Journal of Helminthology (2002) **76,** 311–314 DOI: 10.1079/JOH2002128

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

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Abstract

Serology (ELISA and immunoblot) using native glycoproteins, affinity purified glycoproteins, and a recombinant antigen is known to be highly specific to *Taenia solium* cysticercosis in humans and pigs. These techniques were applied for dogs in the highly endemic area of cysticercosis in Papua (Irian Jaya), Indonesia. Analysis of dog sera by both ELISA and immunoblot revealed 7 of 64 dogs were highly positive. Examination of two sero-positive dogs revealed cysticerci of *T. solium* in the brain and heart of these dogs. Mitochondrial DNA analysis confirmed that they were the same as *T. solium* previously confirmed from pigs and biopsies from local people from Irian Jaya. It is suggested that the life cycle of *T. solium* may be completed not only between humans and pigs but also between humans and dogs.

Introduction

Historically there are several reports that dogs (*Canis familiaris*) may be intermediate hosts of *Taenia solium* based on morphological observations (Meijer, 1933; Verster, 1967; Okolo, 1986). Meijer (1933) reported that 25 of 3831 dogs and 4399 of 11,398 adult pigs in Java, Indonesia were found infected with cysticerci of *T. solium*. Verstar (1967) also reported dogs infected with *T. solium* in Africa, as did Okolo (1986) in 2.7% of 150 dogs examined in eastern Nigeria. A dog brain full of cysticerci of *T. solium* is recorded in the video film and CD (Proyecto Piloto para Estudio del Complejo Teniasis – Cisticercosis en los Andes del Ecuador (Proyecto T/C) and CD-PTC1-PROYECTO T/C – 1999) produced by Universidad Central Del Ecuador (1999). Such information on dogs infected with *T. solium* was based on morphology of scolex with hooklets. Nowadays, it is possible to

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differentiate any taeniid specimens not only by morphology but also by DNA analysis (Bowles & McManus, 1994; Theis *et al.*, 1996; McManus, 1997; Wandra *et al.*, 2000).

Asahikawa Medical College (AMC) has established serodiagnosis of cysticercosis in humans and pigs (Ito *et al.*, 1998, 1999). Based on such serology using ELISA and immunoblot with specific glycoprotein antigens, field surveys of taeniasis/cysticercosis in Papua (Irian Jaya), Indonesia have been undertaken (Simanjuntak *et al.*, 1997; Wandra *et al.*, 2000; Subahar *et al.*, 2001). From field surveys in 2000 and 2001, blood samples were taken from dogs as well as local people and pigs in Wamena, Jayawijaya District, Irian Jaya. In the present paper, the evidence of dogs infected with cysticerci of *T. solium* screened by serology is reported.

Materials and methods

Dog sera

A total of 64 dog serum samples were randomly obtained in 2000 (29 samples) and in 2001 (35 samples) in Wamena, Jayawijaya, Irian Jaya during a field survey of taeniasis/cysticercosis (Wandra *et al.*, 2000; Subahar *et al.*, 2001).

Serology

ELISA and immunoblot (IB) were carried out using glycoproteins (GPs) of a fraction of pH 9.0 purified by preparative isoelectric focusing (GP-ELISA and GP-IB) (Rotofor, BioRad, USA) (Ito *et al.*, 1998, 1999, 2002a). Affinity purified GP (AffGP) was prepared from cyst fluid of *T. solium* metacestodes using an affinity resin column (Hi Trap NHS-activated HP, Amersham Pharmacia Biotech) coupled with monoclonal antibodies against the GP. A recombinant antigen, Ag1V1/Ag2 chimeric protein, (RecAg) was produced according to Sako *et al.* (2000). These AffGP and RecAg were applied for IB only in order to evaluate the usefulness of GP-ELISA. Approximately 1.0 μg ml^{-1} GP was applied for coating ELISA plates (GPs-ELISA, Maxisorp, Nunc, Copenhagen). Approximately 12.5 μg GP, AffGP or RecAg were loaded for two dimensional gel with 6 cm width (mini SDS-PAGE, Tefco, Tokyo), respectively (Sako *et al.*, 2000).

Necropsy of dogs

Two of 7 dogs showing sero-positive response by GP-ELISA and by two or three different IBs were necropsied in Wamena, Papua (Irian Jaya) and the brain, heart and muscles were observed with the naked eye.

Mitochondrial DNA analysis

A partial sequence of 391 bps of CO-I gene (*CO-1*) was analysed and compared with previous data (Okamoto *et al.*, 1995; Wandra *et al.*, 2000).

