

Loop-Mediated Isothermal Amplification Method for Differentiation and Rapid Detection of *Taenia* Species[∇]

Agathe Nkouawa,^{1,3†} Yasuhito Sako,^{1†*} Minoru Nakao,¹ Kazuhiro Nakaya,² and Akira Ito^{1†}

Department of Parasitology¹ and Animal Laboratory for Medical Research,² Asahikawa Medical College, Midorigaoka Higashi 2-1-1-1, Asahikawa, 078-8510 Hokkaido, Japan, and Medical Research Center, Institute of Medical Research and Medicinal Plants Studies (IMPM), Ministry of Scientific Research and Innovation, Yaoundé, Cameroon³

Received 13 August 2008/Returned for modification 14 October 2008/Accepted 6 November 2008

Rapid detection and differentiation of *Taenia* species are required for the control and prevention of taeniasis and cysticercosis in areas where these diseases are endemic. Because of the lower sensitivity and specificity of the conventional diagnosis based on microscopical examination, molecular tools are more reliable for differential diagnosis of these diseases. In this study, we developed and evaluated a loop-mediated isothermal amplification (LAMP) assay for differential diagnosis of infections with *Taenia* species with cathepsin L-like cysteine peptidase (clp) and cytochrome *c* oxidase subunit 1 (cox1) genes. LAMP with primer sets to the cox1 gene could differentiate between three species, and LAMP with primer sets to the clp gene could differentiate *Taenia solium* from *Taenia saginata*/*Taenia asiatica*. Restriction enzyme digestion of the LAMP products from primer set Tsag-clp allowed the differentiation of *Taenia saginata* from *Taenia asiatica*. We demonstrated the high specificity of LAMP by testing known parasite DNA samples extracted from proglottids ($n = 100$) and cysticerci ($n = 68$). LAMP could detect one copy of the target gene or five eggs of *T. asiatica* and *T. saginata* per gram of feces, showing sensitivity similar to that of PCR methods. Furthermore, LAMP could detect parasite DNA in all taeniid egg-positive fecal samples ($n = 6$). Due to the rapid, simple, specific, and sensitive detection of *Taenia* species, the LAMP assays are valuable tools which might be easily applicable for the control and prevention of taeniasis and cysticercosis in countries where these diseases are endemic.

Cestode parasites *Taenia solium*, *Taenia saginata*, and *Taenia asiatica* are the causative agents of taeniasis. Although taeniasis is relatively innocuous, cysticercosis caused by *T. solium* larvae is one of the most severe diseases in humans and remains a complicated health problem in many areas around the world, especially in developing countries (4, 15). Therefore, differentiation of *Taenia* species becomes significant for epidemiological studies and for control of these diseases. Furthermore, it is expected that there are much wider areas in the Asia-Pacific region where the three taeniid species occur sympatrically (3, 5, 23, 24). Diagnosis is mainly performed by microscopic observation of eggs in feces and/or by comparative morphology of proglottids or scolices, but these methods lack both sensitivity and specificity. In order to overcome the lower sensitivity of microscopic diagnosis, various immunological or molecular approaches, including coproantigen and copro-DNA detection methods, have been developed (2, 6, 12, 18, 25). The coproantigen detection method has been shown to be more sensitive than the microscopy method, but it cannot differentiate between *Taenia* species because of it is genus specific, not species specific (2). By contrast, various copro-DNA detection methods using PCR have been developed for sensitive differential detection of taeniid cestodes (6, 12, 18, 25). Although these

techniques provide sensitive and reliable diagnostic results, it is not easy to exploit in the laboratories of developing countries where these diseases are endemic, because PCR requires sophisticated equipment, such as a thermal cycler. Furthermore, *Taq* DNA polymerase is often inactivated by inhibitors present in biological samples, which sometimes cause problems for sensitivity and reproducibility (1, 13).

Recently, a novel nucleic acid amplification method termed loop-mediated isothermal amplification (LAMP) has been developed (17). LAMP employs a DNA polymerase with strand displacement activity and four primers that recognize six sequences on the target DNA. This method amplifies DNA with high specificity, sensitivity, and rapidity under isothermal conditions. Since LAMP is done under isothermal conditions (60 to 65°C), simple incubators, such as a water bath or a block heater, are sufficient for DNA amplification (17). Moreover, a large amount of white precipitate of magnesium pyrophosphate is produced as a byproduct, which enables the visual judgment of amplification by a naked eye (14). Hence, LAMP is a highly sensitive and specific DNA amplification tool suitable for the rapid diagnosis of infectious diseases, including parasitic diseases (9, 20, 22), in a well-equipped laboratory and/or small-scale clinical laboratories and is expected to be highly useful and feasible in the field.

In the present study, we developed and evaluated the LAMP assay with a cathepsin L-like cysteine peptidase (clp) gene of nuclear DNA and a cytochrome *c* oxidase subunit 1 (cox1) gene of mitochondrial DNA for differentiation between and rapid diagnosis of infection with *Taenia* species.

* Corresponding author. Mailing address: Department of Parasitology, Asahikawa Medical College, Midorigaoka Higashi 2-1-1-1, Asahikawa 078-8510, Hokkaido, Japan. Phone: 81-166-68-2422. Fax: 81-166-68-2429. E-mail: yasusako@asahikawa-med.ac.jp.

