# REVIEW

# TOWARDS THE INTERNATIONAL COLLABORATION FOR DETECTION, SURVEILLANCE AND CONTROL OF TAENIASIS/ CYSTICERCOSIS AND ECHINOCOCCOSIS IN ASIA AND THE PACIFIC

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Abstract. Both cysticercosis and echinococcosis are potentially among the most serious helminth zoonoses threatening human health worldwide. However, due to the lack of reliable tools for confirmation or identification of patients or infected animals, epidemiological data are expected to be underestimated. Conversely, sometimes, such data are over estimated due to the lack of specificity. The most important issue for doing field surveys is that they use evidence based science. In this communication, advanced immunological and molecular tools for detection of individuals infected with either metacestodes or adult tapeworms are briefly overviewed, and the applications of such tools for epidemiological surveys in Indonesia, China and other countries are introduced. As immunological tools are based on antigen-antibody responses, there may exist some cross-reactions. Therefore, immunodiagnostic tools are expected to be useful for primary screening, and should be combined with confirmation of direct parasitological evidence (morphology or DNA), and imaging techniques for cysts. As a risk factor for human cysticercosis is the presence of tapeworm carriers, detection of taeniasis cases and differentiation of the three human Taenia species (Taenia solium, T. saginata and T. asiatica) in Asia and the Pacific requires consideration. Similarly, in northwest China, Echinococcus granulosus and E. multilocularis are coendemic and differentiation of these species is required in humans and definitive hosts. It is stressed that combination of several tools for identification of the parasite and for confirmation of diseases is important for obtaining highly reliable data before consideration of control of these zoonoses. Recent projects coordinated by Asahikawa Medical College have concentrated on immunological and molecular diagnostic techniques transferable to colleagues from endemic regions of Asia and the Pacific, and on organization of two international symposia to establish a platform for further collaboration in the future.

## INTRODUCTION

Both cysticercosis and echinococcosis are caused by infection with the metacestode stage of parasites in the genera *Taenia* and *Echinococcus* in family Taeniidae, respectively, and are considered to be among several neglected zoonoses including rabies, brucellosis, anthrax and African sleeping sickness (WHO/DFID-AHP, 2005). There are updated guidelines and reviews for these cestode zoonoses (Eckert *et al*, 2001; Craig and Pawlowski, 2002; Singh and Prabhakar, 2002; Craig and McManus, 2003; Murrell, 2005; Ito *et al*, 2006). Cysticercosis may be involved in one of the soil transmitted helminthiases due to ingestion of eggs of *Taenia solium* "pork tapeworm" as typical in India, where the majority of population is vegetarian, and other developing countries where the living environment is highly contaminated with the eggs from T. solium carriers (Singh et al, 2002). By contrast, taeniasis may also be included in one of the food-borne or meat-borne parasitic zoonoses. When cysticerci in undercooked or uncooked meat of pork and beef, and viscera of pigs are ingested by consumers, they develop into tapeworms of T. solium, T. saginata and T. asiatica, respectively. Among these three human taeniid species, T. solium appears exclusively to cause cysticercosis (Ito et al, 2004). The life cycle of T. solium is the most complicated and serious for human life, since neurocysticercosis (NCC) caused by even a single cysticercus or cysticerci in the brain is the most potentially pathogenic. Cysticercosis is caused by ingestion of eggs released from T. solium tapeworm carriers. It is important to establish reliable specific and sensitive tools for identification of patients or animals infected with cysticerci of T. solium, and for identification of taeniasis patients as the source of environmental contamination. Therefore, differentiation of three human Taenia species is an important public health issue (Ito and Craig, 2003).

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By contrast, echinococcosis is a dead-end parasitic zoonoses where life-cycles are maintained by human activity in relation to domestic animals and/or by prey-predator interaction of the wildlife. When eggs released from the caniid definitive host, mainly dogs and foxes, are ingested by people in endemic areas, some of them may become ill due to development of cystic echinococcosis (cystic hydatidosis) or alveolar echinococcosis (alveolar hydatidosis). The former is caused by Echinococcus granulosus and the latter by E. multilocularis. Recent molecular studies on E. granulosus strongly suggest that it is divided into 10 strains (G1 to G10) but taxonomic re-evaluation suggests that some strains should be elevated to species (Thompson and McManus, 2002; Romig et al, 2006; Nakao et al, in preparation).

