MOLECULAR AND IMMUNOLOGICAL DIAGNOSIS OF TAENIASIS AND CYSTICERCOSIS IN ASIA AND THE PACIFIC

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Abstract. Three human Taenia species, Taenia solium, Taenia saginata, and Taenia asiatica have been found and reported from Asia-Pacific region. T. solium neurocysticercosis (NCC) is one of the neglected and most lethal parasitic diseases worldwide. Therefore, clinical manifestation, neuroimaging, serology, and molecular identification of resected lesions of NCC are briefly overviewed. The difference between T. saginata and T. asiatica, as well as the two genotypes of T. solium is also overviewed, based on mitochondrial DNA analysis. Epidemiological topics from Indonesia, Thailand, and China through our collaboration projects are also overviewed with religious and socio-cultural background information.

INTRODUCTION

The pork tapeworm, Taenia solium, and the beef tapeworm, Taenia saginata, are the two major human taeniid cestodes and the major food-borne cestode zoonoses worldwide (Ito et al, 2003a, 2006a). The third species, Taenia asiatica, has recently been distinguished as an independent species (Eom and Rim, 1993; Hoberg et al, 2000; Eom et al, 2002; Eom, 2006); although there are some contrary opinions (Bowles and McManus, 1994; McManus and Bowles, 1994; Ito et al, 2003a). The number of specimens examined is too small to conclude that there is no hybrid in sympatrically endemic areas (Okamoto et al, 2007).

These three species can develop into adult tapeworms in humans exclusively (Hoberg et al, 2001). The most interesting species of medical and public health importance is T. solium. It has been speculated that T. solium was a human parasite that emerged in Africa several million years ago. Later, the intermediate host shifted from humans to pigs; which means that human cannibalism was essential for completion of the life cycle of this parasite. Molecular analysis of these three species has revealed that T. solium is distant from the other two, and T. saginata and T. asiatica are sister species (Hoberg, 2002; Eom, 2006). The most crucial difference between these two species is not morphological but the intermediate host spectrum. The former develops from an egg into a metacestode, cysticercus, in cattle; whereas, the latter does so in the viscera of pigs (Fan, 1988). As both T. solium and T. asiatica require swine as the intermediate host, a working hypothesis for cysticercosis of T. asiatica was introduced (Ito, 1992). However, it is now established that cysticercosis is exclusively caused by T. solium, because both T. saginata and T. asiatica are sister species, and cysticercosis due to T. saginata does not occur (Ito et al, 2003a).

In this review article, taeniasis/cysticercosis of T. solium is reviewed, initially with molecular and immunological diagnostic tools. Then,
taeniasis of *T. saginata* and *T. asiatica*, as well as the two genotypes of *T. solium*, is discussed.

**TAENIASIS AND CYSTICERCOSIS OF *T. SOLIUM* IN THE ASIA-PACIFIC REGION**

As these all are food-borne zoonotic cestodiasis, it could be expected that these would be eradicable (Schantz *et al.*, 1993a, 1998). However, increases in the population of refugees and/or immigrants through economic, political, or religious problems may often cause poverty and social upheaval. The wave of globalization has accelerated this phenomenon worldwide. Such infections are caused by WAFP (contaminated water, air, food, and people) (Ito, 2007). Among the three human *Taenia* species, the most serious species is *T. solium*. *T. solium* neurocysticercosis is one of the most lethal parasitic diseases worldwide and is on the list of neglected tropical diseases (Ito *et al.*, 2006a, b; Craig *et al.*, 2007). It is found in the majority of developing countries where the people eat pork. However, due to the globalization, outbreaks of *T. solium* cysticercosis have been reported in an orthodox Jewish community in New York (Schantz *et al.*, 1993b), and in Muslim communities in Saudi Arabia (Al Shahrani *et al.*, 2003) and in Kuwait (Hira *et al.*, 2004) through immigrants or visitors from non-Jewish or non-Muslim societies. Recent review articles on the clinical manifestation, neuroimaging, serology, and molecular confirmation of neurocysticercosis have been published (Schantz *et al.*, 1998; Ito *et al.*, 2006a, b). More information is available from these reviews.

**What is *T. solium* neurocysticercosis (NCC)?**

The major clinical manifestations of NCC are epileptic seizures (> 60%) and/or intracranial hypertension (> 30%), and/or meningitis (= 30%). However, these are not specific to NCC. Epilepsy due to NCC is expected, to be the main cause of late-onset epilepsy in developing countries where NCC of *T. solium* is endemic. Epileptic seizures are the most common symptom (70-90%) of NCC. More important clinical background information is that the majority of NCC cases are asymptomatic until the parasite, cysticercus or cysticerci, will be damaged by the host immune responses or by the treatment with metacestocidal drug such as praziquantel (Sarti *et al.*, 1994). Recently, we experienced one asymptomatic NCC patient who harbored *T. saginata* in Bali. This tapeworm carrier was treated with praziquantel, and epileptic seizure attacked within one day (Wandra *et al.*, unpublished). Therefore, the basis for suspecting NCC is the following information: 1) neurologic disorders, 2) neuroimaging, and 3) history of traveling to and/or living in *T. solium* endemic areas. Neuroimaging is not always typical, and approximately only 10% of NCC may show typical imaging (Wilson *et al.*, 1991; White, 1997). Based on such background information suggesting or suspecting NCC, we have to carry out as an additional test, 4) serology, using highly specific antigens (Ito and Craig, 2003; Ito *et al.*, 2006b).