† These authors contributed equally to this work.

∇ Published ahead of print on 12 November 2008.

TABLE 1. *Taenia* species DNA samples used in this study

<i>Taenia</i> species (no. of samples)	Locality	Developmental stage (no. of samples analyzed)
<i>T. saginata</i> (78)	Cambodia	Proglottid (3)
		China
	Indonesia	Cysticercus (1)
		Cysticercus (12) ^a
	Nepal	Proglottid (2)
	Thailand	Proglottid (9)
	Cameroon	Proglottid (1)
	Ethiopia	Proglottid (2)
	Brazil	Cysticercus (2)
		Proglottid (1)
	Ecuador	Proglottid (1)
	Belgium	Proglottid (2)
		Cysticercus (1) ^a
<i>T. asiatica</i> (43)	China	Cysticercus (1) ^a
		Proglottid (10)
	Indonesia	Cysticercus (2)
	Korea	Cysticercus (2) ^a
	Taiwan	Cysticercus (1) ^a
		Proglottid (1)
	Thailand	Proglottid (6)
Philippines	Proglottid (4)	
<i>T. solium</i> (47)	China	Cysticercus (2)
		Proglottid (11)
	India	Cysticercus (11)
	Indonesia	Proglottid (1)
	Nepal	Cysticercus (5)
	Thailand	Proglottid (5)
	Vietnam	Cysticercus (1)
	Cameroon	Cysticercus (3)
	Mozambique	Cysticercus (2)
	South Africa	Cysticercus (1)
	Tanzania	Cysticercus (1)
	Brazil	Cysticercus (1)
	Ecuador	Cysticercus (2)
Mexico	Cysticercus (1)	

^a The cysticercus was developed in NOD/shi-*scid* mice.

MATERIALS AND METHODS

Parasite materials. Cysticerci of *T. solium*, *T. saginata*, or *T. asiatica* were obtained from nonobese diabetic/severe combined immunodeficiency (NOD/shi-*scid*) mice (7, 16) infected by intraperitoneal cavity injection with oncospheres prepared from gravid proglottids of each parasite isolated in Thailand. Genomic DNA was extracted from one cysticercus by using a DNeasy tissue kit (Qiagen, Hilden, Germany).

DNA samples. For evaluation of the LAMP assay, a total of 168 DNA samples extracted from proglottids ($n = 100$) and cysticerci ($n = 68$), including 47 *T. solium* samples, 78 *T. saginata* samples, and 43 *T. asiatica* samples, were examined (Table 1). We used the stored DNA samples previously analyzed by multiplex PCR (25).

Cloning and sequencing of *clp* genes. The *clp* gene of each *Taenia* parasite was cloned by PCR with forward primer 5'-ACATTTTCGTTTCGATCGGTCAT G-3' and reverse primer 5'-TGAACACATGGTTTAAACGTATGG-3'. Primers used were designed from conserved nucleotide sequences between *Echinococcus multilocularis* (21) and *T. solium* (10) *clp* genes to amplify almost the entire gene region. PCR was carried out using high-fidelity polymerase PrimeStar (Takara, Kyoto, Japan) in a final volume of 25 μ l reaction mixture containing 0.2 μ M of each primer, 200 μ M each of deoxynucleoside triphosphate (dNTP), 0.625 units of PrimeStar DNA polymerase, and genomic DNA, as described above. Amplification was performed with 35 cycles of 94°C for 30 s, 60°C for 15 s, and 72°C for 4 min, followed by a final extension at 72°C for 10 min. A single band of approximately 3 kbp was excised from agarose gels by using a NucleoSpin ExTract kit (Macherey-Nagel, Düren, Germany) according to the

instruction manual and was cloned into a pGEM-T vector (Promega, Madison, WI) after the addition of adenine to the ends of the PCR products. The plasmid clone was sequenced on an ABI Prism 310 sequencer (Applied Biosystems, Foster City, CA) with BigDye Terminator version 1.1 (AB Applied Biosystems) and was used as a standard plasmid for determining the specificity and sensitivity of LAMP.

Preparation of standard plasmids for *cox1* genes. The *cox1* gene of each *Taenia* parasite was amplified by PCR using forward primer 5'-ATGAATGTC AAATATTTGT-TAAGTT-3' and reverse primer 5'-CTAAAAGACCATTTT ACACGCGAAT-3' for *T. solium*, forward primer 5'-ATGAGTGTTAAATTT TTATTAAGTT-3' and reverse primer 5'-TTAAACTAAAAAACACGGGC AGGC-3' for *T. saginata*, and forward primer 5'-ATGAGTGTTAAATTTT TTAAGTT-3' and reverse primer 5'-TTAAACTAAAAAACACGAGCAAA C-3' for *T. asiatica*. PCR was carried out as described above, except the annealing temperature was at 58°C and the elongation time was 90 s. The amplified products of each *Taenia* species were cloned into a pGEM-T vector and used as a standard plasmid after being confirmed by sequencing.

LAMP primers. LAMP primers were designed using PrimerExplorer V4 software (<http://primerexplorer.jp/>). The following four oligonucleotide primers were specifically designed to amplify six distinct regions on the target gene: forward inner primer (FIP), backward inner primer (BIP), and two outer primers, F3 and B3 (Table 2). FIP consists of the sense sequence of F2 at the 3' end and the F1c region at the 5' end that is complementary to the F1 region. BIP consists of a B2 region at the 3' end that is complementary to the B2c region and the same sequence as the B1c region at the 5' end (Fig. 1A and 2A).