M Nakao gave a special presentation entitled "Evolution of the genus *Echinococcus* and phylogeography of *E. multilocularis*" at the Asahikawa meeting held on 5-8, July, 2005 (Nakao *et al*, in preparation). Both Romig *et al* (2006) and Nakao *et al*, (in preparation) have a similar view on this issue where in the genotype group as *E. granulosus* sensu stricto (G1 to G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6 to G10). In the current review, we adopt the species name *E. granulosus* for all genotypes (G1 to G10), since taxonomy is not our main purpose of this review.

Most recent work by Xiao and others (2005) has revealed a new species, E. shiquicus from the Qinghai-Tibet plateau in China, which appears to be the most primitive or ancestral species among the three species distributed in the Northern hemisphere (ie E. granulosus, E. multilocularis, E. shiquicus). Alveolar echinococcosis (AE) is caused by E. multilocularis and maintained between foxes and small mammals, whereas cystic echinococcosis (CE) is caused by E. granulosus and maintained mainly between domestic dogs and livestock species. The pathology of AE crucially differs from that of CE and risk factor also may differ between these two diseases. Therefore, to establish differential diagnosis is important not only for identification of patients for suitable treatment, but also to establish a better quality of human life.

This review considers: 1) recent advances in immunological or serological tools for identification of individuals, either humans or animals, infected with these taeniid cestodes; 2) molecular tools for identification of the parasite specimens or isolates. Finally the activities of the Asahikawa Medical College team in relation to these topics in the past three years are also briefly summarized.

## IMMUNODIAGNOSIS OF CYSTICERCOSIS

As reviewed by several authors (Ito 2002; Ito and Craig, 2003, 2004; Schantz, 2006), serology to detect specific antibodies to cysticerci of T. solium has greatly advanced during the past decade. Detection of specific antibodies to metacestode glycoproteins (GP) of T. solium has provided highly specific immunodiagnosis of neurocysticercosis (Gottstein et al, 1986; Parkhouse and Harrison, 1987; Tsang et al, 1989; Ito et al, 1998). Furthermore, recombinant antigens and synthetic peptides of T. solium are now available (Huberr et al, 1999; Chung et al, 1999, 2002; Sako et al, 2000; Greene et al, 2000; Hancock et al, 2003, 2004). One practical issue under debate is whether an ELISA can be produced for neurocysticercosis that is as highly specific and sensitive as glycoprotein-immunoblots. Basically, the answer is "yes" as specific antigens for detection of cysticercosis cases can be readily produced using purified native T. solium antigens using monoclonal or polyclonal antibodies to the specific GP or preparative isoelectric focusing (Ito et al, 1998, 1999; Sako et al, 2000, 2006; Sato et al, 2003). Furthermore, chimeric recombinant antigen of T. solium (TsolAg1V/Ag2) is highly sensitive and specific and the resolution is very similar between ELISA and immunoblot and superior to native antigens (Sako et al, 2000, 2006; Sudewi R et al, in preparation). Another question is "How important is glycosilation for specific T. solium antigens?" Most recent studies by Sato and others (in preparation) have revealed that the T. solium GP epitope appears the same in all endemic areas in the world so far examined. However, the phenotypical picture of glycosilation differs at least between Asian and American/African T. solium metacestodes (Ito et al, 2002). Although T. solium may be broadly divided into two genotypes by mitochondrial DNA sequences ie, an Asian type and Africa/America type (Okamoto et al, 2001; Nakao et al, 2002a), there is no more molecular evidence as to the phenotypic differences in glycosilation between Asian and African/American genotypes.

