*Journal of Helminthology* (2003) **77**, 39–42 DOI: 10.1079/JOH2002146

# *Taenia solium* taeniasis/cysticercosis in Papua, Indonesia in 2001: detection of human worm carriers

**S.S. Margono[1], A. Ito[2]*, M.O. Sato[2], M. Okamoto[3], R. Subahar[1], H. Yamasaki[2], A. Hamid[4], T. Wandra[5], W.H. Purba[5], K. Nakaya[6], M. Ito[7], P.S. Craig[8] and T. Suroso[5]**

[1]Department of Parasitology and [4]Department of Neurology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia; [2]Department of Parasitology and [6]Animal Laboratory for Medical Research, Asahikawa Medical College, Asahikawa, Japan; [3]Department of Laboratory Animal Science, School of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan; [5]Directorate General Communicable Disease Control and Environmental Health, Ministry of Health, Jakarta, Indonesia; [7]Central Institute for Experimental Animals, Kawasaki, Japan; [8]Division of Biological Sciences, School of Environment and Life Sciences, University of Salford, Salford, UK

## Abstract

A preliminary study to detect human worm carriers of *Taenia solium* in Papua (Irian Jaya), Indonesia was carried out using stool examinations for the detection of copro-antigens and adult proglottids after chemotherapy, and confirmation by mitochondrial DNA analysis using expelled proglottids and metacestodes developed in NOD/Shi-*scid* mice from eggs of expelled proglottids. Approximately 8.6% of the local population in Kama (5/58), 1 km from the local capital city centre, Wamena, were confirmed to harbour adult *T. solium* using these techniques.

## Introduction

*Taenia solium* cysticercosis is one of the emerging and re-emerging parasitic diseases worldwide (Craig *et al.*, 1996; Schantz *et al.*, 1998). For the control of this disease, reliable data through accurate diagnostic methods should be available. With the development of computed tomography (CT) scanning and magnetic resonance imaging (MRI), basic physical information on the presence of cystic lesions in the brain could be obtained. More recently, serodiagnosis of almost 100% specificity has been developed (Tsang *et al.*, 1989). In the last decade the gold-standard serodiagnostic method for the detection of cysticercosis/neurocysticercosis (CC/NCC) has been the immunoblot, which has also now been supported by a specific enzyme-linked immunosorbent assay (ELISA) test (Ito *et al.*, 1998, 1999). In most endemic areas in developing countries, image analyses using CT scanning and MRI are not readily available due to the high cost, therefore immunodiagnostic methods have been applied for the detection of CC patients, especially NCC patients (ELISA and immunoblot specific to CC/NCC). In addition, for the detection of taeniasis carriers, the copro-antigen detection method specific for *Taenia* species infections has been used (Allan *et al.*, 1990). Immunodiagnostic tests including ELISA and copro-antigen tests are highly appropriate for epidemiological studies and for the diagnosis of CC/NCC as well as the identification of taeniasis patients in hospitals and within communities (Del Brutto *et al.*, 2001). Subcutaneous

*Author for correspondence
Fax: +81 166 68 2429
E-mail: akiraito@asahikawa-med.ac.jp

nodules from sero-positive persons and adult worms from copro-antigen positive persons can be analysed both by morphology and mitochondrial DNA analysis for confirmation of *T. solium* (Wandra *et al.*, 2000).

The first outbreak of NCC in Irian Jaya (now officially called Papua), Indonesia was reported from the Paniai District, in the central part of Irian Jaya, 30 years ago (Tumada & Margono, 1973). Another outbreak has been described in the Jayawijaya District, in the eastern part of Irian Jaya, near the border to Papua New Guinea, and the new area appears now to be pandemic for CC/NCC (Simanjuntak *et al.*, 1997; Wandra *et al.*, 2000; Subahar *et al.*, 2001; Ito *et al.*, 2002a). In the present study, a summary of preliminary human surveys, especially for the detection of the worm carriers in 2001 is presented.

