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COMPARISON OF CONVENTIONAL TECHNIQUES TO DIFFERENTIATE BETWEEN *TAENIA SOLIUM* AND *TAENIA SAGINATA* AND AN IMPROVED POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM ASSAY USING A MITOCHONDRIAL 12S rDNA FRAGMENT

R. Rodriguez-Hidalgo, D. Geysen*, W. Benítez-Ortiz, S. Geerts†, and J. Brandt†

Laboratorio de Inmunodiagnostico e Investigación, Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito, Ecuador *e-mail: dgeysen@itg.be*

ABSTRACT: Given the constraints of classical diagnostic methods, i.e., morphological and isoenzymatic studies of proglottids, a polymerase chain reaction test complemented with restriction enzyme analysis has been modified by redesigning one of the primers to reduce nonspecific amplifications experienced when using field samples. The use of these new, highly cestode-specific primers and the restriction enzyme *Dde*I led to the development of a diagnostic assay that clearly distinguishes between *Taenia saginata* and *T. solium* proglottids in field samples. This assay confirms the presence of *T. saginata* in Ecuador. DNA amplification of some of these taeniids showed different patterns, suggesting the possibility that strain differences exist. These results demonstrate the need for development of useful molecular assays as reliable tools for epidemiological studies on cestodes.

Humans are the final hosts of *Taenia saginata* and *T. solium*. Usually, gravid proglottids of *T. saginata* leave the intestine actively, whereas those of *T. solium* are passively eliminated with the feces. Schantz (1999) reported a yearly economic loss of about 164 million dollars in Latin America because of the condemnation of the carcasses of pigs and cows, intermediate hosts for these parasites. In addition, *Cysticercus cellulosae*, metacestodes of *T. solium*, can also occur in humans, often leading to neurocysticercosis, a major cause of epilepsy associated with considerable morbidity and mortality (Cruz et al., 1989, 1999). According to Welte (1995), *T. solium* is endemic in most of Latin America, except for Paraguay and Uruguay, whereas *T. saginata* has not been reported from Ecuador or Colombia. Several methods have been described to differentiate between proglottids of the 2 taeniid species. In addition to the absence (*T. saginata*) or presence (*T. solium*) of rostellar hooks on the scolex, Morgan and Hawkins (1949) described a differential method based on the number of uterine branches in gravid segments. They reported that *T. solium* had between 8 and 14 unilateral uterine branches, whereas *T. saginata* had 15–24 branches. But several authors found overlapping numbers, which impeded proper specification (Gemmell et al., 1983; Deluol, 1989; Salfelder et al., 1992; Mayta et al., 2000). Furthermore, mature segments of *T. solium* possess a third accessory ovarian lobe and no vaginal sphincter (Verster, 1969). Le Riche and Sewell (1978) differentiated taeniid cestodes by isoenzyme electrophoresis based on glucose phosphate isomerase (GPI: EC 5.3.1.9.), using either fresh or frozen somata. Kocher et al. (1989) reported that the mitochondrial 12s rDNA is well conserved among *Echinococcus multilocularis*, *T. taeniaeformis*, and *T. martis*, giving a polymerase chain reaction (PCR) fragment of about 440 bp when using cestode-specific primers. González et al. (2000) developed a diagnostic technique based on multiplex PCR with species-specific primers from noncoding DNA fragments, i.e., HDP1 and HDP2 for *T. saginata* and *T. solium*, respectively. Mayta et al. (2000) reported a PCR assay, targeted at the 5.8S ribosomal gene sequence, with primers annealing to the 3′-end of the 18S and 5′-end of the 28S ribosomal regions, respectively. A phylogenetic comparison of 13 taeniid species, based on a 12s rDNA fragment of 300 bp, was carried out by Nickisch-Rosenegk et al. (1999). They reported 50% sequence homology and 30% sequence conservation among the species. This degree of variability was considered useful for interspecies comparisons. These reports formed the basis for the development of a diagnostic tool to differentiate between proglottids of *T. saginata* and those of *T. solium* because all methods were found unsatisfactory when used on field samples on account of the presence of nonspecific, possibly human, amplicons. The development of a diagnostic tool based on these findings is reported herein.

