Recent Advances in Basic and Applied Science for the Control of Taeniasis/Cysticercosis in Asia

A Ito1, T Wandra2, R Subaha1,3, A Hamid4, H Yamasaki1, Y Sako1, W Mamuti1, M Okamoto5, K Nakaya6, M Nakao1, Y Ishikawa1, T Suroso2, PS Craig7 and SS Margono3

1Department of Parasitology and 6Animal Laboratory for Medical Research, Asahikawa Medical College, Asahikawa, Japan; 2Directorate General, Communicable Disease Control and Environmental Health, Ministry of Health and Social Welfare, Jakarta, Indonesia; 3Department of Parasitology and 4Department of Neurology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia; 5Department of Laboratory Animal Science, School of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan; 7Division of Biological Sciences, University of Salford, Manchester, England

Abstract. Detection of seven specific bands by immunoblot (IB) using glycoproteins (GPs) purified by lentil-lectin affinity chromatography has been the gold-standard for neurocysticercosis (NCC) serodiagnosis since 1989. However, due to the presence of contaminants, it was impossible to apply the GPs to ELISA. Our group at Asahikawa Medical College (AMC) succeeded in purifying the GPs by preparative isoelectric focusing; these higher quality GPs were suitable for ELISA. Based on the results of both IB and ELISA testing, developed at AMC for a field survey in Irian Jaya, it became evident that that area had pandemic NCC. We found many NCC patients, pigs full of cysts, and one dog infected with two cysts: these findings were based on serology. Recently, we conducted another survey to detect of the worm carriers of *T. solium*. Three of the 38 local people were positive by copro-antigen specific to *Taenia* species; these three patients expelled segments of *Taenia* spp and these were confirmed as those of *T. solium* by mitochondrial DNA analysis. When viable eggs of any taenid species could be obtained, they can be developed into metacestodes in NOD-scid mice; it then becomes possible to analyze morphological dynamics, metacestode antigenicity, the efficacy of new metacestocidal drugs, and mitochondrial DNA. Mitochondrial DNA analysis of the specimens obtained in Irian Jaya was compared with that of other isolates worldwide. *T. solium* is now divided into two genotypes: the Asian type, and the Africa-American type. Some aspects of the pathological differences between the Asian and Africa-American types and the antigenic components of these two types are discussed.

Introduction

Neurocysticercosis (NCC), caused by the larval stage of the pork tapeworm, *Taenia solium*, is one of the most serious cestode zoonoses and emerging diseases worldwide. In this short review article, some of the recent advances in the basic and applied science of taeniasis and cysticercosis are introduced. This review will consider: the serodiagnosis of NCC; the molecular diagnosis of NCC; the application of these diagnostic methods to an epidemiological survey of cysticercosis/taeniasis in West Papua (Irian Jaya), Indonesia; laboratory techniques for the preparation of parasite material suitable for these diagnostic methods; the prospects for the diagnostic use of animal models.

Correspondence: Akira Ito, Department of Parasitology, Asahikawa Medical College, Midorigaoka-Higashi 2-1-1-1, Asahikawa 078-8510, Japan.
Tel: ++81-166-68-2420; Fax: ++81-166-68-2429
E-mail: akiraito@asahikawa-med.ac.jp

Recent Advances

Serodiagnosis (native and recombinant antigens) in humans and pigs

A summary of essential information regarding contemporary serodiagnosis by immunoblot was by Gottstein et al (1986). This was followed by the characterization of the glycoproteins (GPs) of taenid cestodes by Parkhouse and Harrison (1987). Tsang and others (1989) established a method for the purification of highly specific GPs (7 bands from 8-10kDa up to 50kDa) by lentil-lectin affinity chromatography; Tsang’s serodiagnosis by immunoblot has been the gold standard ever since. However, as the purified GPs have some back ground noise (artefacts of higher molecular weights), it was impossible to apply them to ELISA. Ito et al (1998) succeeded in purifying these antigens, making them virtually free of the background noise that interfered with ELISA, by preparative isoelectric focusing (PIEF). This was the first ELISA system, which reliably differentiated cysticercosis from other diseases, especially the highly cross-reactive alveolar echinococcosis. The GPs purified by Asahikawa Medical College (AMC) group could be used for the
The NCC outbreaks in West Papua (Irian Jaya) in the early 1970s have been thoroughly documented (Tumada and Margono 1973; Gajdusek, 1978; Simanjuntak et al., 1997). Seroepidemiological surveys, using the GPs produced by the AMC group, have been conducted in Irian Jaya (Wandra et al., 2000). It is now clear that serology by immunoblot and ELISA is available for the detection of infected pigs (Ito et al., 1999; Subahar et al., 2001) and dogs (Ito et al., unpublished). We have detected, by serology, one dog with two cerebral T. solium cysts (DNA confirmation). Our most recent field survey was conducted in order to detect the worm carriers of T. solium (local people) in Irian Jaya. In 2001, we found three local people who were copro-antigen positive (of a sample of 38 persons). They all had adult T. solium, the segments of which were confirmed to be those of T. solium by mitochondrial DNA analysis (Okamoto et al., unpublished). We are currently conducting a similar survey in order to detect worm carriers while simultaneously checking for NCC in these carriers by serology. Such a strategy for the detection of NCC cases in humans and animals (pigs and dogs) is likely to be applied in other countries where NCC is endemic (Singh et al., 2002).

A laboratory animal models for cysticercosis

We found that severe combined immunodeficient (scid) mice were highly susceptible to in vitro hatched oncospheres of T. solium and T. saginata asiatica (T. asiatica), which developed into mature metacestodes (Ito et al., 1997a, b; Ito and Ito, 1999). Recently, we found that NOD-scid (non-obese diabetic), severe combined immunodeficient mice showed a much greater susceptibility to experimental infection with not only these two cestodes but also with T. saginata (Ito et al., 2001).

What do mouse models contribute to the understanding of human taeniid infections?

It is very easy to differentiate taeniid cestode segments expelled from patients by mitochondrial DNA analysis or PCR (Bowles and McManus, 1994; Yamasaki et al., in preparation). When viable eggs of these three human taeniid cestodes are available, NOD-scid mice can be infected by in vitro hatched oncospheres; the detailed development of metacestodes can then be analysed. For example, the oncospheres of T. saginata asiatica grow to 2-3mm in diameter in the liver of pigs (Fan, 1988), whereas they grow to 10mm or more in NOD-scid mice within 3-4 months of infection, when their size is similar to that of T. saginata (Ito et al., unpublished). A mouse model system might be expected to: (a) serve as an evaluation system for new drugs, replacing the use of domestic animals (Gonzalez et al., 1998); (b) allow the comparison of the antigenic components of the three human Taenia (T. solium, T. saginata, T. saginata asiatica); (c) assist in the detection of circulating antigens for the monitoring of ongoing infections especially in humans - pigs are usually killed within 6 months of birth and therefore do not need for checking ongoing infection.
ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research (A) (11694259), (B) (10480235, 10557029, 12557024) to A Ito.

REFERENCES


Ito A. Serologic and molecular diagnosis of zoonotic larval cestode infections [review]. Parasitol Int 2002;51:221-35.