## Materials and methods

The village of Kama, in Wamena Kota, a subdistrict of the Jayawijaya District, was chosen for the 2001 study based on a previous survey in 1999 (Wandra *et al.*, 2000; Subahar *et al.*, 2001). The village is located 1 km from the only hospital in Wamena, the capital city. After obtaining ethical approval from the central and local governments, 38 stool samples and 13 serum samples were obtained from 38 persons during January–February 2001, and 20 faecal samples and 15 serum samples were collected from 20 persons in August 2001 (table 1).

Stool samples, collected at random from persons 15 years or older in Kama were examined microscopically for the detection of eggs using the ether-formalin sedimentation method and for the detection of *Taenia* faecal antigens using a copro-antigen detection kit (Taenia-ELISA, Virotech, Germany) (Subahar *et al.*, 2001). All persons, most of them farmers, defecated in the garden, bushes and sometimes in the river and were in the habit of consuming local pork.

When stool samples were found to be positive with the copro-antigen test, each person was admitted to hospital in Wamena on the same day and immediately treated with a single dose of 600 × 2 mg praziquantel, using magnesium sulphate solution as a laxative, both before and after drug treatment.

Both an ELISA and immunoblot using a purified glycoprotein (pH9.0) by preparative isoelectric focusing were carried out for serological confirmation of CC/NCC (Ito *et al.*, 1998, 2002a).

*In vitro* hatched 2500 oncospheres per 0.5 ml sterile PBS prepared from eggs of one adult tapeworm were inoculated into the peritoneal cavity of five non-obese diabetic–severe combined immunodeficiency (NOD/Shi-*scid*) mice (Ito *et al.*, 2001). Mice were killed at 135 days of inoculation and the metacestodes in the peritoneal cavity of each mouse were counted (fig. 1).

Nucleotide sequences of the cytochrome *c* oxidase subunit I gene (*COI*) of mitochondrial DNA was carried out as reported previously (Wandra *et al.*, 2000). Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis was also carried out according to Yamasaki *et al.* (2002).

## Results and Discussion

### *Detection and confirmation of adult worms*

Although a total of 58 faecal samples were examined microscopically for the detection of eggs, *Taenia* eggs were found in only one person, a 50-year-old male (table 1). In contrast, five of 58 stool samples were found to be positive using the copro-antigen test. Proglottids of adult *Taenia* were expelled from the five coproantigen positive persons (three males and two females) after treatment with praziquantel. All adult worms were confirmed to be *T. solium* based on nucleotide sequences of *COI* of mitochondrial DNA as previously reported (data not shown) (Wandra *et al.*, 2000; Okamoto *et al.*, 2001).

### *Antibody response specific to* T. solium *cysticercosis*

From sera of 28 of 58 persons, both ELISA and immunoblot revealed that approximately 32%, including two of five persons who expelled adult *T. solium*, were serologically confirmed to have antibody specific to CC/NCC (table 1). The 50-year-old man, who was copro-antigen positive and simultaneously confirmed to have taeniid eggs, showed a strong positive response to

Table 1. The prevalences (%) of human taeniasis using copro-antigens and neurocysticercosis (NCC) using serology in Wamena, Papua, Indonesia in 2001.

| Date of survey | Number and age of persons examined | Copro-antigen for taeniasis (% positive) | Serology for NCC[a] (% positive) |
|---|---|---|---|
| January–February 2001 | | | |
| | 15 males (15–50 years old) | 2[b]/15 (13.3) | 2/5 (40) |
| | 13 females (15–65 years old) | 1[b]/13 (7.7) | 2/8 (25) |
| | 10 persons with no record of gender | 0/10 | not tested |
| August 2001 | | | |
| | 9 males (30–50 years old) | 1[c]/9 (11.1) | 3/7 (42.9) |
| | 11 females (15–60 years old) | 1[c]/11(9.1) | 2/8 (25) |
| Total | 58 persons | 5[d]/58 (8.6) | 9/28 (32) |