MATERIALS AND METHODS

Parasites

Proglottids and oncospheres were obtained from 25 patients from different regions of Ecuador (highlands: Quito, Imbabura, and Carchi; coastal region: Manabí). These patients were positive by coprology analysis, and parasite material was collected after treatment. In addition, the following reference samples were used: *T. saginata* from a Senegalese patient and *C. bovis* from Ecuador, collected during the present survey. In the absence of adult reference somata of *T. solium*, metacestodes, collected from pigs from Cameroon and from Ecuador, and a specimen from a patient from India were used (Table I). Parasites were identified by GPI isoenzyme electrophoresis (Le Riche and Sewell, 1978) and morphological criteria, i.e., the number of uterine branches in gravid segments, the presence of an accessory ovarian lobe and a vaginal sphincter in mature segments after staining (Morgan and Hawkins, 1949), and the presence of rostellar hooks on the scolices.

Molecular protocols

DNA extraction protocol (slightly modified from Boom et al., 1990) was based on guanidinium (Gu–HCl: 6.0 M Gu–HCl, pH 7.5, and 25 mM ethylenediamine–tetraacetic acid [EDTA]; Life Technologies, Merelbeke, Belgium), followed by complexing on a suspension of diatomaceous earth (Sigma-Aldrich, Bornem, Belgium). The lysis buffer consisted of 60 mM Tris–HCl, pH 7.4, 60 mM EDTA, 10% Tween, 5 mM $MgCl_2$, 1% Triton X-100, and 1.6 M Gu–HCl concentrated 2 times; 250 μl of lysis buffer plus 250 μl of Milli-Q water (Millipore, Brussels, Belgium) and 50 μl of proteinase K (20 mg/ml, Roche Diagnostics, Brussels, Belgium) was added to 3 μl of each sample, i.e., *C. cellulosae* or *Taenia* spp. somata. Samples were incubated in a Thermomixer compact (Eppendorf, Koln, Germany) overnight at 60 C and shaken at 1,400

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* To whom correspondence should be addressed. Prince Leopold Institute of Tropical Medicine, Department of Animal Health, Nationalestraat 155, B-2000 Antwerp, Belgium.

† Prince Leopold Institute of Tropical Medicine, Department of Animal Health, Nationalestraat 155, B-2000 Antwerp, Belgium.

TABLE I. Origin of samples used.

Code	Origin	Date	Host	Code	Origin	Date	Host
E1	Quito/E	17 May 2000	Human	E19	Imbabura/E	27 March 2001	Human
E2	Imbabura/E	14 May 2000	Human	E20	Imbabura/E	27 March 2001	Human
E3	Imbabura/E	18 May 2000	Human	E21	Quito/E	27 March 2001	Human
E4	Quito/E	30 March 2000	Human	E22	Quito/E	27 March 2001	Human
E5	Quito/E	11 April 2000	Human	E23	Quito/E	08 December 1998	Human
E7	Quito/E	17 May 2000	Human	E24	Quito/E	07 April 1999	Human
E10	Imbabura/E	26 June 2000	Human	E25	Quito/E	21 April 1999	Human
E11	Imbabura/E	19 October 2000	Human	E26	Quito/E	30 June 1999	Human
E12	Imbabura/E	16 October 2000	Human	E27	Quito/E	26 October 1999	Human
E13	Manabí/E	14 February 2001	Human	E28	Quito/E	26 April 2001	Human
E14	Imbabura/E	15 February 2001	Human			References samples	
E15	Imbabura/E	20 February 2001	Human	T.sag*	Senegal	16 March 1993	Human
E16	Imbabura/E	15 March 2001	Human	CcE	Ecuador	08 July 1983	Pig
E17	Carchi/E	15 March 2001	Human	CcC	Cameroon	06 December 1985	Pig
E18	Quito/E	15 March 2001	Human	CcH	India	15 December 1981	Human
				Ccb	Ecuador	11 February 2001	Bovine