LAMP reaction. LAMP was carried out in a 25- μ l volume of reaction mixture containing 40 pmol each of FIP and BIP, 5 pmol each of F3 and B3, an 8-U large fragment of *Bst* DNA polymerase (New England Biolabs, Beverly, MA), 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 8 mM MgSO₄, 10 mM (NH₄)₂SO₄, 0.1% Tween 20, 0.8 M betaine (Sigma, St. Louis, MO), and 1.4 mM each of dNTP and the template DNA. The reaction mixture was incubated for 60 min at 63°C for the *clp* gene and at 60°C for the *cox1* gene and heated at 80°C for 5 min. The LAMP products from primer set Tsag-*clp* were digested with the *Hinf*I restriction enzyme (New England Biolabs) for 2 h at 37°C. The LAMP products and restriction enzyme-digested products were electrophoresed on a 2.0% agarose gel and detected by staining with ethidium bromide.

Sensitivity analyses. The sensitivities of the LAMP and PCR methods were assessed using a standard plasmid diluted from 10⁴ copies/reaction to 1. LAMP was performed as previously mentioned. PCR was carried out by using F3 and B3 primers of each LAMP primer set. PCRs were performed in a 25- μ l volume of reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 0.2 μ M of each primer, 0.2 mM each of dNTP, diluted standard plasmid, and 0.5 units of *Taq* DNA polymerase (ExTaq; Takara), and cycling conditions were 30 s at 94°C (first cycle, 2 min at 94°C), 30 s at 60°C (*clp* gene) and 58°C (*cox1* gene), and 30 s at 72°C for 30 cycles. The PCR products were electrophoresed on a 2.0% agarose gel and detected by staining with ethidium bromide.

DNA extraction from fecal samples. In order to evaluate the detection limit of taeniid DNA in fecal samples, 1 gram of feces from a noninfected volunteer was mixed with 5, 10, 20, 30, 40, or 50 *T. asiatica* or *T. saginata* eggs, and DNA was extracted by using a QIAamp DNA stool mini kit (Qiagen) after egg disruption treatment with glass beads (19). In addition, taeniid egg-positive or -negative fecal samples obtained in Indonesia and Thailand were used to assess LAMP, and DNA samples were extracted by the same procedure. LAMP was performed as previously described except that the reaction was performed for 90 min.

Nucleotide sequence accession numbers. The nucleotide sequence data of the *clp* genes cloned in this study are available in the GenBank, EMBL, and DDBJ databases under accession numbers AB441815 (*T. solium*), AB441816 (*T. saginata*), and AB441817 (*T. asiatica*).

RESULTS AND DISCUSSION

The standard plasmid of each gene was constructed to facilitate initial evaluation and optimization of LAMP. The LAMP products are detected as a ladder of multiple bands on the gel due to the formation of a mixture of stem-loop DNAs with various stem lengths and cauliflower-like structures, with multiple loops formed by annealing between alternately inverted repeats of the target sequence in the same strand (17). Application of the LAMP assay with each specific primer set to each

TABLE 2. LAMP primer sets

Species	Primer set	Primer type ^a	Sequence (5' to 3')	Target
<i>T. saginata</i>	Tsag-cox1	F3	TCGGCAAATATTTAATTCCTTTG	cox1 gene
		B3	AAATTCTAGACGCACCCG	
		FIP	GCCATCGAAGGAATCAATAACCAATCTGATTGAATTTACCCCG	
		BIP	GACTTTTTATCCGCCTTTGTCTGCAATGCAACGAAAACATCAAGA	
<i>T. asiatica</i>	Tasi-cox1	F3	GATTTTCTTTTTTTTGGATGCCCA	
		B3	TTGAAAATAATGACGACGACA	
		FIP	AGGCAAGTTTAAATCAGATAACCCATTTTGATAGGTGGTTTTGGTAA	
		BIP	GTGGTTGTTGATTCTTCAATAGTTTAAGGCGGATAAAAAAGTTCAC	
<i>T. solium</i>	Tsol-cox1	F3	CCTATTTTAATTGGAGGTTTTGG	
		B3	CTACCCCACTTCTCTTGA	
		FIP	CAACCATGCACTTAAAGCATTCAAATTCATTGATAAGAGGATTATCGG	
		BIP	GGATGTGTTTAGGCGCTGGTACAACGAAGATGATAAAGGTG	
<i>T. saginata</i>	Tsag-clp	F3	GGAAGTCAAAAATCAGGTGAG	clp gene
		B3	CGCTGATAGCTAGGCTAAC	
<i>T. asiatica</i>		FIP	CTCAGTCCCACCAATCCATTTCAAATCTTCATTATGCTGCGTTAC	
		BIP	GCACCGTGTCATTGGTAAATTTGGTGGAGCTTACTAAGCTCTATCG	
<i>T. solium</i>	Tsol-clp	F3	GAGGTCAAAAATCAGGTGAGAT	
		B3	AATGCTCCTGACTTGGTT	
		FIP	AGGTGCTTTCACAATAGTCCCCTGCGTCATAGGTCTTGC	
		BIP	TAGTCGTTGCTTCGATAGAGCTCGCTGATATCTAGGCTAATGCTG	

^a FIP consists of the sense sequence of F2 at the 3' end and the F1c region at the 5' end that is complementary to the F1 region. BIP consists of a B2 region at the 3' end that is complementary to the B2c region and the same sequence as the B1c region at the 5' end.

