THE DIFFERENTIATION OF A NEWLY DESCRIBED ASIAN TAENIID FROM *TAENIA SAGINATA* USING ENZYMATICALLY AMPLIFIED NON-TRANSCRIBED RIBOSOMAL DNA REPEAT SEQUENCES

Dante S Zarlenga

US Department Of Agriculture, Agricultural Research Service,
Biosystematic Parasitology Laboratory,
Beltsville, Maryland, USA.

Abstract. A newly described Asian taeniid which is morphologically indistinguishable from adult *Taenia saginata* has been identified in the aboriginal population of Taiwan. Hybridization patterns of restriction enzyme digested genomic DNA isolated from "Taiwan" *Taenia* and *Taenia saginata* revealed distinct variations between these cestodes. We have demonstrated by Southern blot analysis of ribosomal DNA fragments that Taiwan *Taenia* and *T. saginata* differ in a 2.4 kb fragment present in Bam HI digested DNA from *T. saginata* but absent from Taiwan *Taenia* DNA. The unique 2.4 kb sequence from *T. saginata*, as well as a partially homologous 3.1 kb fragment found in both Taiwan *Taenia* and *T. saginata*, contain sequences shown to be complementary to the 3' -end of the large ribosomal DNA subunit and to a large portion of the non-transcribed ribosomal DNA repeat. These fragments were subcloned into pUC 13 plasmid DNA, restriction enzyme mapped and partially sequenced. Two oligonucleotides complementary to regions on both the 2.4 kb and the 3.1 kb fragments were synthesized which generate 1.0 kb and 0.29 kb fragments specific for Taiwan *Taenia* and *T. saginata*, respectively, when used as primers during enzymatic amplification of cestode genomic DNA. Using this technique, we have been able to determine the identity of either cestode from a single proglottid with less than 200 ng of genomic DNA per reaction and further demonstrate that Taiwan *Taenia* exists in other parts of Eastern Asia.

INTRODUCTION

Cestode infections have been observed in the aboriginal population of Taiwan for at least 70 years since it was first reported by Oi (1915). More recent prevalence studies (Fan 1983; Chan *et al*, 1987; Chung *et al*, 1987) have indicated regional infection rates exceeding 17%. The causative agent of Taiwan taeniasis was determined to be *T. saginata* based upon morphological characteristics of the adult worm; however, the lack of detection of bovine cysticercosis in Taiwan, as well as the absence of beef in the diets of infected individuals, suggested the need for re-examining the causative agent (Huang *et al*, 1966; Huang, 1967; Kuntz and Lawless, 1966). Results from this re-examination suggested swine as the intermediate host of Taiwan *Taenia* (Chan *et al*, 1987; Chung *et al*, 1987). Interestingly, cysticerci of Taiwan *Taenia* were found only within the livers of the infected swine (Fan *et al*, 1986) and were morphologically different from those of *T. saginata*. Chao and Fan (1986) concluded that Taiwan *Taenia* and *T. saginata* were not the same; though no reliable method was yet available to differentiate the adult worms. Herein, we demonstrate the utility of recombinant DNA methodology in the study of Taiwan *Taenia* and further show the applicability of enzymatically amplified rDNA intergenic sequences to the differentiation of adult worms of Taiwan *Taenia* and *T. saginata*.

MATERIALS AND METHODS

Taenia proglottids were obtained as fixed material in 70% ethanol. Genomic DNA for agarose gel electrophoresis was liberated by SDS:proteinase K digestion (Zarlenga *et al*, 1991), treated for 30 minutes with DNase-free RNase (5 µg/ml) then extracted with buffer equilibrated phenol:chloroform and ethanol precipitated. Total cestode DNA (1–3 µg) was digested with the appropriate restriction enzyme

(10 U/μg DNA), separated by agarose gel electrophoresis then transferred to Nytran membranes according to the method of Southern (1975). Total RNA was isolated by guanidinium isothiocyanate:cesium trifluoroacetate isopycnic centrifugation (Zarlenga and Gamble, 1987) then labeled with [γ-^{32}P]ATP (3000 Ci/mmole) and T4 polynucleotide kinase. Plasmid probes were generated by nick translation (Rigby *et al*, 1977). Hybridizations and membrane washes were performed as described (Zarlenga *et al*, 1991).

Genomic DNAs (10 μg) from Taiwan *Taenia* and *T. saginata* were digested with restriction enzyme Bam HI, separated by agarose gel electrophoresis, and the regions containing the 3.1 kb rDNA fragments from both cestode DNAs and the unique 2.4 kb rDNA fragment from *T. saginata* were purified and subcloned into pUC 13 DNA according to Struhl (1985). *E. coli* HB101 transformed cells containing the 3.1 kb rDNA fragments from Taiwan *Taenia* and *T. saginata* (designated pTTr 3.1 and pTSgr 3.1, respectively), and the 2.4 kb fragment from *T. saginata* (designated pTSgr 2.4) were identified by colony screening (Grunstein and Hogness, 1975) using [γ-^{32}P]ATP kinased total RNA. Recombinant plasmids were mapped and partial sequence data determined using Sequenase™ (US Biochemicals).