* T.sag = *T. saginata*; E = Ecuador; Cc = Metacestode of *T. solium*; Cb = Metacestode of *T. saginata; H = human; C = Cameroon.*

rpm. Then, 40 µl of diatomaceous earth suspension was added, mixed vigorously for 2 sec, and incubated for 1 hr at 37 C. The suspension was centrifuged for 20 sec at 12,000 *g* and the pellet resuspended in 900 µl 70% ethanol (v/v) at 4 C. This was centrifuged again for 20 sec and the supernatant discarded. This process was repeated once in ethanol and again in 900 µl acetone. Finally, the pellet was dried in a Thermoblock Dri-bath (Barnstead/Thermolyne, Dubuque, Iowa) at 50 C for 20 min; then, 90 µl 10 mM Tris plus 1 mM EDTA, pH 8, was added, followed by incubation for 20 min at 60 C while shaking at 1,000 rpm in the Thermomixer. After centrifugation, 50 µl of the supernatant was transferred to a new Eppendorf tube. This was kept at 4 C or stored at −20 C until use for PCR amplification.

Polymerase chain reaction: PCR was performed in a total volume of 25 µl containing 5 µl of *Taenia* sp. DNA as template and 20 µl of PCR mix containing 4.3 µl of Milli-Q water; 12.5 µl buffer (20 mM Tris–HCl, pH 8.4, 100 mM KCl, 0.2% Triton X-100, 3.3 mM $MgCl_2$); 2 µl of a mix of the 4 deoxynucleotide triphosphates (dNTP, final concentration 0.2 mM); 0.4 µl of each primer and 0.4 U of *Taq*-polymerase Silverstar, 50 U/µl (Eurogentec, Seraing, Belgium). The amplification was performed in a PHC-3 Thermal cycler (Techne, Cambridge, U.K.) with an initial denaturation step at 94 C for 4 min, followed by 40 cycles consisting of 94 C for 1 min, 58 C for 1.5 min, and 72 C for 2 min. Five microliters of the amplified products together with a Gene Ruler™ (MBI Fermentas, GmbH, St.Leon-Rot, Germany) marker of 100 bp was separated by electrophoresis, using a Mupid-21 system (Eurogentec) in 2% agarose w/v and 0.04 M Tris-acetate plus 0.002 M EDTA buffer for 20 min at 100 V. The gel was stained with ethidium bromide (Sigma-Aldrich) for 30 min and the DNA products visualized using ultraviolet light.

Primers: The primers T60F and T375R, described by Nickisch-Rosenegk et al. (1999), and the primers TM12SR and ITMTR2, developed during this study, were used to amplify a region from the 12s rDNA gene (Fig. 1). Primers were designed with the aid of the following programs: Amplify (W. R. Engels, 1993) and Right Primer, version M1.25 (R. Isaac, Biodesk, 1994). The BLAST program (GenBank, http://www.ncbi.nlm.gov/) was used to check the specificity of the primers against all DNA sequences present in GenBank.

DNA band purification: When a nonspecific band was observed, the 300-bp band was purified by cutting the DNA segment out of the gel using the Wizard® DNA Clean-up kit (Promega, Leiden, The Netherlands) following the manufacturer's protocol.

Restriction fragment length polymorphism: Restriction fragment length polymorphism (RFLP) digestion was performed according to the manufacturer's specifications (Life Technologies) using 10 U *Dde*I/µg DNA, plus 6 µl of amplified DNA in a total volume of 15 µl. Tubes containing the reaction mixture were incubated for 4 hr at 37 C. Six microliters of the digested product was mixed with 2 µl of the loading buffer and transferred onto a 10% polyacrylamide gel. A marker of 100 bp was included for size identification of the bands. DNA was separated by a Mighty Small horizontal electrophoresis apparatus (Amercham Pharmacia Biotech, Roosendaal, The Netherlands) in TBE (88 mM Tris, 89 mM boric acid, 2 mM EDTA).

