immediately conducted after taking blood tests, and RDTs were conducted in accordance with the manufacturer's instructions.

When parasites were detected microscopically, the species was confirmed by nested PCR. The small subunit rRNA (SSU rRNA) from the nuclear DNA of the four human malaria parasite species were amplified by nested PCR using respective primer sets for *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The nested PCR condition and the species specific primers followed Kimura's method [13]. To detect *P. knowlesi* DNA, the SSU rRNA from nuclear DNA and cytochrome *b* from mitochondrial DNA of *P. knowlesi* were amplified by nested PCR. The sequences of the PCR products were determined using an ABI 3130xl Genetic Analyzer (Applied Biosystems, CA, USA) after TA cloning. *P. knowlesi* specific primer set, the condition of the nested PCR and DNA sequencing were the same as those used in our previous study [14].

Statistical analysis was performed by Stata ver.11 (Stata Corp, Texas, USA). The chi-square and Fisher's exact probability tests were used to compare the characteristics of *P. ovale* malaria and *P. vivax* malaria for dichotomous variables, and the Student's *t*-test was used to compare continuous variables. A difference of $P < 0.05$ was considered significant.

Results

Microscopic examination was performed for diagnosis in all of the malaria cases. A total of 153 cases of malaria were observed between January 2004 and June 2013, 113 of which were found among Japanese travellers. Of the latter, there were 78 cases of *P. falciparum* malaria (including 1 case of co-infection with *P. malariae* malaria and 1 case of co-infection with *P. vivax* malaria confirmed by PCR), 22 cases of *P. vivax* malaria (including 1 case of co-infected with *P. falciparum* malaria confirmed by PCR), 11 cases of *P. ovale* malaria, 3 cases of *P. malariae* malaria, and 1 case of *P. knowlesi* malaria. RDTs were performed in 67, 21, 10, 2, 1 and 2 cases among the mono-infection cases of *P. falciparum* malaria, *P. vivax* malaria, *P. ovale* malaria, *P. malariae* malaria, *P. knowlesi* malaria and co-infection cases, respectively. The cases confirmed by PCR were 11, 19, 10, 3 and 1 cases in *P. falciparum* malaria, *P. vivax* malaria, *P. ovale* malaria, *P. malariae* malaria and *P. knowlesi* malaria, respectively (Table 1).

We focused on a comparison of *P. ovale* malaria mono-infection cases and *P. vivax* malaria mono-infection cases. There were 10 cases of *P. ovale* malaria (mean age, 27.8 years; male-to-female ratio, 2.3:1) and 18 cases of *P. vivax* (mean age, 29.8 years; male-to-female ratio, 2.6:1). These patients constituted the final study population. The results of RDTs were evaluated for 9 patients with *P. ovale* malaria and 17 patients with *P. vivax* malaria (excluding 1 co-infected case).

Among the 18 cases of *P. vivax* malaria, 13 (72.2%) had been infected in Asia, 4 (22.2%) in South America, and 1 (5.6%) in Africa (Republic of Rwanda) (Table 2). All of the 10 cases of *P. ovale* malaria had been infected in Africa (Table 3). The average parasitemia in the cases of *P. ovale* malaria was significantly lower than that in

Table 1. The characteristics of 153 malaria cases from microscopic analysis

		Japanese Travellers	RDTs	PCR
Total	153	113	103	42
Mono-Infections				
P. falciparum		76	67	9
P. vivax		21	21	18
P. ovale		11	10	10
P. malariae		2	2	2
P. knowlesi		1	1	1
Co-Infections				
P. falciparum + *P. malariae*		1	1	1
P. falciparum + *P. vivax*		1	1	1

Table 2. The characteristics of the cases of *P. vivax* malaria

No.	Year	Country	Type of RDTs	Result of RDTs (T1/T2, P.f, Pan)*	Parasitemia (%)	Result of PCR
1	2004	Banuatu	BN	+/+	0.15	Pf, Pv
2	2006	Rwanda	BN	−/+	0.18	Pv
3	2007	Indonesia	BN	−/+	0.20	Pv
4	2007	Indonesia	SDMA	−/+	ND	Pv
5	2007	PNG	BN	−/+	0.20	Pv
6	2007	Malaysia	BN	−/−	0.005	Pv
7	2007	Brasil	BN	−/+	0.79	Pv
8	2008	India	SDMA	−/+	0.06	Pv
9	2008	India	SDMA	−/+	0.40	Pv
10	2009	PNG	SDMA	−/+	0.13	Pv
11	2009	Equador	SDMA	−/+	0.15	Pv
12	2009	Thailanda, India, Nepal, Bangladesh	BN	−/+	0.06	Pv
13	2009	French Guiana	SDMA	−/+	0.34	Pv
14	2010	Sudan	SDMA	−/+	0.22	Pv
15	2010	Brasil	BN	−/+	0.10	Pv
16	2010	Indonesia	BN	−/+	0.09	Pv
17	2010	India	BN	−/+	0.33	Pv
18	2011	PNG	BN	−/+	0.02	Pv