The polymerase chain reaction (PCR) was performed on a Perkin-Elmer/Cetus Thermo Cycler as described elsewhere (Nishikawa *et al*, 1989; Zarlenga *et al*, 1991) using 0.2 μg of genomic DNA. Amplified samples (1 μl) from each reaction were analyzed by agarose gel electrophoresis.

RESULTS

Southern blots using Bam HI digested genomic DNAs demonstrated that Taiwan *Taenia* and *T. saginata* rDNA banding patterns were identical, except for a *T. saginata* specific fragment hybridizing at 2.4 kb which was not present in Taiwan *Taenia* DNA (Fig 1). Subsequent Southern blots of restriction enzyme digested Taiwan *Taenia* and *T. saginata* DNAs screened with the cloned 2.4 kb fragment from *T. saginata* (pTSgr 2.4) demonstrated strong hybridization with the 3.1 kb rDNA fragment from both Taiwan *Taenia* and *T. saginata* DNAs, as well as the homologous 2.4 kb fragment in *T. saginata*, with less intense hybridization occurring with several lower molecular weight bands in *T. saginata* DNA only (Fig 1). Consequently, the comigrating 3.1 kb rDNA fragments were subcloned from Taiwan *Taenia* and *T. saginata* and designated pTTr 3.1 and pTSgr 3.1, respectively. The mapped sequences demonstrated that pTTr 3.1 and pTSgr 3.1 were essentially identical (Fig 2) and very similar to pTSgr 2.4.

Partial sequence data was obtained from all 3 subcloned fragments in the region where the restriction maps of pTTr 3.1 and pTSgr 3.1 were shown to differ from that of pTSgr 2.4 (Fig 3A). The sequences of each fragment were similar

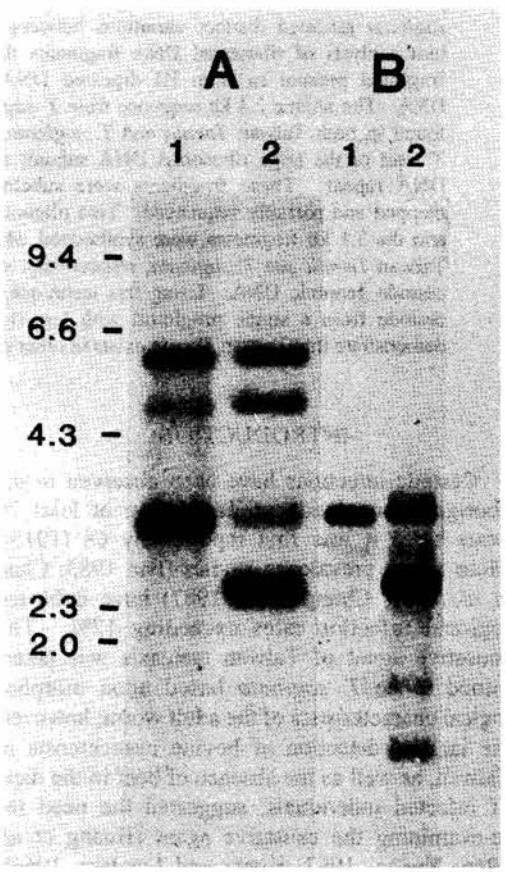

Fig 1 – Southern blots of Bam HI digested genomic DNA from Taiwan *Taenia* (lane 1) and *T. saginata* (lane 2) probed with (A) [γ-^{32}P] ATP-kinased total RNA or (B) [α ^{32}P] dCTP labeled pTSgr 2.4. Molecular weight size markers are shown in kb.

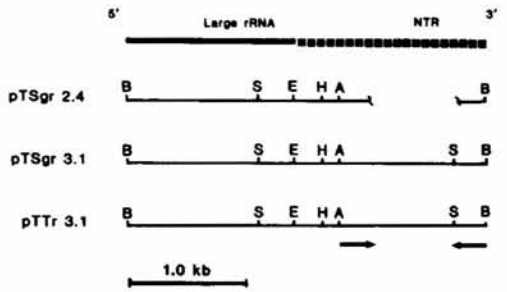

Fig 2 – Restriction enzyme maps of cloned inserts from pTSgr 2.4, pTSgr 3.1 and pTTr 3.1 representing the coding regions (solid line) and non-transcribed repeat sequences (broken line) from the 3' -end of the large rDNA subunit. Arrows indicate the regions from which sequence data was generated. The location of the missing 0.7 kb fragment in pTSgr 2.4 is represented by the open line. NTR = non-transcribed repeat, B = Bam HI, E=Eco RI, A = Acc I, H = Hinc II and S = Sst I.