The gel was stained using a commercial kit, Plus one (Amercham Pharmacia Biotech), and preserved under plastic foil.

RESULTS

Totally 25 specimens were analyzed. Primers T60F and T375R amplified a fragment of about 360 bp of the mitochondrial 12s rDNA gene and a second, nonspecific, 650-bp fragment. The latter was present in most of the analyzed taeniid specimens. The sizes of PCR fragments with these primers are presented in Figure 1. The optimal temperature for annealing was determined to be 58 C using a Robocycler gradient 40 (Stratagene, Amsterdam, The Netherlands).

Figure 2 shows the alignment of the fragment described by Nickisch-Rosenegk et al. (1999) and Nakao et al. (2000), falling within the 440-bp mitochondrial fragment for cestodes as described by Kocher et al. (1989). On the other hand, primer T375R is part of Nakao's fragment (Nakao et al., 2000). A comparison of the T375R primer sequence as described by Kocher at al. (1989) and Nakao et al. (2000) revealed a nucleotide difference in the third and 27th bp.

A new primer, ITM12SR (derived from T375R), was synthesized with the following sequence: 5′-AATCGAGGGTGACGGGCGGTGTGTACA-3′. The new combination of primers (T60F and ITM12SR) yields the same results as the original combination (T60F and T375R) retaining the second fragment, which is probably nonspecific for all taeniids (results not shown).

When unpurified amplicons are used for RFLP, a very complex pattern emerges. Although it is possible to distinguish the taeniid species by their patterns (not shown), the process is unsatisfactory for routine use. Therefore, an investigation was undertaken to determine whether it would be possible to redesign one or both of the PCR primers to eliminate the nonspecific amplicons. A new primer, ITMTR2 (5′-TGACGGGCGGTG

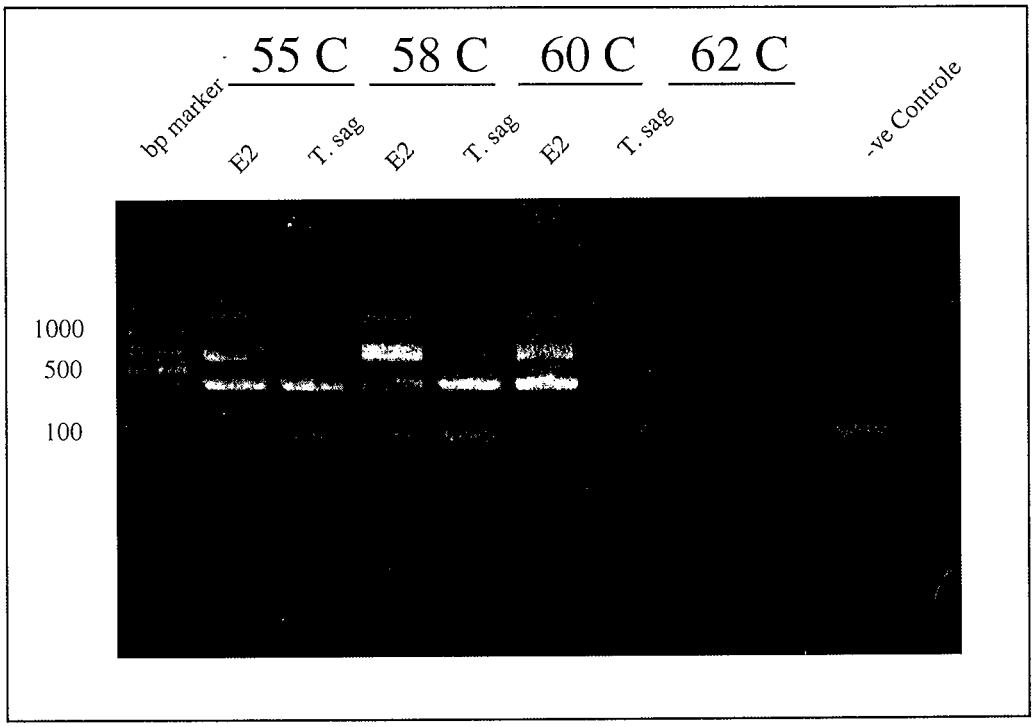

FIGURE 1. Two samples (E2 is field sample E2 and T. sag is *Taenia saginata*) were used to determine the optimal temperature for annealing using the T60F and T375R primer pair. At 58 C, DNA fragments were clearly observed. Sample E2 shows 2 fragments; the larger is probably nonspecific. Analysis is performed on a 2% agarose gel and staining is with ethidium bromide. The marker is a 100-bp DNA ladder.