**Gold standards for serology to detect NCC**

The gold standard for the detection of NCC is immunoblot using purified glycoproteins (GPs) (Tsang *et al.*, 1989; Ito *et al.*, 1998; Chung *et al.*, 1999; Sako *et al.*, 2000; Green *et al.*, 2000; Hancock *et al.*, 2003). Research groups at CDC, Atlanta, USA and Asahikawa Medical College (AMC), Japan have developed immunoblots that use purified GPs, using different tools for purification of GPs. CDC applied lentil-lectin affinity chromatography (Tsang *et al.*, 1989); whereas, AMC did isoelectric focusing (Ito *et al.*, 1998) to detect specific antibodies. The most interesting and crucial difference in resolution between the two groups was that CDC could recommend immunoblots only; whereas, AMC did isoelectric focusing (Ito *et al.*, 1998) to detect specific antibodies.
(Ito et al., 1998; Sako et al., 2000; Sato et al., 2003, 2006). Resolution from recombinant chimeric antigens was similar to or better than that from native GPs, and more than 94% of confirmed NCC cases were easily detected with no cross reactions from highly cross reactive echinococcosis, either cystic or alveolar (Sako et al., 2000).

However, the serology is not always perfect. The sensitivity is 30-60% in solitary NCC cases (Wilson et al., 1991). Therefore, it is not certain that we can detect specific antibodies in NCC cases with a solitary cyst (Ito et al., 1999; Ohsaki et al., 1999; Ishikawa et al., 2007).

We can expect no or poor antibody responses from NCC cases in the following cases: 1) immunodeficiency, 2) single cyst, 3) inactive, calcified lesions only, or 4) other species that may cause zoonotic cysticercosis. In such cases, molecular identification becomes crucial (Ito, 2002; Ito and Craig, 2003; Ito et al., 2006b).

An alternative approach is to detect circulating antigens in patients or domestic animals using monoclonal antibodies by ELISA only. Thus far, we know it is rather complicated to draw the cutoff values using mathematics. Very small differences in OD values have to be evaluated for the differentiation of patients or animals infected with T. solium cysticerci. It may often cause false positives from non-infected pigs in developed country in Europe due to how the cutoff values were drawn. There is a crucial difference from antibody-ELISA, where we can differentiate NCC cases from other diseases even by the naked eye (Sato et al., 2003). There is no real blind test to evaluate the specificity or sensitivity of such tools (Dorny et al., 2004). Such tools to detect antigens (antigen-ELISA) are based on the use of monoclonal antibodies, not to the cysticercus of T. solium but to that of T. saginata, and they are used to detect cross-reactive components of T. solium infection. To date, there is no visual immunological, biochemical, or molecular information on the cross-reactive components. Such tools to detect circulating antigens are therefore expected to be useful for monitoring of progression (Ito and Craig, 2004). If an antigen-ELISA is established based on monoclonal antibodies to species-specific components of T. solium, its usefulness is expected to be great, not only for monitoring of progression, but also for diagnosis (Ito and Craig, 2004).

**Molecular identification of a cysticercus or cysticerci in histopathological specimens**

Surgery is an option recommended in urgent cases of malignant tumors or other diseases in the brain with acute severe symptoms, including neurologic disorders. When NCC cases are admitted without any information from neuroimaging and/or serology supporting NCC, they may have surgical options (Ito et al., 1999; Ohsaki et al., 1999; Ishikawa et al., 2007). Histopathological examination is still the gold standard to confirm rostellar hooks and suckers unique to taeniid species. However, it is not always easy to confirm hooks. In such cases, it is not critical to diagnose them as T. solium infections. Such cases were sometimes described as T. saginata (Pawlowski and Schultz 1972; Šlais, 1973). There could be rare cysticercosis cases caused by other rare zoonotic species, such as T. taeniaeformis, T. hydatigena, T. ovis, T. serialis, T. multiceps, T. crassiceps, or others. Therefore, molecular approaches using such specimens are now crucial to identify the species (Yamasaki et al., 2004a, b, 2006; Ito et al., 2006b). Development of hooklets is highly variable, including no hook in SCID mice experimentally infected with oncospheres of T. solium (Margono et al., 2003; Ito et al., unpublished). As T. solium worldwide can be differentiated into two genotypes (Okamoto et al., 2001; Nakao et al., 2002), we can differentiate Asian and African/American genotypes. Furthermore, two polymorphisms in both genotypes have also been found (Campbell et al., 2006; Sudewi et al., 2008).
How to detect carriers of *Taenia* spp