Results and Discussion

Based on the serology (ELISA) available for humans and pigs (Ito *et al.*, 1998, 1999), initially two of 29 dogs sampled in 2000 were sero-positive by both GP-ELISA (left half in fig. 1) (OD value: 0.135 and 0.069, cut-off value 0.06) and GP-IB (fig. 2a). Immunoblot results showed 2, 2 and 6 dogs positive using GP, AffGP and RecAg, respectively. Although background noise appeared in GP-IB (fig. 2a), the noise was cleared when AffGP (fig. 2b) and RecAg (fig. 2c) were used. The presence of only one diagnostic band using RecAg facilitated the judgment of positives and negatives. Necropsy of one dog (OD value 0.135) with positive IB results using all three antigens revealed two cysts from the brain. Confirmation of *T. solium* was made by morphological observation, measurement of the hooks and mitochondrial DNA analysis. The average measurements of large and small hooks were 162.1 μm (n = 14) and 111.2 μm (n = 8), respectively (fig. 3), and were similar to those of *T. solium* (Verster, 1967; Theis *et al.*, 1996). The partial sequence of *CO-1* was identical to that previously confirmed as Asian genotype of *T. solium* (Wandra *et al.*, 2000; Okamoto *et al.*, 2001; Nakao *et al.*,

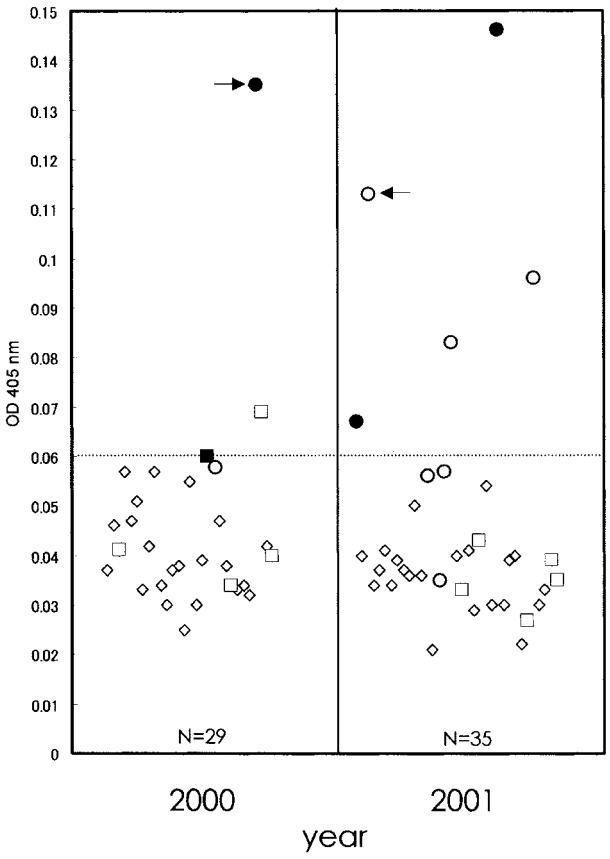

Fig. 1. Summary of ELISA results using glycoproteins (GP) (OD values at 405 nm) and immunoblots using three different antigens (GP, AffGP and RecAg) for 29 dogs in 2000 (left half) and 35 dogs in 2001 (right half). Closed and open circles are sero-positive dogs by three antigens (●) and by two antigens (GP and RecAg) (○), respectively. Dog samples positive by GP-IB only (■), positive by RecAg-IB only (□), GP-IB negative (◇). Arrows indicate dogs which were observed and from which cysticerci of *Taenia solium* were recovered after necropsy.

https://doi.org/10.1079/JOH2002128 Published online by Cambridge University Press