TCTACATGAGTTA-3′), was developed using part of the ITM12SR primer sequence. Analysis of this primer by the GenBank program BLAST did not reveal homology to sequences of human or intestinal parasite origin. The amplification result of the mitochondrial 12s rDNA segment gave a clean single fragment of 360 bp (Fig. 3).

The RFLP results (Fig. 4) of this amplicon showed a clear arrangement of the fragments without confounding bands caused by the presence of nonspecific segments. Nine samples (E3, E11, E12, E14, E16, E17, E18, E20, and CcE) corresponded to the profile of *T. solium*, whereas the other 17 specimens plus the reference samples showed a profile typical of *T. saginata*. Minor differences in the low-intensity band profiles around the 200-bp mark were seen in samples E21 and E19 when compared with samples E13 and E15 (Fig. 4).

These results corresponded entirely with the GPI zymograms of the 25 specimens and with all the morphological criteria for all but specimen E18. GPI patterns showed the proximal band of *T. saginata* extracts in a more anodal position than that of *T. solium* extracts, as described by Le Riche and Sewell (1978). As presented in Table II, sample E18 showed all the characteristics of *T. solium*, except for having a *T. saginata*–like unarmed scolex and an active migration (observation of at least 1 active migration of a gravid segment through the anal sphincter). Oncospheres of this tapeworm proved to be infective for a pig, yielding cysticerci with armed scolices. The GPI pattern of E18 corresponded with *T. solium*. As such, of the 25 specimens analyzed, 17 were identified as *T. saginata* and 8 samples as *T. solium* on the basis of their overall morphology, GPI-zymograms, and PCR.

DISCUSSION

In phylogenetic studies on several cestode species, Nickisch-Rosenegk et al. (1999) used universal primers T60F and T375R to analyze a 12s rDNA segment. These authors reported sequence differences between these 2 species but did not aim at a practical application for differentiating at species level. In the present study, the same primers with the same PCR protocol were used, and a differential diagnosis between the 2 taeniids could be made through RFLP. Amplification of this segment with T60F and T375R produced a fragment of about 360 bp, together with a weaker nonspecific band of about 650 bp (Fig. 1). A BLAST search of the primers ITM12SR and T375R

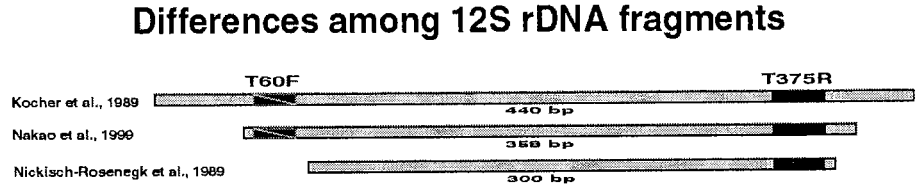

FIGURE 2. Sizes of the mitochondrial 12S rDNA fragments, with the location of the T60F and T375R primers.