Another immunodiagnostic strategy is to detect circulating antigens in human cysticercosis. Monoclonal antibody was raised against antigens of *T. saginata* rather than *T. solium* (Parkhouse and Harrison 1987; Brandt *et al*, 1992). The specificity may be based on the cross reaction between *T. saginata* and *T. solium*. Detection of circulating antigens may be expected to be more useful for monitoring of prognosis or post-treatment prevalence in human NCC (Dorny *et al*, 2004a) rather than for immunodiagnosis of cysticercosis independent of other information such as image data etc. (Ito and Craig, 2004). Currently there has not been a blind test to compare the reliability of detection of serum antibodies (antibody-ELISA) *versus* circulating serum antigens (antigen-ELISA) for human or porcine cysticercosis (Dorny *et al*, 2004b). WHO or FAO should arrange such comparative blind studies in order to establish gold standards available in developed and developing countries, including the cost effectiveness and the potential for in-house development rather than use of commercially available kits.

Serology to detect anti-cysticercosis antibodies is available not only for humans but also for pigs and dogs (Ito *et al*, 1998, 1999; 2002, 2004; Sako *et al*, 2000; Wandra *et al*, 2000; Subahar *et al*, 2001; Margono *et al*, 2003; Sato *et al*, 2003). The most important issue is not only to compare different tests but rather develop a more rigid molecular basis of such tools (Huberr *et al*, 1999; Chung *et al*, 1999, 2002; Sako *et al*, 2000; Greene *et al*, 2000; Hankock *et al*, 2003, 2004) and eventually provide kits or specific reagents available for other groups (Ito *et al*, 2006).

## IMMUNODIAGNOSIS OF TAENIASIS

In order to prevent cysticercosis, treatment of T. solium tapeworm carriers is one of the most important issues (Schantz et al, 1993; Pawlowski et al, 2005, Pawlowski, 2006). Currently, the best way for detection of tapeworm carriers is by copro-ELISA to detect antigens in fecal samples (Allan et al, 1990, 1992, 1996; reviewed by Allan and Craig, 2006). The copro-ELISA is essentially Taenia specific and therefore does not effectively differentiate the three human Taenia species. However, copro-ELISA is highly useful in endemic areas, since the sensitivity is almost 100% for detection of taeniasis carriers and fecal samples may be much easier to be collected in endemic areas than blood samples. There was a report to stress species-specific serology for human T. solium taeniasis, however, there was no clear evidence shown for illustration of the specificity differences between the two or among the three human Taenia species (Wilkins et al, 1999). Nakao and others in Asahikawa (unpublished) have also developed a serological approach using a recombinant protein from an adult T. solium stage specific HLBP (hydrophobic ligand binding proteins) for detection of taeniasis carriers.

# FIELD APPLICATIONS (TAENIASIS/ CYSTICERCOSIS) IN INDONESIA AND IN CHINA

Field surveys have been undertaken in Indonesia on the three human *Taenia* species (*T. solium*, *T. saginata*, *T. asiatica*). One project is on Samosir island in Toba Lake, North Sumatra, where the Batak, ethnic group used to frequently eat uncooked viscera of pigs with blood (raw sang sang) and where T. asiatica taeniasis appeared to be very common in the 1970's (Kosin et al, 1972; Cross et al, 1976; Fan et al, 1992). Nowadays, the local people tend to eat cooked sang sang. However, T. asiatica is still relatively common upto 2.5 % (6/240). Risk factors include meat sellers themselves who sell uncooked viscera and meat and often eat raw liver during slaughter or preparation of carcass for selling (Wandra et al, unpublished). In Bali, both taeniasis and cysticercosis of T. solium were historically common and a public health problem (Simanjuntak et al, 1997; Margono et al, 2005). However, recent surveys have revealed that T. saginata taeniasis is now much more common due to changes in eating habits by the predominant Hindi people in Bali (Sutisna et al, 1999; Wandra et al, 2006a, b). In contrast, T. solium is highly endemic in Papua (formerly Irian Jaya) and the cause of a major public health problem (reviewed by Ito et al, 2004, 2005; Margono et al, 2006).