Taenia cox1 gene resulted in successful amplification of the target gene from the respective parasite genomic DNA (Fig. 1B). On the other hand, because of high nucleotide sequence similarity between the clp genes of *T. saginata* and *T. asiatica*, we could not design a specific primer set to differentiate between these two *Taenia* species but could design specific primer sets Tsol-clp and Tsag-clp to differentiate *T. solium* from *T. saginata* and *T. asiatica* and differentiate *T. saginata*/*T. asiatica* from *T. solium*, respectively. However, the recognition site GAGTC for the restriction enzyme *Hinf*I in the amplified region of the *T. asiatica* clp gene (Fig. 2A) enables us to differentiate *T. asiatica* from *T. saginata*. In this case, restriction enzyme digestion of the LAMP products from *T. asiatica* genomic DNA with primer set Tsag-clp produces three bands with the predicted sizes of 179, 217, and 255 bp, because, unlike the PCR products, the LAMP products are characteristic structures with inverted repeats of the target sequence. In fact, three bands that agreed with the predicted size in the restriction enzyme digestion of the LAMP products from *T. asiatica* genomic DNA with primer set Tsag-clp were detected (Fig. 2B, lane 6).

Analytical specificity of the LAMP assays. In order to evaluate the specificity of the LAMP assays, the known parasite DNA samples prepared from proglottids and cysticerci were examined. Because LAMP requires four primers that recognize six different sequences on a target sequence, the target sequence specificity of the LAMP reaction appears to be high. Indeed, LAMP with primer sets to the cox1 or clp gene specifically amplified each respective target gene, with a species-specific detection. The results obtained by LAMP with cox1 primer sets were consistent with those obtained by multiplex PCR with the cox1 gene, whereas two DNA sam-

ples extracted from proglottids, which were identified as being *T. saginata* by both LAMP and multiplex PCR with cox1 primer sets, were detected as *T. asiatica* by LAMP with clp primer sets (Table 3). The size of bands produced by restriction enzyme digestion indicated that these two LAMP products were specifically derived from the *T. asiatica* clp gene (data not shown). After cloning of the clp gene from these two DNA samples by PCR with primer set Tsag-clp F3 and B3 and sequencing, we found a single nucleotide substitution, T to C, as indicated in Fig. 2A, which leads to the appearance of the *Hinf*I recognition site in the region between B1c and B2 and is identical to the nucleotide substitution in *T. asiatica*. Thus, the nucleotide substitution in the clp gene of these two *T. saginata* samples may lead to the different result from the diagnosis based on the cox1 gene. Alternatively, the possibility that these two DNA samples were obtained from a hybrid parasite having *T. asiatica* nuclear DNA and *T. saginata* mitochondrial DNA could not be ruled out, because samples of these parasite materials were collected from areas where both parasites exist sympatrically in China and Thailand, respectively, and these materials were identified by only mitochondrial DNA (3, 8). Exactly two tapeworms obtained in Thailand where three human *Taenia* species were confirmed to be sympatrically occurring (3) are concluded to be the hybrids of *T. saginata* and *T. asiatica* (M. Okamoto, M. Nakao, M. T. Anantaphruti, J. Waikagul, and A. Ito, unpublished data). More-detailed characterizations of these two DNA samples must be performed using another nuclear DNA marker, in addition to analyses of longer nucleotide sequences of the clp genes. These results demonstrated that the LAMP methods with each primer set designed in this study can specifically