[a] ELISA and immunoblot using the same antigen of pH 9.0 purified by preparative isoelectric focusing (Ito *et al.*, 1998, 2002a).
[b] Did not provide serum samples.
[c] Simultaneously seropositive for NCC.
[d] Proglottids or whole worms expelled were confirmed to be *Taenia solium* by analysis of *COI* of mitochondrial DNA.

https://doi.org/10.1079/JOH2002146 Published online by Cambridge University Press

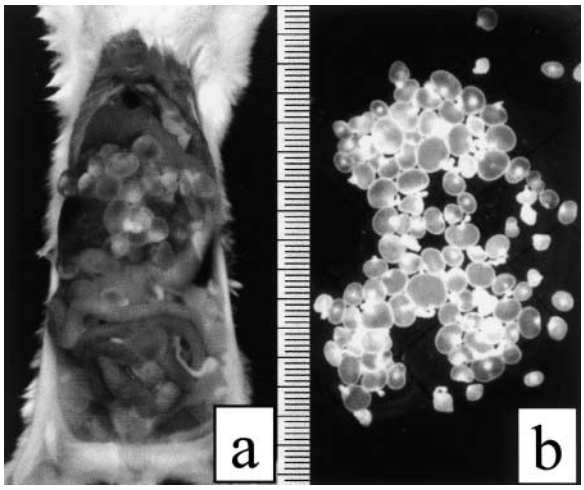


Fig. 1. (a) A NOD/Shi-*scid* mouse harbouring metacestodes of *Taenia solium* 135 days after peritoneal injection of *in vitro* hatched oncospheres. (b) A total of 106 metacestodes recovered from the peritoneal cavity.

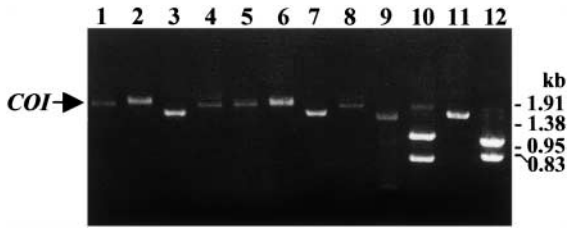


Fig. 2. Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis using the COI gene of *Taenia* cysticercus from Indonesia. 1.8 kb-COI genes were amplified by PCR using mitochondrial DNA prepared from one cysticercus each with hooklets (lanes 1–4) and without hooks (lanes 5–8). Genes were tested with *Taq*I (lanes 1 and 5), *Bam*HI (lanes 2 and 6), *Nco*I (lanes 3 and 7) or *Dde*I (lanes 4 and 8) unique for *T. saginata*, *T. asiatica*, Asian and American/African genotypes of *T. solium*, and then electrophoresed on a 0.9% agarose gel. The COI gene from the Asian genotype of *T. solium* was digested with only *Nco*I (lanes 3 and 7). An arrow indicates undigested *COI*. For comparison, RFLP profiles of COI genes from *T. saginata*, *T. asiatica*, Asian and American/African genotypes of *T. solium* are shown in lanes 9, 10, 11 and 12, respectively.

CC/NCC by both the ELISA (OD value: 0.101) and immunoblot, whereas a 29-year-old woman who was copro-antigen positive showed a relatively weak response using ELISA (OD value: 0.052, vs. cut off OD value: 0.030) and immunoblot. However, both persons were also exposed to eggs, which caused CC/NCC, as well as to cysticerci, which previously resulted in intestinal taeniasis, but no subcutaneous nodules were detected in these two persons following clinical examination or palpation. No serum samples were available from the remaining three copro-antigen positive persons.