PNG: Papua New Guinea; BN: Binax NOW® malaria; SDMA: SD malaria antigen®; ND: not described; Pf: *Plasmodium falciparum*; Pv: *Plasmodium vivax*.
*T1: the positive band of Histidine rich protein 2 and T2: the positive band of aldolase for BN, P.f: the positive band of *Plasmodiun falciparum* lactate dehydrogenase (LDH) and Pan: the positive band of Plasmodium LDH, for SDMA.

Table 3. The characteristics of the cases of *P. ovale* malaria

No.	Year	Country	Type of RDTs	Result of RDTs (T1/T2, P.f, Pan)*	Parasitemia (%)	PCR
1	2004	Ghana	BN	−/−	0.030	Po
2	2005	Senegal	BN	−/−	0.060	Po
3	2006	Uganda	ND	ND	0.040	Po
4	2007	Uganda	BN	−/−	0.040	Po
5	2008	Uganda	SDMA	−/−	0.001	Po
6	2009	Ghana	SDMA	−/+	0.080	Po
7	2010	Uganda	SDMA	−/+	0.004	Po
8	2010	Ghana	SDMA	−/−	0.012	Po
9	2012	Uganda	BN	−/−	0.042	Po
10	2013	Uganda	BN	−/−	0.003	Po

BN: Binax NOW® malaria; SDMA: SD malaria antigen®; M: male; F: female; ND: not described, Po: *Plasmodium ovale*.
*T1: the positive band of Histidine rich protein 2 and T2: the positive band of aldolase for BN, P.f: the positive band of *Plasmodiun falciparum* lactate dehydrogenase (LDH) and Pan: the positive band of Plasmodium LDH, for SDMA.

P. vivax malaria (P = 0.002). The sensitivity of RDTs for *P. ovale* malaria was also significantly lower than that for *P. vivax* malaria (P < 0.001). The sensitivity of SDMA for *P. vivax* malaria and *P. ovale* malaria was 100% (7/7) and 50% (2/4), respectively. The sensitivity of BN for *P. vivax* malaria was 90.0% (9/10), but it was ineffective in detecting the cases of *P. ovale* malaria (0/5; Table 4). There were only 3 cases of *P. malariae* malaria, one of which showed co-infection with *P. falciparum*. The other 2 cases of *P. malariae* malaria were mono-infection of *P. malariae*. In the mono-infection cases, one case of *P. malariae* malaria showed a positive line detecting pLDH of SDMA while the other case showed a positive line detecting aldolase of BN.

Table 4. The comparison between *P. vivax* malaria cases and *P. ovale* malaria cases

	P. vivax malaria	*P. ovale* malaria	P value
Number of patients	18	10	
Age (mean ± SD)	29.8 ± 12.0	27.8 ± 7.0	0.326
Sex (male)	13	7	0.900
Areas to travel			
Africa	1	10	
Asia, Oceania	13	0	< 0.001
South America	4	0	
Average parasitemia (%)	0.229	0.031	0.002
Positive by RDTs (%)	16/17 (94.1)	2/9 (22.2)	< 0.001
BN (%)	9/10 (90.0)	0/5 (0)	< 0.001
SDMA (%)	7/7 (100)	2/4 (50.0)	0.039

The data are presented as number of patients, unless otherwise specified.
P. vivax: *Plasmodium vivax*; *P. ovale*: *Plasmodium ovale*; RDT: rapid diagnostic test; BN: Binax NOW® malaria; SDMA: SD malaria antigen®

Discussion

RDTs are generally useful for diagnosing malaria infection [3–5]. However, the sensitivity of RDTs depends upon the species of malaria parasite. The highest sensitivity was observed for *P. falciparum* malaria (78.8–99.1%) [5, 11, 15–18], followed by *P. vivax* malaria (77.6–96%), *P. ovale* malaria (18.4–80.0%), and *P. malariae* malaria (21.4–47.0%) [3–5, 19, 20]. For non-falciparum malaria, RDT sensitivity was particularly low for *P. ovale* and *P. malariae*, a finding that may be attributable to the low parasitemia [3–5], the difference in targeted antigens [6, 9, 10], or the genetic variability between the infected parasites [21, 22].