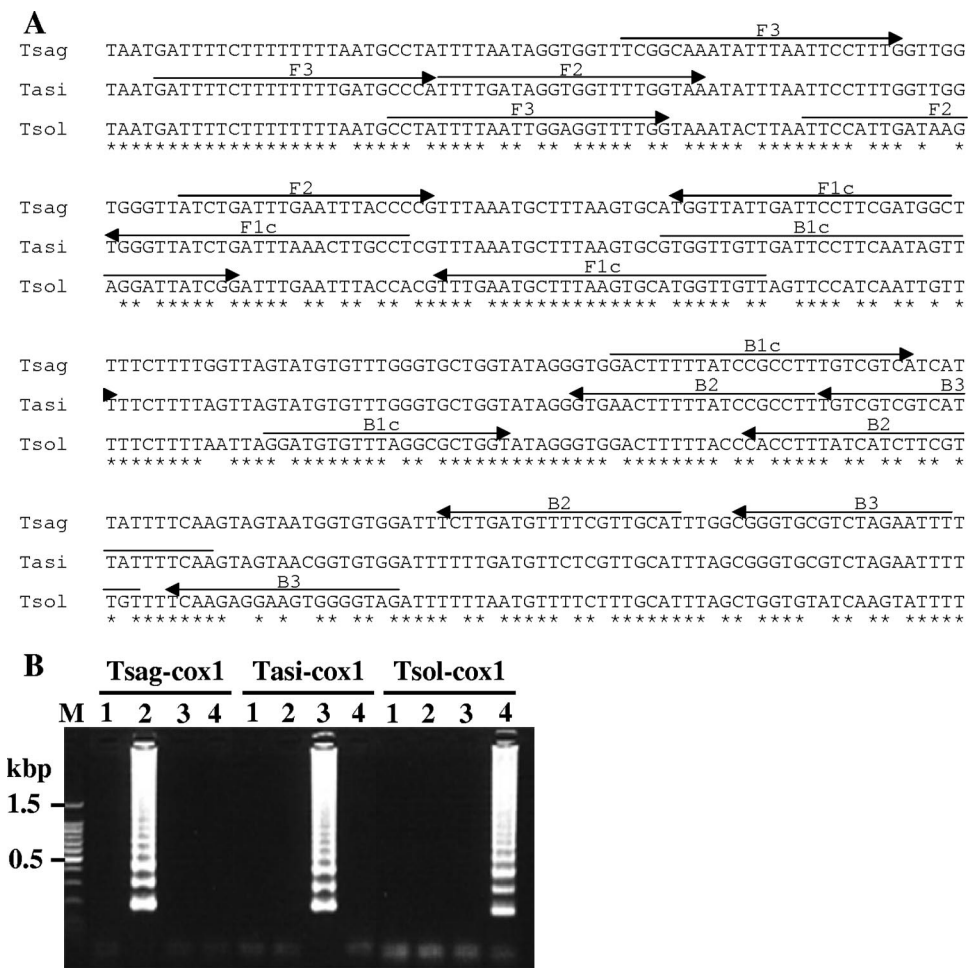


FIG. 1. Nucleotide sequence alignment of the target region of *cox1* genes (A) and LAMP results (B). (A) The locations of the primer recognition sites are indicated by arrows. (B) The LAMP products were run on a 2% agarose gel. Lane M, 100-bp DNA ladder marker (Promega); lane 1, negative control; lane 2, *T. saginata* genomic DNA; lane 3, *T. asiatica* genomic DNA; lane 4, *T. solium* genomic DNA; Tsag-cox1, results of LAMP with primer set Tsag-cox1; Tasi-cox1, results of LAMP with primer set Tasi-cox1; Tsol-cox1, results of LAMP with primer set Tsol-cox1.

amplify the target gene and are applicable to a differential diagnosis of infections with *Taenia* species.

Analytical sensitivity of the LAMP assay. Tenfold serial dilution of each standard plasmid was used to determine the lower detection limits for LAMP and PCR, and the sensitivities of the two methods were compared. In this study, F3 and B3 primers of each LAMP primer set were utilized for PCR. Figure 3A shows results of LAMP and PCR with primer set Tsol-clp. Both LAMP and PCR detected up to one copy of target gene/reaction, which indicated no difference in sensitivity between the two methods. LAMP and PCR with other primer sets provided the same results as LAMP with primer set Tsol-clp (data not shown).

Next, the detection limit of taeniid eggs in feces was evaluated using *T. asiatica* and *T. saginata* eggs (Fig. 3B). At least five eggs per gram of feces was sufficient for taeniid eggs to be detected by LAMP with primer sets Tasi-cox1 and Tsag-cox1, whereas more than 10 eggs/g of feces was needed to be detected by LAMP with primer set Tsag-clp (data not shown). The differences between the *cox1* gene and the *clp* gene in the detection sensitivity may be responsible for the

number of copies of each target gene within the samples, since a large number of mitochondrial DNA exists in a cell, one feature to be selected as a target DNA for detection. Several detection methods for *Taenia* species in feces based on PCR techniques have been reported. The multiplex PCR method with mitochondrial DNA (25), the PCR-restriction fragment length polymorphism method with mitochondrial DNA (18), and the nested-PCR method with the *Tso31* gene encoding the *T. solium* oncosphere-specific protein (12) have been reported to show detection limits of 5 eggs/g of feces, 17 eggs/g of feces, and 40 eggs/g of feces, respectively. It seems that more eggs are needed for detection when the nuclear gene is chosen as a diagnostic gene marker, although the amplification efficiency of each PCR method varies. In this study, *T. solium* eggs were not available, but a similar sensitivity might be expected because all LAMP primer sets could amplify one copy of the target gene when using pure plasmid DNA samples.

Differential detection of *Taenia* species in fecal samples. Furthermore, in order to assess the LAMP method for the detection of copro-DNA, we investigated fecal samples that