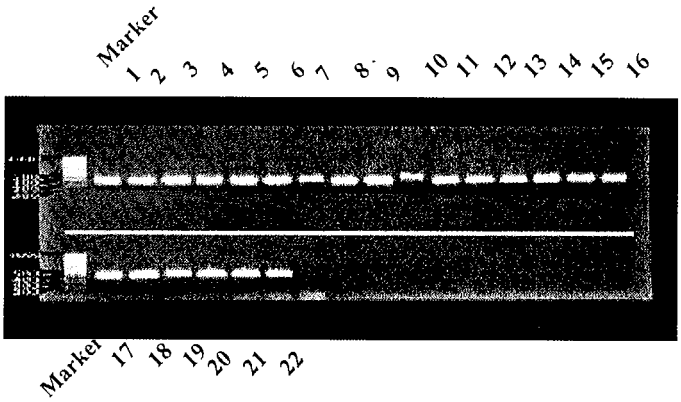

FIGURE 3. PCR results corresponding to the different field samples (E1–E22) as described in Table I. Amplification of the mitochondrial 12s rDNA segment from taeniids, using primers T60F and ITMTR2. Analysis is performed on a 2% agarose gel and stained with ethidium bromide. The marker is a 100-bp DNA ladder.

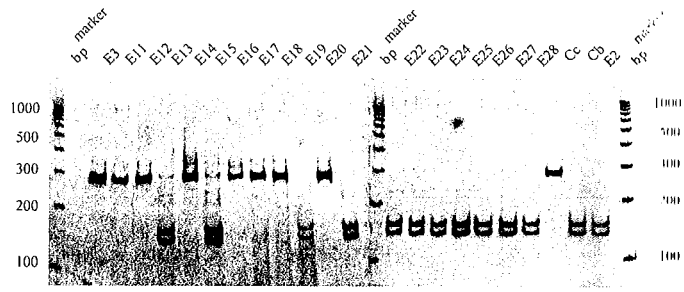

FIGURE 4. Restriction results corresponding to the different field samples (E3; E11–E21 and E2, E22–E28 and CcE and ECb) as described in Table I. *Dde*I enzymatic restriction of the 12s rDNA segment amplified with the primers T60F and ITMTR2. High single bands (±300 bp) correspond with *Taenia saginata* profiles, whereas the low double bands (±150 bp) are *T. solium* profiles. Nonspecific bands are absent. Analysis is performed on a 10% polyacrylamide gel, and staining is with a Silver Kit. The marker is a 100-bp DNA ladder.

against all sequences in the GenBank database revealed the possibility of amplification of taeniid mitochondrial fragments and DNA from human mitochondria. Because adults of *Taenia* spp. live in the human intestine, host DNA contamination is a real possibility and could explain the nonspecific amplification. Subsequent BLAST searches permitted the design of the primer ITMTR2, which has no homology to human DNA or any other mitochondrial DNA except that of taeniid mitochondrial origin. The new primer pair, T60F–ITMTR2, amplified a 360 bp fragment without the occurrence of the nonspecific band (Fig. 3). When analyzed using RFLP with *Dde*I digestion, the pattern of fragments obtained was identical to that predicted by using GeneJockey II. PCR results correlated well with the morphological characteristics. One of the samples (E18) had morphological characteristics of both taeniids, i.e., an unarmed scolex but a typical *T. solium* reproductive system. But the PCR result, GPI isoenzyme pattern, and the experimental infection of a pig

TABLE II. Speciation of 25 specimens of *Taenia* spp. based on morphological features, i.e., absence or presence of rostellar hooks, maximum number of unilateral uterine branches for putative *T. solium*, minimum number for putative *T. saginata*, number of ovarian lobes and presence, or absence, of a vaginal sphincter, and mode of migration of gravid segments.

Specification	Scolex	Uterine branches	Ovarian lobes	Vaginal sphincter	Migration	Code
T. saginata	NO*	22	NO	Present	Active	E1
T. saginata	NO	22	NO	NO	Active	E2
T. saginata	NO	19	NO	NO	Active	E4
T. saginata	NO	18	NO	NO	Active	E5
T. saginata	NO	18	NO	NO	Active	E7
T. saginata	NO	17	NO	Present	Active	E10
T. saginata	NO	17	2	Present	Active	E13
T. saginata	NO	18	NO	NO	Active	E15
T. saginata	NO	17	2	Present	Active	E19
T. saginata	Unarmed	17	2	NO	Active	E21
T. saginata	Unarmed	17	NO	NO	Active	E22
T. saginata	NO	17	NO	NO	Active	E23
T. saginata	NO	18	NO	NO	Active	E24
T. saginata	NO	17	NO	NO	Active	E25
T. saginata	NO	16	NO	NO	Active	E26
T. saginata	NO	16	NO	NO	Active	E27
T. saginata	NO	17	NO	NO	Active	E28
T. sol/T. sag	Unarmed	10	NO	Absent	Active	E18
T. solium	NO	9	NO	NO	Passive	E3
T. solium	NO	9	NO	NO	Passive	E11
T. solium	NO	10	NO	NO	Passive	E12
T. solium	Armed	9	3	Absent	Passive	E14
T. solium	NO	8	3	NO	Passive	E16
T. solium	NO	9	3	NO	Passive	E17
T. solium	Armed	10	2	Absent	Passive	E20

