

Detection of *Opisthorchis viverrini* in Human Stool Specimens by PCR

Opisthorchiasis is a liver fluke infection, found mainly in Southeast Asia (8) but increasingly in developed countries due to an influx of Asian immigrants (7). The diagnostic methods are based on the demonstration of eggs in stools, although there are still difficulties in distinguishing eggs from heterophyid and leicthodendriid parasites (2). We have successfully developed a PCR-based method for detection of *Opisthorchis viverrini* in experimentally infected hamsters (6). The aim of the present study was to determine the value of PCR for detection of *O. viverrini* eggs in human feces.

Stool specimens from 85 parasite-infected individuals, as confirmed by the formalin-ether technique, were collected from Srinagarind Hospital, Faculty of Medicine, Khon Kaen University. The specimens were composed of 40 *O. viverrini* parasites, 10 minute intestinal flukes, 10 hookworms, 10 echinostomes, 7 *Taenia* spp. parasites, 3 *Strongyloides stercoralis* parasites, 2 *Entamoeba coli* parasites, and 3 specimens from individuals with mixed infections. To evaluate the sensitivity of the method, normal stool specimens were spiked with either 1, 10, 25, 50, 100, 200, 500, and 1,000 *O. viverrini* eggs or 0.02, 0.2, 2, 20, and 200 pg and 2 ng of genomic DNA, respectively. The sensitivity of the detection for stool specimens was compared with that for Stoll's egg count technique (5).

To extract the DNA from stool specimens, 100 mg of feces in 1 ml of sterile distilled water was extracted with 200 µl of ether and centrifuged at 6,500 × g for 2 min. The pellet was mixed with 500 µl of 0.5 N NaOH and left for 1 h at room temperature. After being autoclaved, the supernatant was collected and invertedly mixed with 250 µl of 3 M sodium acetate and 500 µl of absolute ethanol before being cleaned up with the QIAamp DNA Mini kit (Qiagen, Hilden, Germany), and 10 µl was used in the PCR (6).

The sensitivity test in the spiking experiment demonstrated that the limit of our PCR detection is 200 eggs or 200 pg of genomic DNA. The expected 330-bp product and its doublet of 660 bp were in 32 of the 40 fecal specimens (80.0%) from patients with *O. viverrini* (Table 1). Compared with that of Stoll's egg count, the sensitivity of the PCR was found to be

100, 68.2, and 50% in the fecal specimens containing >1,000, 200 to 1,000, and <200 eggs per g of feces, respectively. Our method gave one false positive (97.8% specificity) in the specimen containing eggs of echinostomes (Table 1). The positive and negative predictive values are 96.9 and 84.6%, respectively.

Our PCR method is sensitive and highly specific, with no amplification occurring when samples contained small intestinal flukes or other parasitic eggs. It was more sensitive than the previous method using a DNA probe as a diagnostic tool (3). The detection limit of 200 eggs can be assumed to be produced by one adult fluke, since an adult worm can release 50 to 200 eggs per g of feces (1, 4). The sensitivity value of PCR is somewhat lower than those of the formalin-ether and Stoll egg count techniques. This may be caused by the use of low amounts of stool sample (0.1 g for PCR and 2 and 1 g for formalin-ether and Stoll's egg count, respectively). However, mild infection (200 to 1,000 eggs per g of feces) could be easily missed by Stoll's dilution technique (4), whereas our technique gave a 68.2% positive rate. The false-positive result occurring for a specimen containing eggs of echinostomes may not have been an actual positive result, since the specimen was the only one positive among the 10 stool samples containing echinostomes.

Thus, the results in this study suggest that our PCR method is a suitable tool for detection of *O. viverrini* infection, especially in a large number of samples at one time. With some improvement in sample preparation, it will be very useful for sensitive and specific diagnosis and epidemiological studies and offers a potential means for detection of the parasite in the intermediate hosts.

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TABLE 1. Results of PCR and Stoll's egg count compared to formalin-ether technique

Diagnosis for stool specimen (n) ^a	No. of specimens with indicated PCR result by egg count group ^b					
	Positive			Negative		
	<200	200–1,000	>1,000	<200	200–1,000	>1,000
<i>O. viverrini</i> (40)	1	15	16	1	7	0
Other parasitic infections (45)	0	3 ^c	0	28	8	6

^a Diagnosis was performed based on the formalin-ether technique.

^b Egg count was performed by Stoll's technique and expressed as eggs per gram of feces.

^c One specimen was diagnosed as an echinostome, and the others were diagnosed as mixed infections with *Taenia* spp. and *O. viverrini*.

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