RESEARCH NOTE

PLASMODIUM OVALE INFECTIONS DETECTED BY PCR ASSAY IN LAO PDR

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Abstract. *Plasmodium ovale* infection was demonstrated in 5 out of 143 inhabitants in a village in Lao PDR by blood microscopy and PCR assay. Although the specimen confirmed to be positive for *P. ovale* by microscopical examination was only one, the target sequences in the 18S rRNA genes of malaria parasite detected in all of the five cases were consisted with those of *P. ovale* by the PCR assay. This is the first report concerning the presence of so many cases with *P. ovale* infection in Lao PDR.

Plasmodium ovale malaria is a common disease among humans in tropical Africa but has been known to be a rare infection in Asian regions (Miller and Warrell, 1990; Lysenko and Beljaev, 1969). Although several cases infected with *P. ovale* have been reported in four countries (China, Myanmar, Vietnam, and Thailand) surrounding Lao PDR (Yao and Wu, 1941; Somboon and Sivasomboon, 1983; Gleason *et al*, 1970; Cardigan and Desowitz, 1969), there was only one reported case in Lao PDR until now (Karnasuta *et al*, 1997).

As an antimalaria activity in a rural village (Phavang V) in Khammouane Province, a southeastern province of Lao PDR, an active survey for malaria by microscopical examination and PCR assay was conducted on 143 inhabitants in July, 1997. Microscopical diagnosis for malaria was carried out by routine examination of thick and thin blood smears stained by Giemsa.

PCR assays were also carried out on the same blood specimens as described by Kimura *et al* (1997). After extraction and purification of malaria DNA from blood samples, a small region of the *Plasmodium* 18S rRNA genes was universally amplified by primary PCR, using genus-specific primers for malaria parasites. The first PCR products were further amplified for species diagnosis by the nested PCR with species-specific primers for four human malaria parasites. The nested PCR products were electrophoresed separately and stained with ethidium bromide to see the bands by UV light.

The positive rates resulted from the microscopical examination and the PCR assay are summarized in Table 1. Among the 143 blood specimens, 49 specimens were demonstrated to be positive for malaria parasites by microscopy and as many as 87 specimens were positive by the PCR assay. The PCR assay detected five specimens positive for *P. ovale*; they were two specimens with *P. ovale* single infection and three with mixed infection, as shown in Fig 1. *P. ovale* malaria was identified morphologically in one Giemsa-stained blood smear (Fig 2); infected cells were generally distorted and fringed, trophozoites were compact. The presence of *P. ovale*, however, could not be confirmed microscopically in the remaining four specimens in spite of careful re-examination, probably because of very low parasite density.

The target sequences of all five specimens detected by PCR assay were further analyzed for DNA sequence of *P. ovale* using the genus-specific and the *P. ovale*-specific reverse primers. The sequences of all five specimens were consistent with those of *P. ovale* (Fig 3) ; three of them were found to be normal types while the others were the same variant type found in Vietnam and Thailand (Kimura *et al*, 1997; Kawamoto *et al*, 1996; Zhou *et al*, 1998).

The PCR assay is very sensitive in the detection of malaria parasites, as evidenced by the observation that the method was about 2 times more effective in detecting malaria infection than micros-

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Table 1
Malaria species detected by Giemsa staining and PCR assay among the inhabitants in Phavang village.

Method	No. positive	No. specimens positive for:								
		F	V	O	FV	FM	VO	FVM	FVO	FVMO
Giemsa staining	49	44	2	1	2	0	0	0	0	0
PCR assay	87	54	7	2	19	1	1	1	1	1

F: *P. falciparum*, V: *P. vivax* M: *P. malariae*, O: *P. ovale*

Fig 1–*P. ovale*-positive cases identified by nested PCR. By microscopical examination, No.219 (6y,♂) and 346 (45y,♀) were negative for malaria parasite, No.235 (60y,♂) was positve with *P. ovale*, No.360 (10y,♀) was positive for *P. falciparum*, and No.365 (8y,♀) was positive for both *P. falciparum* and *P. vivax*. F = *P. falciparum*, V = *P. vivax*, M = *P. malariae*, O = *P. ovale*, m= DNA size marker.

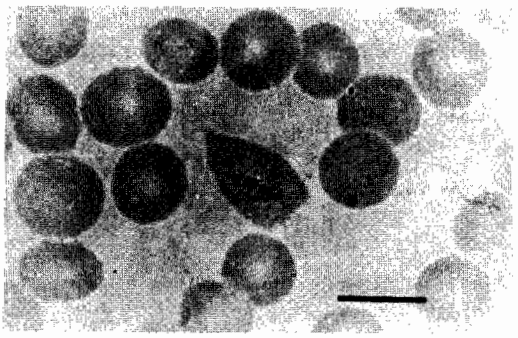

Fig 2–*P. ovale* -infected erythrocyte in Giemsa stained thin blood smear. Fig shows a trophozoite. The infected erythrocyte is distorted in sharp and fringed. A bar = 10 μm.

No. 5′/3′

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219   ACGATCAGATACCGTCGTAATCTTAACCATAAACTATGCCGACTAGGTTTTGGATGAA
235   ..........................................................
346   ..........................................................
360   ..........................................................
365   ..........................................................

219   AGATTTTTAAATAAGAAAATTCCTTTT--GGAAATTTCTTAGATTGCTTCCTTCAGT
235   .A.....................T--.............................
346   .C.....................CGG.............................
360   .G.....................CGG.............................
365   .C.....................CGG.............................
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Fig 3–Partial sequences of the 18S rRNA gene from *P. ovale*-positive cases detected by nested PCR. Sequences are alinged with reference to that of sample No. 219. Nucleotide identities are indicated by dots, and the lack of the corresponding nucleotide is indicated by dashes. The positions of the genus-specific forward (upper) and *P. ovale*-specific reverse (lower) primers are underlined.

copy in the present study. Additionally, the PCR assay is effective for the correct identification of malaria species. The present study reported five cases with *P. ovale* infection in a village, indicating that *P. ovale* infection is not a rare malaria infection in the country.

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