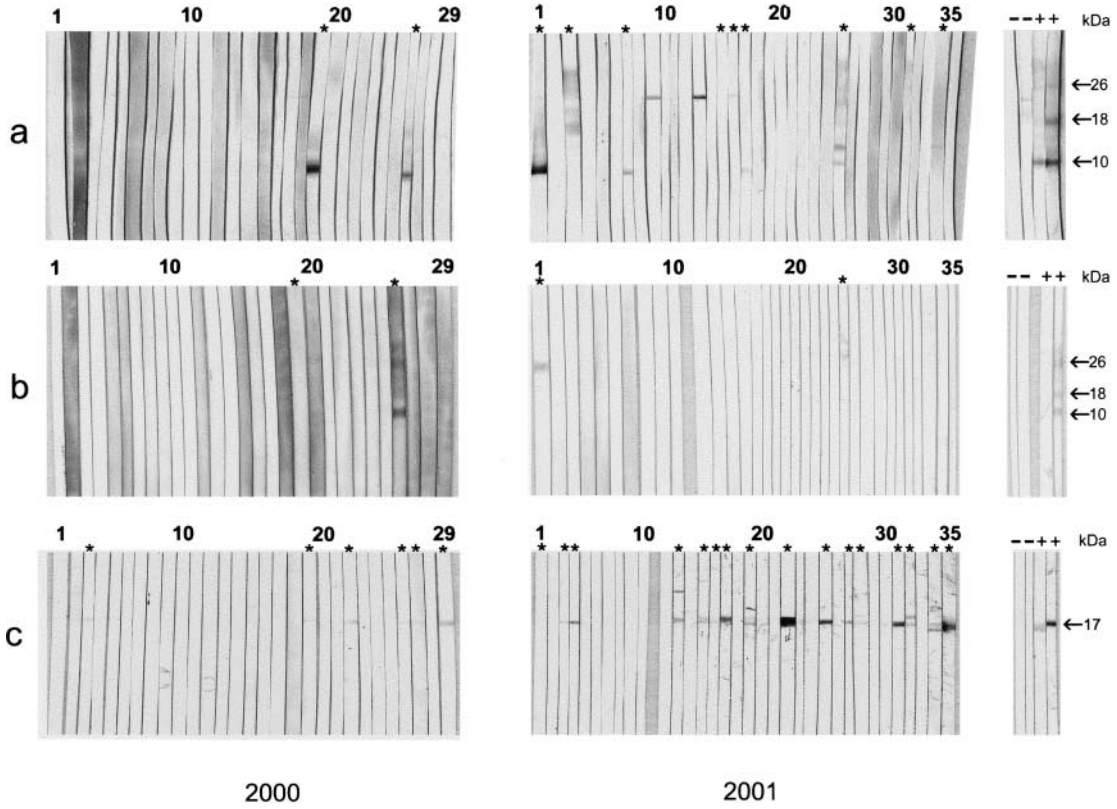

Fig. 2. Immunoblot figures of 64 dogs using GP (a), AffGP (b) and RecAg (c). Asterisks (*) indicate positives. Arrows indicate diagnostic bands.

2002; Ito *et al.*, 2002b; DDBJ/EMBL/GeneBank Accession No. AB066488).

Five of 35 dogs sampled in 2001 showed OD values of 0.067, 0.083, 0.096, 0.113 and 0.146 (cut-off value 0.060) in GP-ELISA (right half in fig. 1). Necropsy of one of the five dogs (OD value 0.113) revealed one cyst in the cardiac tissue. Figure 2 shows 9, 2 and 16 dogs positive in GP-IB, AffGP-IB and RecAg-IB, respectively. Five of 8 dogs positive in GP-IB were simultaneously GP-ELISA positive. Three others were negative but close to the cut-off value in GP-ELISA. Two of the five dogs were IB positive by all three different antigens (closed circles in fig. 1, asterisks in fig. 2) but three other dogs were positive by both GP- and RecAg-IB (open circles in fig. 1, asterisks in fig. 2).

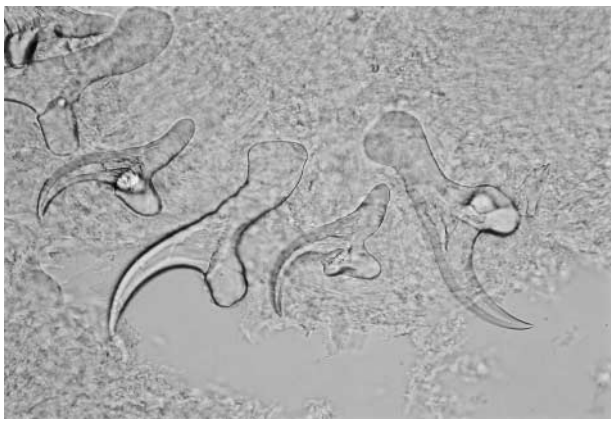

Fig. 3. Hooks (large 162.1 μm and small 111.2 μm) of a cysticercus of *Taenia solium* recovered from the brain of a single dog, which showed high responses to glycoproteins by both ELISA and immunoblot. Half of this cyst was used for mitochondrial DNA analysis.

Under the cut-off value, 8 of 64 dogs were positive in RecAg-IB (open squares in fig. 1) and 4 dogs were positive in both GP-IB and RecAg-IB (open circles in fig. 1). Therefore, it is necessary to evaluate the specificity of RecAg-IB and the sensitivity of AffGP-IB for the detection of infected dogs. It is expected that the low sensitivity of AffGP-IB may be cleared if more antigens are loaded. Nevertheless, all dogs positive by three or two antigens are highly expected to have been exposed to eggs of *T. solium*. Moreover, it is expected that GP-ELISA is highly reliable and simpler than these IBs. Both GP-ELISA and GP-IB confirmed to be highly reliable for human and porcine cysticercosis established at AMC (Ito *et al.*, 1998, 1999; Wandra *et al.*, 2000; Subahar *et al.*, 2001) are also available for detection of dogs infected with *T. solium*.

The evidence obtained from serology for the detection of specific IgG responses to *T. solium* metacestodes, mitochondrial DNA analysis and morphometric analysis

https://doi.org/10.1079/JOH2002128 Published online by Cambridge University Press

of worms recovered in necropsy reveals that dogs may become naturally infected with cysticerci of *T. solium*, and suggests that the life cycle of the parasite may be completed not only between humans and pigs but also between humans and dogs. Although it is unlikely that humans acquire infection of *T. solium* adult worms through eating dog meat or brain compared with eating pork meat or brain, human infection with *T. solium* is possible where the custom of eating dog meat in endemic areas still occurs (Okolo, 1986).

This is the first report which shows that *T. solium* cysticerci in dogs can be detected serologically and where canine derived cysts have been confirmed to be *T. solium* based on morphological and mitochondrial DNA analysis.

Acknowledgements

This study was supported in part by grants-in-aid for Scientific Research (A) and (B) (10480235, 11694259, 12557024, 14256001) from the Japan Society of Promotion of Science to A. Ito and by Japan Health Science Foundation to S.S. Margono.

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(Accepted 5 June 2002)