The specific identification of the three *Taenia* species after expulsion of tapeworms, based on case detection by anamnesis and copro-ELISA tests, can be carried out using mitochondrial DNA sequencing and multiplex PCR (Wandra *et al*, 2000, 2003, 2006a; Ito *et al*, 2002; Yamasaki *et al*, 2002, 2004; Margono *et al*, 2003).

Similar field surveys on taeniasis/cysticercosis have also recently been initiated in Qinghai and Sichuan Provinces in China with several collaborating groups (Yamasaki *et al*, 2004, 2006; Wang *et al*, unpublished; Li *et al*, unpublished).

# MOLECULAR APPROACHES FOR TAENIASIS AND CYSTICERCOSIS

Based on the whole sequence of mitochondrial DNA of the human *Taenia* species by Australian and Japanese groups (Bowles and McManus, 1994; Nakao *et al*, 2000, 2002a, b, 2003a, unpublished; reviewed by McManus *et al*, 2004), it is now possible using PCR to specifically identify individuals harboring taeniid cysts, taeniid eggs, proglottids or whole worms, and from copro-ELISA positive fecal samples (Mayta *et al*, 2002; Yamasaki *et al*, 2002, 2004). Yamasaki and others (2004) found that frozen human fecal samples upto 10 years old may still be useful for DNA analysis.

# REEVALUATION OF THE DISTRIBUTION OF T. SAGINATA AND T. ASIATICA IN ASIA AND THE PACIFIC

Many parasitologists recognized the very strange observation of why apparently *T. saginata* only occurred where local people ate pork rather then beef (reviewed by Ito et al, 2003, 2004). Now, it has been confirmed that these cases were due to a new species, T. asiatica (Fan, 1988; Chao et al, 1988; Eom and Rim, 2001; reviewed by Simanjuntak et al, 1997; Hoberg et al, 2000; Hoberg, 2002, 2006; Ito et al, 2003, 2004; Eom, 2006). As stressed by Ito and others (2003), all human Taenia specimens in Asia and the Pacific region which were regarded as T. saginata in the 20th century, should be re-evaluated. However, most of these historical specimens were fixed in formalin and therefore rather difficult to extract sufficient DNA. Such specimens should in future be kept in ethanol for molecular identification and for public health education. Such a strategy may also be applied for T. saginata recovered from parts of Russia where people eat reindeer (commented by Ito, 2006).

#### IMMUNODIAGNOSIS OF ECHINOCOCCOSIS

There have been several recent good review articles on the serological diagnosis of echinococcosis, both CE and AE (Gottstein, 1992; Pawlowski et al, 2001; Siles-Lucas and Gottstein, 2001; Ito, 2002; McManus et al, 2003; Zhang et al, 2003). Recent work by Sako and others (2002) revealed that all candidate E. multilocularis protein antigens (EM10, EMII/3, EMII/3-10, EM4, Em18) reported by several groups independently (Vogel et al, 1988; Muller et al, 1989; Hemmings and McManus, 1991; Frosch et al, 1994; Felleisen and Gottstein, 1993; Ito et al, 1993, 1995, 1999) were in fact smaller components of the same protein, EM10. Among these, Em18 is the smallest component and has the lowest homology with human ERM (ezrin-radexin-moesin) proteins. Therefore, it is expected that Em18 may be more specific compared with the parent proteins (Fujimoto et al, 2005). Recent blind testing of French and German patient sera have shown that Em18 serology is highly specific and sensitive and the only good candidate antigen which can detect antibodies in AE cases that could not be detected with other antigens or serological tests (Breson-Hadni et al, in preparation; Kern et al, in preparation).

Antigen B is a major lipoprotein component present in cysts of *E. granulosus*. More than 95% of clinically expressed or advanced CE patient sera may become positive against Antigen B. Recombinant Antigen B has also been successfully produced by several groups using either *E. granulosus* or interestingly *E. multilocularis* RNA (Chemale *et al*, 2001; Gonzalez and Cachau, 2003; Arend *et al*, 2004; Mamuti *et al*, 2004). Mamuti and others (2006a) revealed developmental stage specific gene expression of different subunits of Antigen B, 8 kDa (reviewed by Mamuti *et al*, 2006b).