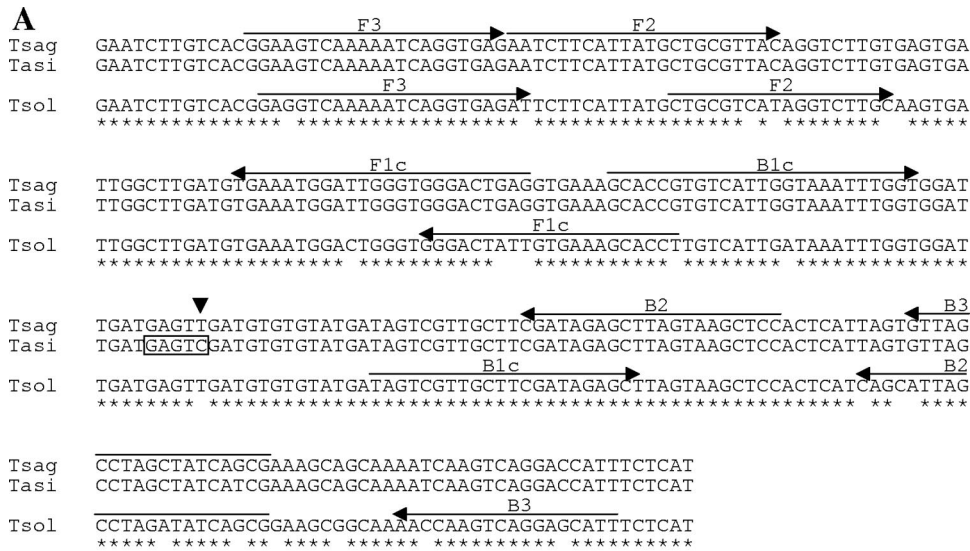


FIG. 2. Nucleotide sequence alignment of the target region of *clp* genes (A) and LAMP results (B). (A) The locations of the primer recognition sites are indicated by arrows and the restriction enzyme *Hinf*I recognition site in the LAMP products of *T. asiatica* *clp* gene is boxed. The single nucleotide substitution, C to T, in the *T. saginata* sequence is indicated by an arrowhead. (B) The LAMP products and *Hinf*I digestion products were run on a 2% agarose gel. Lane M, 100-bp DNA ladder marker (Promega); lane 1, negative control; lane 2, *T. saginata* genomic DNA; lane 3, *T. asiatica* genomic DNA; lane 4, *T. solium* genomic DNA; lane 5, *Hinf*I digestion of LAMP products from *T. saginata* genomic DNA with primer set Tsag-clp; lane 6, *Hinf*I digestion of LAMP products from *T. asiatica* genomic DNA with primer set Tsag-clp; Tsag-clp, results of LAMP with primer set Tsag-clp; D, results of *Hinf*I digestion; Tsol-clp, results of LAMP with primer set Tsol-clp. The DNA fragments generated after digestion with *Hinf*I are indicated by arrowheads.

were positive ($n = 6$) or negative ($n = 10$) for taeniid eggs by microscopy and collected in Indonesia and Thailand (Fig. 4). Out of six taeniid egg-positive fecal samples, three specimens from Indonesia tested positive with primer sets Tsag-cox1 (Fig. 4A, lanes 15 to 17) and Tsag-clp (data not shown), and the remaining specimens from Thailand were positive with primer sets Tasi-cox1 (Fig. 4B, lanes 12 to 14) and Tsag-clp (data not shown). No differences in detection be-

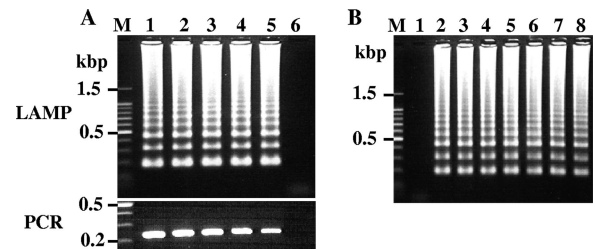


FIG. 3. (A) Comparison of detection sensitivities of LAMP and PCR with Tsol-clp primer set. The standard plasmids were serially diluted from 10^4 copies per reaction to 1 copy per reaction and amplified by LAMP (upper panel) and PCR (lower panel). The F3 and B3 primers of the Tsol-clp primer set were used in the PCR. The LAMP reactions were carried out for 60 min. Lane M, 100-bp DNA ladder marker (Promega); lanes 1 to 5 represent 10^4 , 10^3 , 10^2 , 10, and 1 copy(ies)/reaction, respectively; lane 6, negative control. (B) Detection limits of target genes by LAMP with Tasi-cox1 primer set against DNA samples prepared from feces containing various numbers of *T. asiatica* eggs. The LAMP reactions were carried out for 90 min. Lane M, 100-bp DNA ladder marker; lane 1, negative control; lane 2, *T. asiatica* genomic DNA as a positive control; lanes 3 to 8 represent 5, 10, 20, 30, 40, and 50 *T. asiatica* eggs/g of feces, respectively.

TABLE 3. Analytical specificity of the LAMP assays^a

<i>Taenia</i> species (no. of samples)	No. of samples (%) detected by LAMP with indicated primer set	
	cox1	clp
<i>T. saginata</i> (78)	78 (100)	76 (97.4) ^b
<i>T. asiatica</i> (43)	43 (100)	43 (100)
<i>T. solium</i> (47)	47 (100)	47 (100)

^a *Taenia* species were confirmed by multiplex PCR with *cox1* genes (25).

^b Two samples were detected as *T. asiatica* by LAMP.