Fig 3A – Partial DNA sequence data from pTSgr 2.4, pTSgr 3.1 and pTTr 3.1 as outlined in Fig 2. Primers oTAEr$_1$ and oTAEf$_1$ which were used for PCR amplification are designated by arrows where oTAEr$_1$ is complementary to the sequence shown above. The location of the primers is also approximated by the arrows in Fig 2. The location of the 0.7 kb sequence insert within pTSgr 3.1 and pTTr 3.1 is marked by asterisks (**). Base sequences were identical between pTSgr 2.4, pTSgr 3.1 and pTTr 3.1 unless otherwise indicated.

in the region proximal to the 3' Bam HI site after which pTTr 3.1 and pTSgr 3.1 diverged from pTSgr 2.4 proximal to the 3' Sst I site. It was determined that a 0.7 kb internal fragment that was present in pTSgr 3.1 and pTTr 3.1

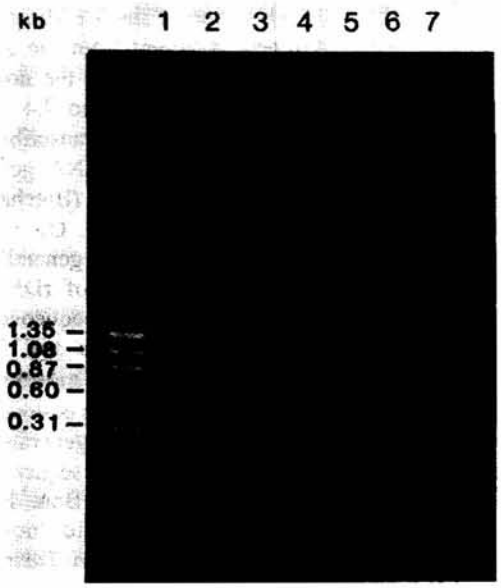

Fig 3B – Agarose gel electrophoresis of PCR products from geographical isolates of Taiwan *Taenia* and *T. saginata*. Reaction products were separated on a 0.8% gel then visualized by ethidium bromide staining. (1) *Taenia* (Taiwan); (2) *Taenia* (Korea); (3) *T. saginata* (Kenya); (4,5,6) *T. saginata* (Ethiopia); (7) *T. saginata* (Belgium).

was absent from pTSgr 2.4. Further analysis showed that beyond the 0.7 kb insert, the sequences of all three subcloned fragments were once again similar. Sequence data were used to generate two primers, designated oTAE(f_1) and oTAE(r_1), that flank the 0.7 kb sequences (Fig 3A) and that could be used for PCR analysis. PCR subsequently performed using these two primers and parasite genomic DNA extracted from geographical isolates of Taiwan *Taenia* and *T. saginata* generated 1.0 kb and 0.29, kb fragments, respectively (Fig 3B). Results demonstrated the potential utility of PCR analysis in the diagnosis of infection and the differentiation of these two closely related taeniids.

DISCUSSION

In this study, we developed molecular markers for Taiwan *Taenia* and *T. saginata*. We concluded, based on partial sequence analysis of the 2.4 and 3.1 kb rDNA fragments from *T.*

saginata, that a 0.7 kb region of the 3.1 kb non-transcribed rDNA repeat is absent from the 2.4 kb sequence. It is not unexpected that the non-homologous portions of the 3.1 kb and 2.4 kb fragments might occur within the non-transcribed sequences since this region of the rRNA gene repeat is known to be highly variable (Botchan *et al*, 1977; Long and Dawid, 1980). On the other hand, given the variability that generally exists within the intergenic regions of rDNA repeats, strong similarities between sequences from this region of Taiwan *Taenia* and *T. saginata* DNAs are consistent with morphological data which suggest that these two cestodes are closely related. PCR primers were generated that exploit the absence of the 0.7 kb sequence in the *T. saginata* specific 2.4 kb Bam HI fragment in order to devise a rapid, non-isotopic method to differentiate Taiwan *Taenia* from *T. saginata*. Following PCR amplification, as expected, a single 1.0 kb sequence was synthesized from Taiwan *Taenia* genomic DNA corresponding to the 3.1 kb Bam HI rDNA fragment; however, only one intense band 0.29 kb in length was generated from *T. saginata* genomic DNA with the other predicted 1.0 kb fragment appearing only upon overloading the gel with sample. Thus, these different and cestode specific PCR fragments could be used for distinguishing Taiwan *Taenia* from *T. saginata*. Southern blot data suggest that the 1.0 kb and 0.29 kb fragments are not equally represented as PCR products because of a large difference in relative abundance between the 3.1 kb and 2.4 kb Bam HI fragments within the genome of *T. saginata* (Fig 1). Because of this difference in relative abundance, it is likely that as the percentage of the 0.29 kb fragment increases in the reaction mixture with subsequent PCR cycles, the product competes for available primer sites within the 3.1 kb fragment, and subsequently interferes with the synthesis of the 1.0 kb sequence. Results presented here, nevertheless, offer the only reliable means to adequately differentiate between the adult stages of these two taeniids and further demonstrate that Taiwan *Taenia* is not confined to Taiwan but exists in other parts of Eastern Asia (Fan *et al*, 1988) where its consideration as *T. saginata* has likely occurred.