# MOLECULAR APPROACHES FOR ECHINOCOCCOSIS

#### Mitochondrial DNA (mtDNA)

Copro-DNA tests were established by several groups in Europe (Deplazes *et al*, 1996; Mathis *et al*, 1996; Dinkel *et al*, 1998, 2004; Abbasi *et al*, 2003). McManus's group in Australia was a pioneer for molecular phylogenic studies on mtDNA of platyhelminths including *E. granulosus* (G1) and *T. asiatica* (Bowles and McManus, 1994; reviewed by McManus *et al*, 2004). MtDNA sequences of *E. multilocularis* and *T. solium* were fully analyzed by Nakao and others (2002a, b, 2003a).

# **Microsatellite DNA**

Microsatellite DNA analysis of E. multilocularis (Bretagnue et al, 1996) was applied for demonstration of outcrossing in adult E. multilocularis in Hokkaido, Japan (Nakao et al, 2003b). Although mtDNA of E. multilocularis in Hokkaido has no polymorphism (Nakao et al, unpublished), microsatellite DNA analysis revealed polymorphism. Two microsatellites were isolated from a genomic library of E. multilocularis. The microsatellites, designated EMms1 and EMms2 consisted of a tandem repeat of a CAC trinucleoside unit. Southern blot hybridization suggested that each of them was a single locus. Using fox-derived wild tapeworms (N = 104), PCR amplification of microsatellites was performed to assess the usefulness of these loci. Four alleles of EMms1 and two alleles of EMms2 were found. The heterozygosities observed were 10.6% in EMms1 and 7.7% in EMms2 of adult worms in wild foxes captured from several cities between two geographically close cities where no heterozygosity was observed. These results suggested that both selfing and outcrossing occur in the adult stage of E. multilocularis.

The evolutionary changes of microsatellite DNA is much faster than mtDNA and is expected to be useful for tracing the polymorphism of parasite population at different scales and to measure parasite dispersal (Bart *et al*, 2006).

#### A new species, Echinococcus shiquicus

Through mtDNA analysis of isolates of *E.* multilocularis and *E. granulosus* from Chinese collaborating groups, a new species was discovered by mtDNA studies (Xiao *et al*, 2005; reviewed by Xiao *et al*, 2006). This species has been recovered to date only from Tibetan foxes and plateau pika on the eastern Tibet plateau in northwest Sichuan and south Qinghai Provinces. The zoonotic potential, if any, of *E. shiquicus* is unknown.

# TOWARD INTERNATIONAL COLLABORATION AND COOPERATION

#### Seminars for transfer of technology

Based on the advanced tools developed originally at AMC group, we wish to provide such diagnostic tools for those who are willing to collaborate on these cestode zoonoses. As a result of a special fund from the Ministry of Education, Japan (MEXT), Ito and collaborators were able to start several seminars from 2003 for transfer of technology. Seminars have already been organized on 4 occasions and a total of 26 trainees from 13 developing countries and 6 expert lecturers have been invited.

#### International symposia in 2003 and 2005

MEXT fully supported two international symposia on cestode zoonoses. The first one was on echinococcosis held in Bangkok at the 4<sup>th</sup> International seminar on Food-Borne Parasitic Zoonoses in 2003 (see Southeast Asian J Trop Med Public Health 2004, 35 (supplement). The second one was on taeniasis/ cysticercosis and echinococcosis held in Asahikawa in 2005 [see Parasitol Int 2006 (supplement)].

#### CONCLUSION

Sustainable collaboration with local researchers is essential research and development and for better resolution of diagnostic tests for these cestode zoonoses especially in Asia and the Pacific Region. It is hoped that ultimately control programs will incorporate some of the above developments that are already available for clinical, epidemiological and surveillance issue.

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