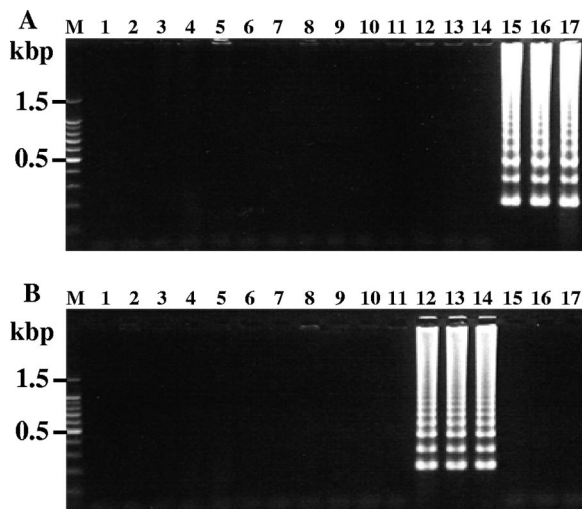


FIG. 4. Differential detection of *Taenia* species in fecal samples. Each genomic DNA extracted from taeniid egg-negative fecal samples collected in Indonesia (lanes 2 to 11) and taeniid egg-positive fecal samples collected in Thailand (lanes 12 to 14) and Indonesia (lanes 15 to 17) was examined by LAMP. The LAMP reactions were carried out for 90 min. The LAMP results with primer sets Tsag-cox1 (A) and Tasi-cox1 (B) are shown. Lane M, 100-bp DNA ladder marker (Promega); lane 1, negative control.

tween nuclear DNA and mitochondrial DNA were observed. The results obtained by LAMP were consistent with those of multiplex PCR. No positive results were observed with the taeniid egg-negative samples.

Our results demonstrated that the LAMP method has high sensitivity and specificity for differential detection of *Taenia* species. Compared to PCR, LAMP has the advantages of reaction simplicity and cost-effectiveness. LAMP does not need sophisticated and expensive equipments, and a simple water bath or a heat block is sufficient to furnish a constant temperature for reactions requiring only 1 to 2 h. Another useful feature of LAMP is that a white precipitate of magnesium pyrophosphate leading to turbidity of reaction mixtures as a byproduct of gene amplification makes it easy to distinguish positive samples from negative samples. In fact, we could discriminate between positive and negative samples by a naked eye in all cases (data not shown). Although limited numbers of clinical specimens were analyzed in the present study, it has been shown that the LAMP method has the potential for use in the differential diagnoses of infections with *Taenia* species that could be easily applicable in countries where taeniasis/cysticercosis are endemic for better control and prevention of these diseases.

ACKNOWLEDGMENTS

Fecal and/or parasite samples used for this study were obtained through joint projects on epidemiological studies of taeniasis/cysticercosis in Indonesia, Thailand, and China (3, 11, 24). We sincerely thank T. Wandra, J. Waikagul, and T. Y. Li for their great help with these samplings.

This work was supported by the leadership in science and technology in Asia from the Ministry of Education, Culture, Sports, Science and Technology, Japan; International Collaboration Research Fund from the Japan Society for the Promotion of Science

(JSPS) (17256002); and JSPS-Asia/Africa Science Platform Fund to A.I.