REFERENCES

Botchan P, Reeder RH, Dawid IB. Restriction analysis of the nontranscribed spacers of *Xenopus laevis* ribosomal DNA. *Cell* 1977; 11:599-605.

Chan CH, Fan PC, Chung WC, *et al*. Studies on taeniasis in Taiwan. I. Prevalence of taeniasis among aborigines in Wulai District, Taipei County, Northern Taiwan. Proceedings of the 1st Sino-American Symposium on Biotechnology and Parasitic Diseases, 1987; 1:65-77.

Chao D, Fan PC. Larval stage of a possible new species of tapeworm from Taiwan aborigines. *Chin Bioscience* 1986; 27:1-6.

Chung WC, Fan PC, Chan CH, *et al*. Studies on taeniasis in Taiwan. II. Prevalence of taeniasis among aborigines in Lanyu (Orchid Island) District, Taitung County, Eastern Taiwan, with reference to domestic pigs (Lanyu strain) which can be considered as the intermediate host of Taiwan *Taenia*(?). Proceedings of the 1st Sino-American Symposium on Biotechnology and Parasitic Diseases 1987; 1:78-90.

Fan PC. Taeniasis in Taiwan. Proceedings of the 10th International Congress of the World Association for the Advancement of Veterinary Parasitology, Perth, Australia, 1983; 105.

Fan PC, Chan CH, Chung WC, *et al*. Studies on taeniasis in Taiwan. IV. Current status of epidemiological, experimental and morphological aspects of Taiwan *Taenia*. Proceedings of the Sino-Japanese Symposium on Parasitic Zoonoses 1986, Osaka, Japan 1986; 100-7.

Fan PC, Chung WC, Lin CY, et al. Studies on taeniasis in Taiwan. VI. Is *Taenia saginata* from Taiwan, Korea, and Indonesia a new species. *Chin J Parasitol* 1988; 1:56-70.

Grunstein M, Hogness DS. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. *Proc Natl Acad Sci USA* 1975; 72:3961-5.

Huang SW. Studies on *Taenia* species prevalent among the aborigines in Wulai District, Taiwan. II. On the species of *Taenia*. *Bull Inst Zool, Acad Sin* 1967; 6:29-34.

Huang SW, Lin CY, Khaw OK. Studies on *Taenia* species prevalent among aborigines in Wulai District, Taiwan. I. On the parasitological fauna of the aborigines in Wulai District. *Bull Inst Zool Aca Sin* 1966; 5:87-91.

Kuntz RE, Lawless DK. Intestinal parasites of people of Taiwan. Intestinal parasites of aborigines (Yami) of Lanyu (Orchid Island). *J Formosan Med Assoc* 1966; 65:287-93.

Long EO, Dawid IB. Repeated genes in eukaryotes. *Ann Rev Biochem* 1980; 49:727-64.

Nishikawa BK, Fowlkes DM, Kay BK. Convenient uses of polymerase chain reaction in analyzing recombinant cDNA clones. *Biotechniques* 1989; 7:730-5.

Oi T. Examination of eggs of intestinal parasites in Central Taiwan. *J Formosan Med Assoc* 1915; 154:816-25.

Rigby PWJ, Dieckmann M, Rhodes C, Berg P. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J Mol Biol* 1977; 113:237-51.

Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975; 98:503-17.

Struhl K. A rapid method for creating recombinant DNA molecules. *Biotechniques* 1985; 3:452-3.

Zarlenga DS, Gamble HR. Simultaneous isolation of preparative amounts of RNA and DNA from *Trichinella spiralis* by cesium trifluoroacetate isopycnic centrifugation. *Anal Biochem* 1987; 162:569-74.

Zarlenga DS, McManus DP, Fan PC, Cross JH. Characterization and detection of a newly described Asian taeniid using cloned ribosomal DNA fragments and sequence amplification by the polymerase chain reaction. *Exp Parasitol* 1991; 72:174-83.