### *Metacestodes developed in NOD/Shi-*scid *mice*

Figure 1 shows fully developed cysticerci 135 days (4.5 months) after inoculation in NOD/Shi-*scid* mice. A range of 57–144 metacestodes were recovered from each mouse resulting in a mean of 100.6. Morphological observations of 135-day-old metacestodes revealed that the development of hooklets was highly variable, ranging from individuals with no hooks, 1–14 small hooks only, large hooks and a small number of small hooks, and with a complete complement of small and large hooks (data not shown). It is considered that small hooks of approximately 110 $\mu$m length are formed at first and these grow into large hooks of approximately 160 $\mu$m. Not only adult proglottids but also metacestodes grown in NOD/Shi-*scid* mice were confirmed to be *T. solium* by mitochondrial DNA analysis as previously reported (Wandra *et al.*, 2000). Figure 2 shows PCR–RFLP profiles of *COI* of single metacestode each with or without hooks. On the basis of the nucleotide sequence of *COI* from the Asian genotype of *T. solium* (Okamoto *et al.*, 2001; Nakao *et al.*, 2002), the gene can be digested with *Nco*I, generating 1.5 and 0.3 kb fragments. This was confirmed by base excision sequence scanning (BESS) T-base analysis (data not shown) (Yamasaki *et al.*, 2002).

As expected, the prevalence of 32% of CC/NCC in Kama Village, located very close to the capital city Wamena, was lower than that in the rural area of Assologaima (46%) (Wandra *et al.*, 2000; Subahar *et al.*, 2001). Nevertheless, a relatively high prevalence (8.6%) of taeniasis carriers was detected and confirmed. Therefore, the present study has shown that the application of immunodiagnostic methods for the detection of human cysticercosis and/or taeniasis combined with molecular approaches and the use of an animal model for obtaining metacestodes is necessary for the identification of *T. solium*, as was the case for the identification of this species in dogs (Ito *et al.*, 2002b,c). Future studies should be undertaken on schoolchildren for the early detection, early treatment and local community-based sustainable education of taeniasis/cysticercosis.

## Acknowledgements

This study was supported in part by grants-in-aid for Scientific Research (A) and (B) (12557024, 14256001) from the Japan Society of Promotion of Science to A. Ito and by Japan Health Science Foundation to S.S. Margono.

## References

**Allan, J.C., Avila, G., Garcia Noval, J., Flisser, A. & Craig, P.S.** (1990) Immunodiagnosis of taeniasis by coproantigen detection. *Parasitology* **101**, 473–477.

**Craig, P.S., Rogan, M.T. & Allan, J.C.** (1996) Detection, screening and community epidemiology of taeniid cestode zoonoses: cystic echinococcosis, alveolar echinococcosis and neurocysticercosis. *Advances in Parasitology* **38**, 169–250.

**Del Brutto, O.H., Rajshekhar, V., White, A.C. Jr., Tsang, V.C.W., Nash, T.E., Takayanagui, O.M., Schantz, P.M., Evans, C.A.W., Flisser, A., Correa, D., Botero, D., Allan, J.C., Sarti, E., Gonzalez, A.E., Gilman, R.H. & Garcia, H.H.** (2001) Proposed diagnostic criteria for neurocysticercosis. *Neurology* **57**, 177–183.

**Ito, A., Plancarte, A., Ma, L., Kong, Y., Flisser, A., Cho,**

https://doi.org/10.1079/JOH2002146 Published online by Cambridge University Press

**S.Y., Liu, Y.H., Kamhawi, S., Lightowlers, M.W. & Schantz, P.M.** (1998) Novel antigens for neurocysticercosis: a simple method for preparation and evaluation for serodiagnosis. *American Journal of Tropical Medicine and Hygiene* **59**, 291–294.

**Ito, A., Plancarte, A., Nakao, M., Nakaya, K., Ikejima, T., Piao, Z.X., Kanazawa, T. & Margono, S.S.** (1999) ELISA and immunoblot using purified glycoproteins for serodiagnosis of cysticercosis in pigs naturally infected with *Taenia solium*. *Journal of Helminthology* **73**, 363–365.

**Ito, A., Nakaya, K., Sako, Y., Nakao, M. & Ito, M.** (2001) NOD-*scid* mouse as an experimental animal model for cysticercosis. *Southeast Asian Journal of Tropical Medicine and Public Health* **32** (Suppl 2), 83–89.