REFERENCES

1. Abu Al-Soud, W., and P. Rådström. 2000. Effects of amplification facilitators on diagnostic PCR in the presence of blood, feces, and meat. *J. Clin. Microbiol.* **38**:4463–4470.
2. Allan, J. C., G. Avila, J. Garcia-Noval, A. Flisser, and P. S. Craig. 1990. Immunodiagnosis of taeniasis by coproantigen detection. *Parasitology* **101**: 473–477.
3. Anantaphruti, M. T., H. Yamasaki, M. Nakao, J. Waikagul, D. Watthanakulpanich, S. Nuamtanong, W. Maipanich, S. Pubampen, S. Sanguankiat, C. Muennoo, K. Nakaya, M. O. Sato, Y. Sako, M. Okamoto, and A. Ito. 2007. Sympatric occurrence of *Taenia solium*, *T. saginata*, and *T. asiatica*, Thailand. *Emerg. Infect. Dis.* **13**:1413–1416.
4. Bern, C., H. H. Garcia, C. Evans, A. E. Gonzalez, M. Verastegui, V. C. W. Tsang, and R. H. Gilman. 1996. Magnitude of the disease burden from neurocysticercosis in a developing country. *Curr. Infect. Dis.* **29**:1203–1209.
5. Eom, K. S., H. K. Jeon, Y. Kong, U. W. Hwang, Y. Yang, X. Li, L. Xu, Z. Feng, Z. S. Pawlowski, and H. J. Rim. 2002. Identification of *Taenia asiatica* in China: molecular, morphological, and epidemiological analysis of a Luzhai isolate. *J. Parasitol.* **88**:758–764.
6. González, L. M., E. Montero, L. J. S. Harrison, R. M. E. Parkhouse, and T. Garate. 2000. Differential diagnosis of *Taenia saginata* and *Taenia solium* infection by PCR. *J. Clin. Microbiol.* **38**:737–744.
7. Ito, A., K. Nakaya, Y. Sako, M. Nakao, and M. Ito. 2001. NOD-*scid* mouse as an experimental animal model for cysticercosis. *Southeast Asian J. Trop. Med. Public Health* **32**:85–89.
8. Ito, A., M. Nakao, T. Wandra, T. Suroso, M. Okamoto, H. Yamasaki, Y. Sako, and K. Nakaya. 2005. Taeniasis and cysticercosis in Asia and the Pacific: present state of knowledge and perspectives. *Southeast Asian J. Trop. Med. Public Health* **36**:123–130.
9. Kuboki, N., N. Inoue, T. Sakurai, F. D. Cello, D. J. Grab, H. Suzuki, C. Sugimoto, and I. Igarashi. 2003. Loop-mediated isothermal amplification for detection of African trypanosomes. *J. Clin. Microbiol.* **41**:5517–5524.
10. Li, A. H., S. U. Moon, Y. K. Park, B. K. Na, M. G. Hwang, C. M. Oh, S. H. Cho, Y. Kong, T. S. Kim, and P. R. Chung. 2006. Identification and characterization of a cathepsin L-like cysteine protease from *Taenia solium* metacystode. *Vet. Parasitol.* **141**:251–259.
11. Li, T., P. S. Craig, A. Ito, X. Chen, D. Qiu, J. Qiu, M. O. Sato, T. Wandra, H. Bradshaw, L. Li, Y. Yang, and Q. Wang. 2006. Taeniasis/cysticercosis in a Tibetan population in Sichuan province, China. *Acta Trop.* **100**:223–231.
12. Mayta, H., R. H. Gilman, E. Prendergast, J. P. Castillo, Y. O. Tinoco, H. H. Garcia, A. E. Gonzalez, and C. R. Sterling. 2008. Nested PCR for the specific diagnosis of *Taenia solium* taeniasis. *J. Clin. Microbiol.* **46**:286–289.
13. Monteiro, L., D. Bonnemaïson, A. Vekris, K. G. Petry, J. Bonnet, R. Vidal, J. Cabrita, and F. Mégraud. 1997. Complex polysaccharides as PCR inhibitors in feces: *Helicobacter pylori* model. *J. Clin. Microbiol.* **35**:995–998.
14. Mori, Y., K. Nagamine, N. Tomita, and T. Notomi. 2001. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochem. Biophys. Res. Commun.* **289**:150–154.
15. Murell, K. D. 2005. Epidemiology of taeniasis and cysticercosis, p. 32–44. In K. D. Murell (ed.), WHO/FAO/OIE guidelines for the surveillance, prevention and control of taeniasis/cysticercosis. OIE, Paris, France.
16. Nakaya, K., W. Mamuti, N. Xiao, M. O. Sato, T. Wandra, M. Nakao, Y. Sako, H. Yamasaki, Y. Ishikawa, P. S. Craig, P. M. Schantz, and A. Ito. 2006. Usefulness of severe combined immunodeficiency (*scid*) and inbred mice for studies of cysticercosis and echinococcosis. *Parasitol. Int.* **55**(Suppl.):S91–S97.
17. Notomi, T., H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, and T. Hase. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **28**:e63.
18. Nunes, C. M., A. K. K. Dias, F. E. F. Dias, S. M. Aoki, H. B. D. Paula, L. G. F. Lima, and J. F. Garcia. 2005. *Taenia saginata*: differential diagnosis of human taeniasis by polymerase chain reaction-restriction fragment length polymorphism assay. *Exp. Parasitol.* **110**:412–415.
19. Nunes, C. M., L. G. F. Lima, C. S. Manoel, R. N. Pereira, M. M. Nakano, and J. F. Garcia. 2006. Technical report: fecal specimens preparation methods for PCR diagnosis of human taeniasis. *Rev. Inst. Med. Trop. Sao Paulo* **48**:45–47.
20. Poon, L. L. M., B. W. Y. Wong, E. H. T. Ma, K. H. Chan, L. M. C. Chow, W. Abeyewickreme, N. Tangpukdee, K. Y. Yuen, Y. Guan, S. Looareesuwan, and J. S. M. Peiris. 2006. Sensitive and inexpensive molecular test for falciparum malaria: detecting *Plasmodium falciparum* DNA directly from heat-treated blood by loop-mediated isothermal amplification. *Clin. Chem.* **52**:303–306.
21. Sako, Y., H. Yamasaki, K. Nakaya, M. Nakao, and A. Ito. 2007. Cloning and characterization of cathepsin L-like peptidases of *Echinococcus multilocularis* metacystodes. *Mol. Biochem. Parasitol.* **154**:181–189.
22. Savan, R., T. Kono, T. Itami, and M. Sakai. 2005. Loop-mediated isothermal

- amplification: an emerging technology for detection of fish and shellfish pathogens. *J. Fish Dis.* **28**:573–581.
23. **Sudewi, A. A., T. Wandra, A. Artha, A. Nkouawa, and A. Ito.** 2008. *Taenia solium* cysticercosis in Bali, Indonesia: serology and mtDNA analysis. *Trans. R. Soc. Trop. Med. Hyg.* **102**:96–98.
24. **Wandra, T., P. Sutisna, N. S. Dharmawan, S. S. Margono, R. Sudewi, T. Suroso, P. S. Craig, and A. Ito.** 2006. High prevalence of *Taenia saginata* taeniasis and status of *Taenia solium* cysticercosis in Bali, Indonesia, 2002–2004. *Trans. R. Soc. Trop. Med. Hyg.* **100**:346–353.
25. **Yamasaki, H., J. C. Allan, M. O. Sato, M. Nakao, Y. Sako, K. Nakaya, D. Qiu, W. Mamuti, P. S. Craig, and A. Ito.** 2004. DNA differential diagnosis of taeniasis and cysticercosis by multiplex PCR. *J. Clin. Microbiol.* **42**:548–553.