Low parasitemia was associated with false-negative results from the RDTs regardless of malaria species [4, 5]. Moreover, since reinfection and semi-immune status generally cause low parasitemia [23], those influences need to be eliminated. Travellers who visiting families and relatives and expatriates living in malaria endemic areas were excluded from our study because they were at a higher risk of reinfection. The parasitemia in *P. ovale* malaria was consequently as low as that in immune patients and was significantly lower than that in *P. vivax* malaria. This finding, along with those in previous reports [23], suggests that *P. ovale* malaria presents with low parasitemia even in non-immune travellers, which may result in false-negative results from the RDTs.

As mentioned above, *P. malariae* and *P. ovale* malaria may be difficult to diagnose by RDTs due to the low parasitemia [7, 8, 11]. Recently, Houzé et al. [24] reported that the new RDT, Clearview, has improved sensitivity for *P. malariae* malaria but that the sensitivity for *P. ovale* malaria was not improved. Moreover, even a case of *P. ovale* malaria with relatively high parasitemia (21,150 parasites/μL) resulted in a false-negative RDT result in their study, implying some reason for false-negative results other than low parasitemia in *P. ovale* malaria. The difference in target antigens from parasites, such as HRP2 and PfLDH for *P. falciparum* or pLDH and aldolase for pan-malaria species, can also influence the sensitivity of RDTs [6]. Detecting *P. ovale* and *P. vivax*, two RDTs (BN for aldolase detection and SDMA for pLDH detection) were used in our study. SDMA showed relatively good sensitivity for both *P. vivax* and *P. ovale* as compared to BN, probably because pLDH-based RDTs generally perform better than aldolase-based RDTs [20]. Bigaillon et al. [6] also reported the ineffectiveness of BN in detecting *P. ovale* malaria, which they suggested was due to low aldolase production by *P. ovale* malaria [10]. Aldolase-based RDTs generally show low sensitivity not only for *P. ovale* malaria but also for other types of malaria [9], but low sensitivity was also observed for pLDH-based RDTs [10–12, 16, 17, 24].

Although the genetic variability of HRP2 was related to the low sensitivity of RDTs for *P. falciparum* malaria [21], it was reported that the genetic variability of pLDH [22] and aldolase [25, 26] did not explain the relatively poor performance of RDTs for the detection of *P. falciparum*, *P. vivax*, and *P. malariae*. Talman et al. [22] reported that *P. ovale* exhibited three different types of amino acid sequence (named O1, O2 and O3), which may contribute to the relatively poor detection of *P. ovale*. The sensitivity of RDTs is still insufficient for accurate diagnosis of *P. ovale* malaria regardless of the type of antigens. Therefore, microscopic examination is preferable for the definitive diagnosis of *P. ovale* malaria.

The sensitivity of RDTs was not high enough to accurately diagnose *P. ovale* malaria in Japanese travellers who were never infected with malaria previously. Thus, microscopic examination is required to ensure that *P. ovale* malaria is not overlooked.

Conflict of Interests

All the authors declare that they have no conflict of interests.

Acknowledgments

The authors gratefully acknowledge Kanako Komaki-

Yasuda, Kazuhiko Yano, and Shungo Kano at the Department of Tropical Medicine and Malaria, Research Institute, National Center for Global Health and Medicine, Japan, for their technical expertise in microscopy.

This work was partly supported by funding from the Research on Emerging and Re-emerging Infectious Diseases by the Ministry of Health, Labor, and Welfare, Japan (H23-shinkou-ippann-006 and H25-iryougijutsu-shitei-012).