**Ito, A., Sako, Y., Nakao, M. & Nakaya, K.** (2002a) Neurocysticercosis in Asia: serology/seroepidemiology in humans and pigs. pp. 25–31 *in* Craig, P.S. & Pawlowski, Z. (*Eds*) *Cestode zoonoses: echinococcosis and cysticercosis – an emergent and global problem*. Amsterdam, IOS Press.

**Ito, A., Nakao, M., Okamoto, M., Sako, Y. & Yamasaki, H.** (2002b) Mitochondrial DNA of *Taenia solium*: from basic to applied science. pp. 47–55 *in* Singh, G. & Prabhakar, S. (*Eds*) Taenia solium *cysticercosis*. Wallingford, Oxon, CABI Publishing.

**Ito, A., Putra, M.I., Subahar, R., Sato, M.O., Okamoto, M., Sako, Y., Nakao, M., Yamasaki, H., Nakaya, K., Craig, P.S. & Margono, S.S.** (2002c) Dogs as alternative intermediate hosts of *Taenia solium* in Papua (Irian Jaya), Indonesia confirmed by highly specific ELISA and immunoblot using native and recombinant antigens and mitochondrial DNA analysis. *Journal of Helminthology* **76**, 311–314.

**Nakao, M., Okamoto, M., Sako, Y., Yamasaki, H., Nakaya, K. & Ito, A.** (2002) A phylogenetic hypothesis for the distribution of two genotypes of the pork tapeworm *Taenia solium* worldwide. *Parasitology* **124**, 657–662.

**Okamoto, M., Nakao, M., Sako, Y. & Ito, A.** (2001) Molecular variation of *Taenia solium* in the world. *Southeast Asian Journal of Tropical Medicine and Public Health* **32** (Suppl 2), 90–93.

**Schantz, P.M., Wilkins, P.P. & Tsang, V.C.W.** (1998) Immigrants, imaging, and immunoblots: the emergence of neurocysticercosis as a significant public health problem. pp. 213–242 *in* Scheld, W.M., Craig, W.A., Hughes, J.M. (*Eds*) *Emerging infections 2*. Washington DC, ASM Press.

**Simanjuntak, G.M., Margono, S.S., Okamoto, M. & Ito, A.** (1997) Taeniasis/cysticercosis in Indonesia as an emerging disease. *Parasitology Today* **13**, 130–132.

**Subahar, R., Hamid, A., Purba, W., Wandra, T., Karma, C., Sako, Y., Margono, S.S., Craig, P.S. & Ito, A.** (2001) *Taenia solium* infection in Irian Jaya (West Papua), Indonesia: a pilot serological survey of human and porcine cysticercosis in Jayawijaya District. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 388–390.

**Tsang, V.C., Brand, J.A. & Boyer, A.E.** (1989) An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *Journal of Infectious Diseases* **159**, 50–59.

**Tumada, L.R. & Margono, S.S.** (1973) Cysticercosis in the area of the Wissel Lakes, West Irian. *Southeast Asian Journal of Tropical Medicine and Public Health* **3**, 371–376.

**Wandra, T., Subahar, R., Simanjuntak, G.M., Margono, S.S., Suroso, T., Okamoto, M., Nakao, M., Sako, Y., Nakaya, K., Schantz, P.M. & Ito, A.** (2000) Resurgence of cases of epileptic seizures and burns associated with cysticercosis in Assologaima, Jayawijaya, Irian Jaya, Indonesia, 1991–95. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 46–50.

**Yamasaki, H., Nakao, M., Sako, Y., Nakaya, K., Sato, M.O., Mamuti, W., Okamoto, M. & Ito, A.** (2002) DNA differential diagnosis of human taeniid cestodes: base excision sequence scanning T-base reader analysis using mitochondrial genes. *Journal of Clinical Microbiology* 3818–3821.

(Accepted 6 September 2002)