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***Taenia solium* cysticercosis in Bali, Indonesia: serology and mtDNA analysis**

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Running title: mtDNA of *Taenia solium*, Bali

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Summary

An active *Taenia solium* cysticercosis case in Bali, Indonesia was followed up by CT scan and serology for monitoring of prognosis. Serology using semi-purified glycoprotein and recombinant antigens showed drastic drop in titers after calcification of the cysts. Three paraffin-embedded cysts for histopathological confirmations from three patients were used for mitochondrial DNA analysis. The sequences of *cox 1* gene from *T. solium* cysticerci from Bali differed from those in Papua and other Asian countries.

1. Introduction

Taenia solium cysticercosis is rather un-common in Indonesia, since the majority is Muslim. However, cysticercosis occurs in some areas or islands, where most people are either Christians or Hindi (Wandra et al., 2003; Ito et al., 2006). It has been revealed that cysticercosis is very serious public health issue in Papua (= former Irian Jaya, Indonesia) (Wandra et al., 2003). It has been conceived that *T. solium* has been introduced into Papua from Bali from 1969 when Papua was governed by Indonesia (Wandra et al., 2003). However, there is no molecular evidence to discuss this hypothesis.

In Bali, taeniasis either due to *T. saginata* or *T. solium* has been found to be distributed in all nine districts: Historically, cysticercosis was rather common but has sporadically been found at least past three decades. It is crucial difference from taeniasis of *T. saginata* now rather common in Bali (Wandra et al., 2006). Unfortunately, there is no cysticercus specimen of *T. solium* fixed in ethanol from Bali. However, if histopathological paraffin-embedded specimens are available, we can analyze mitochondrial DNA (Yamasaki et al., 2005; Ito et al., 2006). Recently, we could obtain three paraffin-embedded cysticercus specimens from three patients in Bali.

In this short report, *cox 1* gene sequences of mitochondrial DNA was analyzed using the

specimens from Bali and compared with those from Papua and several other Asian countries. A follow-up study of a patient who showed good serological dynamics for detection of active cysticercosis was also briefly discussed, since serology to detect active cysticercosis is still under debate.

2. Serological Follow-up of active cysticercosis

A 36-year-old Balinese man, from a rural village, Gianyar District, southern part of Bali, Indonesia, was diagnosed as a disseminated cysticercosis in June 2003. A computed tomography scan of his brain showed multiple active lesions in the left frontal lobe and parieto-occipital region. Histopathological examination of nodule resected from the tongue in Jan 2004 revealed a characteristic structure of taeniid cysticercus. After chemotherapy with albendazole, only calcified lesions were detected in February 2006. Serological examinations by both ELISA and immunoblots using highly specific semi-purified glycoprotein antigens (pH 8.1) for detection of active cysticercosis (Sako et al., 2000) were strong positive in June 2003 and January 2004 but weak positive in February 2006. OD values in ELISA using the same antigens increased from 0.221 (June 2003) to 0.254 (January 2004) and dropped to 0.093 (February 2006) (cut off=0.051). Such dynamic antibody responses through treatment for three years were also confirmed by ELISA using recombinant chimeric antigen (Sako et al., 2000). OD values were 0.411 (June 2003), 0.528 (January 2004) and 0.165 (February 2006) (cut-off = 0.093).

3. Mitochondrial DNA

Three paraffin-embedded nodules from 36, 36 and 26 years old men in Bali were available for mitochondrial DNA analysis. DNA was extracted from thin sections (4 sections with 5- μ m thickness) using a DEXPAT kit (TaKaRa Shuzo, Shiga, Japan) (Yamasaki et al.,

2005). In brief, 10 drops of lysis solution provided from the kit were added into paraffin sections in the microtube, and lysed at 100C for 2 hr. After centrifugation, the resultant supernatants (~100 µl) were used as template DNA samples for polymerase chain reaction (PCR). For the amplification of a mitochondrial cytochrome *c* oxidase subunit 1 (*coxI*), PCR was performed using forward (5'-ATGACTAATATATTTTCTCGTAC-3', positions 520-542) and reverse primers (5'-ATTAACACATAAACCTCGGGA-3', positions 740-720) according to the method described previously (Yamasaki et al., 2005). The PCR-amplified *coxI* was run on a 10% polyacrylamide gel and DNA sequencing was performed on an ABI PRISM 310 Genetic Analyzer.

CoxI fragment of 224-bp was successfully amplified from this sample as well as additional two other samples from two other patients in Bali. DNA sequencing of the PCR product revealed the cysticercus *T. solium* Asian genotype based on the differential nucleotide at positions 690 and 723 (Fig. 1). In addition, the nucleotide at position 650 was thymine (T) instead of cytosine (C). So far, the nucleotide at 650, T, is unique to *T. solium* isolates from Bali, Indonesia.

4. Discussion

This serological follow-up study revealed a good correlation between neuroimaging and serology through curative chemotherapy. The results strongly suggested that the serology applied for this case was highly useful for detection of active cysticercosis and monitoring of prognosis (Sako et al., 2000; Ito et al., 2006).

Due to the historical episode that *T. solium* in Papua was introduced from Bali from 1969 when Papua was governed by Indonesia (Wandra et al., 2003, 2006), we have been interested in comparison of *T. solium* in Bali and in Papua. Mitochondrial DNA analysis using 3 paraffin-embedded specimens for histopathological confirmations of 3 patients

revealed that *T. solium* isolated from Bali differed from isolates from Papua, Indonesia and all other Asian countries, Thailand, India and China at position 650 using the *cox1* fragment of 224-bp. African/American genotypes differed from Asian ones at positions 690 and 723 (Fig. 1). So far, we could have checked only three metacestodes of *T. solium* resected from three patients in Bali. Therefore, further similar studies based on the full sequencing of mitochondrial DNA genes using more number of *T. solium* specimens from Bali and Papua and also from other areas in Indonesia such as East Nusa Tenggara, located between Bali and Papua, where cysticercosis is recognized not rare (Wandra et al., unpublished), are necessary for phylogeographic considerations. Further subtyping of *T. solium* in Asia and/or America/Africa genotypes may be interesting for tracking back where cysticercosis patients from non-endemic countries were exposed to eggs of *T. solium* in endemic countries.

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Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

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Ethical approval

Not required.

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Fig. 1. Comparison of nucleotide sequences of *cox1* of the *T. solium* isolate from a Balinese cysticercosis patient and those of *cox1* from known human *Taenia* cestodes. Nucleotides at positions 690 and 723 serve as differential markers for two genotypes of *T.*

solium, *T. saginata* and *T. asiatica* (Yamasaki et al., 2005). Thymine base at position 650 is unique to *T. solium* isolated from Bali, Indonesia. The nucleotide sequences of *T. solium* from Bali (Indonesia), Papua (Indonesia), Thailand, India, China, Mexico and Mozambique are from AB271234, AB066488, AB066487, AB066489, AB066485, AB066490 and AB066493, respectively. .