References

1. WHO: Guidelines for the treatment of malaria. 2nd edition. Geneva: World Health Organization; 2010.
2. Kimura M, Miyake H, Kim HS, et al. Species-specific PCR detection of malaria parasites by microtiter plate hybridization: clinical study with malaria patients. J Clin Microbiol 1995; 33: 2342–2346.
3. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev 2002; 15: 66–78.
4. McMorrow ML, Aidoo M, Kachur SP. Malaria rapid diagnostic tests in elimination settings—can they find the last parasite? Clin Microbiol Infect 2011; 17: 1624–1631.
5. Marx A, Pewsner D, Egger M, et al. Meta-analysis: accuracy of rapid tests for malaria in travelers returning from endemic areas. Ann Intern Med 2005; 142: 836–846.
6. Bigaillon C, Fontan E, Cavallo JD, et al. Ineffectiveness of the Binax NOW malaria test for diagnosis of Plasmodium ovale malaria. J Clin Microbiol 2005; 43: 1011.
7. Farcas GA, Zhong KJ, Lovegrove FE, et al. Evaluation of the Binax NOW ICT test versus polymerase chain reaction and microscopy for the detection of malaria in returned travelers. Am J Trop Med Hyg 2003; 69: 589–592.
8. Grobusch MP, Hanscheid T, Zoller T, et al. Rapid immunochromatographic malarial antigen detection unreliable for detecting Plasmodium malariae and Plasmodium ovale. Eur J Clin Microbiol Infect Dis 2002; 21: 818–820.
9. Nkrumah B, Acquah SE, Ibrahim L, et al. Comparative evaluation of two rapid field tests for malaria diagnosis: Partec Rapid Malaria Test® and Binax Now® Malaria Rapid Diagnostic Test. BMC Infect Dis 2011; 11: 143.
10. Mason DP, Kawamoto F, Lin K, et al. A comparison of two rapid field immunochromatographic tests to expert microscopy in the diagnosis of malaria. Acta Trop 2002; 82: 51–59.
11. Maltha J, Gillet P, Bottieau E, et al. Evaluation of a rapid diagnostic test (CareStart Malaria HRP-2/pLDH (Pf/pan) Combo Test) for the diagnosis of malaria in a reference setting. Malar J 2010; 9: 171.
12. Gillet P, van Dijk DP, Bottieau E, et al. Test characteristics of the SD FK80 Plasmodium falciparum/Plasmodium vivax malaria rapid diagnostic test in a non-endemic setting. Malar J 2009; 8: 262.
13. Kimura M, Kaneko O, Liu Q, et al. Identification of the four species of human malaria parasites by nested PCR that targets variant sequences in the small subunit rRNA gene. Parasitol Int 1997; 46: 91–95.
14. Tanizaki R, Ujiie M, Kato Y, et al. First case of *Plasmodium knowlesi* infection in a Japanese traveller returning from Malaysia. Malar J 2013; 12: 128.
15. Maltha J, Gillet P, Jacobs J. Malaria rapid diagnostic tests in endemic settings. Clin Microbiol Infect 2013; 19: 399–407.
16. Van der Palen M, Gillet P, Bottieau E, et al. Test characteristics of two rapid antigen detection tests (SD FK50 and SD FK60) for the diagnosis of malaria in returned travellers. Malar J 2009; 8: 90.
17. Maltha J, Gillet P, Cnops L, et al. Evaluation of the rapid diagnostic test SDFK40 (Pf-pLDH/pan-pLDH) for the diagnosis of malaria in a non-endemic setting. Malar J 2011; 10: 7.
18. Maltha J, Gillet P, Jacobs J. Malaria rapid diagnostic tests in travel medicine. Clin Microbiol Infect 2013; 19: 408–415.
19. Abba K, Deeks JJ, Olliaro P, et al. Rapid diagnostic tests for diagnosing uncomplicated P. falciparum malaria in endemic countries. Cochrane Database Syst Rev 2011: CD008122.
20. Barber BE, William T, Grigg MJ, et al. Evaluation of the sensitivity of a pLDH-based and an aldolase-based rapid diagnostic test for diagnosis of uncomplicated and severe malaria caused by PCR-confirmed Plasmodium knowlesi, Plasmodium falciparum, and Plasmodium vivax. J Clin Microbiol 2013; 51: 1118–1123.
21. Baker J, McCarthy J, Gatton M, et al. Genetic diversity of Plasmodium falciparum histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. J Infect Dis 2005; 192: 870–877.
22. Talman AM, Duval L, Legrand E, et al. Evaluation of the intra- and inter-specific genetic variability of Plasmodium lactate dehydrogenase. Malar J 2007; 6: 140.
23. Collins WE, Jeffery GM. Plasmodium ovale: parasite and disease. Clin Microbiol Rev 2005; 18: 570–581.
24. Houze S, Hubert V, Cohen DP, et al. Evaluation of the Clearview(R) Malaria pLDH Malaria Rapid Diagnostic Test in a non-endemic setting. Malar J 2011; 10: 284.
25. Lee N, Baker J, Bell D, et al. Assessing the genetic diversity of the aldolase genes of Plasmodium falciparum and Plasmodium vivax and its potential effect on performance of aldolase-detecting rapid diagnostic tests. J Clin Microbiol 2006; 44: 4547–4549.
26. Cho CH, Nam MH, Kim JS, et al. Genetic variability in Plasmodium vivax aldolase gene in Korean isolates and the sensitivity of the Binax Now malaria test. Trop Med Int Health 2011; 16: 223–226.