* NO = No observation possible (disintegration of material or scolices not recuperated).

were all conclusive for *T. solium*. Apparent size differences in the amplicons from samples E16 (*T. solium*) and E19 (*T. saginata*) and minor profile characteristics in *T. saginata* samples E21 and E19 could be because of strain differences. This possibility requires a considerable amount of further investigation and will be explored in future studies. The specificity of the primers described in this study enabled easy differentiation of adult *Taenia* spp. This test is faster, less complex, and easier to interpret than the test described by González et al. (2000), which is a method based on 3 primers in a multiplex PCR. On the other hand, Mayta et al. (2000), using 3 restriction enzymes (*Alu*I, *Dde*I, and *Mbo*I), could clearly distinguish *T. solium* from *T. saginata*. These authors used primers to amplify a region of the 5.8s rDNA, corresponding to a fragment of approximately 1,300 bp. In the present study, the 5.8s rDNA segment was also amplified, using the same protocol and primers as Mayta et al. (2000). But the lack of specificity of the primers, the low sensitivity of the test (about 80% of the samples showed positive amplification), the complexity of fragments in the RFLP, and the size of the segment amplified are the main disadvantages of the study of Mayta et al. (2000) in comparison with the method proposed in the present study.

In conclusion, the present work contradicts the general assumption that *T. saginata* is no longer present in Ecuador. In spite of being a notifiable disease in Ecuador, there are no official reports of bovine cysticercosis except for the documented evidence by Briones (1969). Consequently, because of the major importance of *T. solium*, all cases of taeniasis are assumed to be caused by the latter. The improved PCR-RFLP assay, amplifying a fragment of the mitochondrial 12s rDNA, clearly differentiated between *T. solium* and *T. saginata* and will be able to confirm the presence of strains or new species of *Taenia* in Ecuador or Latin America in future studies. This is of major importance in epidemiological studies of human cysticercosis and will be invaluable in evaluating possible zoonotic implications. Differentiation on the basis of morphological criteria may be the least expensive method but is hindered by difficulties in the acquisition of suitable somata for clear diagnosis. As shown in Table II, the difficulty of recovering mature segments and scolices or the advanced disintegration did not often allow proper identification. As for the gravid proglottids, the number of uterine branches in *T. saginata* and *T. solium* seems not to overlap in Ecuador, a conclusion which should be further substantiated by a higher number of samples. But relying on a single morphological feature, e.g., the observation of a *Taenia* sp. without rostellar hooks, may lead to a false conclusion. This is evidenced by the fact that the carrier of E18, in spite of the *T. saginata*–like scolex, was nevertheless subjected to cysticercosis screening, a routine procedure for *T. solium*–carriers, resulting in a positive CT-scan for neurocysticercosis.

PCR-based differentiation is not difficult and has a high sensitivity and specificity. But the high technological demands, i.e., expensive infrastructure and the price per sample, are obstacles preventing the general use of this method.

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LITERATURE CITED

BOOM, R., C. J. A. SOL, M. M. M. SALIMANS, C. L. JANSEL, P. M. E. WERTHEIM-VAN DILLEN, AND J. VAN DER NOORDAA. 1990. Rapid and simple method for purification of nucleic acids. Journal of Clinical Microbiology **28:** 495–503.

BRIONES, G. M. 1969. Investigación de *Cysticercus bovis*, en el ganado vacuno sacrificado en el matadero de la ciudad de Portoviejo. Tesis de Doctor en Medicina Veterinaria y Zootecnia. Facultad de Medicina Veterinaria y Zootecnia. Universidad Técnica de Manabi, Ecuador, 144 p.

CRUZ, M., A. DAVIS, H. DIXON, Z. S. PAWLOWSKI, AND J. PROAÑO. 1989. Operational studies on the control of *Taenia solium* taeniasis cysticercosis in Ecuador. Bulletin of World Health Organization **67:** 401–407.

———, P. M. SCHANTZ, I. CRUZ, P. ESPINOSA, P. M. PREUX, A. CRUZ, W. BENITEZ, V. C. W. TSANG, J. FERMOSO, AND M. DUMAS. 1999. Epilepsy and neurocysticercosis in an Andean community. International Journal of Epidemiology **28:** 799–803.

DELUOL, A. M. 1989. Atlas de parasitologie. Tome III-Helminthes. Editions Varia, Paris, France, 99 p.

ENGELS, W. R. 1993. Contributing software to the internet: the *amplify* program. Trends in Biochem Sciences **18:** 448–450.

GEMMELL, M., Z. MATYAS, Z. PAWLOWSKI, AND E. J. L. SOULSBY. 1983. Guidelines for surveillance prevention and control of taeniasis/cysticercosis. World Health Organization (VPH/83.49), Geneva, Switzerland, 207 p.

GONZÁLEZ, L., E. MONTERO, L. HARRISON, M. PARKHOUSE, AND T. GARATE. 2000. Differential diagnosis of *Taenia saginata* and *Taenia solium* infection by PCR. Journal of Clinical Microbiology **38:** 737–744.

ISAAC, R. 1994. Right primer program, down-loadable at http://endeavor.med.nyu.edu/download/molbio/rightPrimer.sci.bin

KOCHER, T. D., W. K. THOMAS, A. MAYER, S. V. EDWARDS, S. PÄÄBO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the USA **86:** 6196–6200.

LE RICHE, P., AND M. SEWELL. 1978. Differentiation of taeniid cestodes by enzyme electrophoresis. International Journal for Parasitology **8:** 479–483.

MAYTA, H., A. TALLEY, R. GILMAN, J. JIMENEZ, M. VERASTEGUI, M. RUIZ, H. H. GARCIA, AND A. GONZALEZ. 2000. Differentiating *Taenia solium* and *Taenia saginata*-infections by simple hematoxylin-eosin staining and PCR-restriction enzyme analysis. Journal of Clinical Microbiology **38:** 133–137.

MORGAN, B., AND P. HAWKINS. 1949. Veterinary helminthology. Burgess Publishing Company, Minneapolis, Minnesota, 400 p.

NAKAO, M., Y. SAKO, N. YOKOYAMA, M. FUKUNAGA, AND A. ITO. 2000. Mitochondrial genetic code in cestodes. Molecular and Biochemical Parasitology **111:** 415–424.

NICKISCH-ROSENEGK, M., R. SILVA-GONZALEZ, AND R. LUCIUS. 1999. Modification of universal 12 rDNA primers for specific amplification of contaminated *Taenia* spp. (cestoda) gDNA enabling phylogenetic studies. Parasitology Research **85:** 819–825.

SALFELDER, K., T. DE LISCANO, AND E. SAVERTEIG. 1992. Atlas of parasitic pathology. Kluwer Academic Publishers. Dordrecht, The Netherlands, 191 p.

SCHANTZ, P. 1999. *Taenia solium In* Taeniasis/Cysticercosis, H. H. García, and S. M. Martínez M. (eds.). Editorial Universo Lima, Peru, p. 215–217.

VERSTER, A. 1969. A taxonomic revision of the genus *Taenia* Linnaeus, 1758 s.str. Onderstepoort Journal of Veterinary Research **36:** 3–58.

WELTE, V. R. 1995. Animal health yearbook 1994. Food and Agriculture Organization, Rome, Italy, 279 p.