For the prevention of cysticercosis of *T. solium* in human and for production of safe meat or food without the cysticerci of *T. saginata, T. asiatica*, or *T. solium*, detection of taeniasis carriers is essential to block the lifecycle of these human *Taenia* spp (Schantz *et al.*, 1993b, 1998; Schantz, 2006; Craig and Ito, 2007). There are several tools to detect taeniasis carriers. The most popular tool is copro-ELISA test (Allan *et al.*, 1992, 1996; Allan and Craig, 2006). However, it is not a species-specific molecular tool, as the copro-DNA test (Yamasaki *et al.*, 2004a) that has been introduced for the confirmative identification of the species. The detection of antibodies specific to adult *Taenia* (Wilkins *et al.*, 1999; Nakao *et al.*, unpublished) has also been reported, although the species specificity has not always been well evaluated. The most reliable detection of taeniasis carriers is the history of expulsion of gravid proglottids, especially in *T. saginata* and *T. asiatica* (Wandra *et al.*, 2006a). It may also be useful for *T. solium* (Flisser *et al.*, 2005).

Molecular differentiation of three taeniid species in humans

Based on mitochondrial (mt) DNA analysis, it is relatively easy to differentiate between these three species (Okamoto *et al.*, 2001; Nakao *et al.*, 2002; Yamasaki *et al.*, 2004a). Now, nuclear DNA sequencing data are also available for the differentiation of these three species (Okamoto *et al.*, unpublished).

**TAENIA SAGINATA AND T. ASIATICA IN ASIA-PACIFIC REGIONS**

Many researchers working in Asia-Pacific have long recognized a curious phenomenon (Yokogawa, 1935; Huang *et al.*, 1966; Kosin *et al.*, 1972; Fan, 1988). This puzzle was that adult taeniid tapeworms expelled from people in Asia-Pacific regions seemed to be *T. saginata*, the beef tapeworm, although these people ate pork rather than beef (Simanjuntak *et al.*, 1997; Ito *et al.*, 2003a). Now it has become clear that it was due to a third species, *T. asiatica* (Eom, 2006). It is rather difficult to differentiate these two species morphologically; although

Fig 1- Fully developed cysticerci of *T. asiatica* (a) and *T. saginata* (b) in NOD/Shi-scid mice 3 months after subcutaneous and intraperitoneal injections of *in vitro* hatched oncospheres, respectively.
there is a crucial difference in the intermediate host animals as mentioned above. Usually, *T. saginata* cysticercus is much bigger than that of *T. asiatica*. Some different morphological characteristics of the cysticercus of these two species may become clearer when we obtain cysticerci from immunodeficient mice (Fig 1) (Nakaya et al., 2006). The cyst wall is much thinner in the cysticerci of *T. asiatica* compared with *T. saginata*. Through our collaboration projects, *T. asiatica* has been confirmed from Taiwan, China, Korea, Indonesia, Philippines, Vietnam, and Thailand (Ito et al., unpublished).

**TAENIASIS AND CYSTICERCOSIS IN ASIA-PACIFIC REGIONS**

**In Indonesia**

We have been working in three regions in Indonesia: Papua (Irian Jaya), Bali, and Sumatra (Wandra et al., 2006a, b). In Papua *T. solium* NCC is a very serious public health problem, because the majority of the people in Papua are Christian. As pigs and dogs have free access to human feces, we checked dog sera and found that more than 10% of dogs showed antibody responses to *T. solium* GPs and recombinant antigens (Sato et al., 2003). Approximately 50% of adult population was expected to have been exposed to the eggs of *T. solium* (Wandra et al., 2000, 2003; Subahar et al., 2001; Ito et al., 2002, 2004; Margono et al., 2003, 2005, 2006). Dogs as well as humans and swine have been confirmed to be infected with the cysticerci of *T. solium* by molecular and morphological identification of the cysts obtained, based on serological screenings (Subahar et al., 2001; Ito et al., 2002; Sato et al., 2003; Margono et al., 2006).

Most recent work on comparison of mtDNA of *T. solium* from Bali and Papua suggests that *T. solium* in Bali and Papua might have different origins (Sudewi et al., 2008); although it has been conceived that *T. solium* in Papua was introduced from Bali (Gadjusek, 1978; Simanjuntak et al., 1997). To date, there has been no record of *T. saginata* or *T. asiatica* in Papua.

*T. solium* has historically been well recognized in Bali; however it is now sporadic (Wandra et al., 2006a; Sudewi et al., 2008). By contrast, *T. saginata* is rather common in Bali. The majority of Balinese are Hindu but differ from people in India or Nepal because they eat beef. The Balinese like to eat uncooked or undercooked minced beef (*beef lawar*), or minced pork with fresh blood (*pork lawar*), but do not eat uncooked or undercooked viscera. Therefore, there has been no human case of *T. asiatica* (Margono et al., 2006; Wandra et al., 2006a). Recent fieldwork, in August 2007, in Bali found unique dual infections of *T. saginata* and *T. solium* (asymptomatic NCC) (Wandra et al., unpublished).

*T. asiatica* is well known among the Batak people on Samosir Island in North Sumatra. They are Christians and eat uncooked or undercooked pork with viscera, and dog meat with viscera. There was the only record of *T. solium* over 20 years ago (Wandra et al., 2006b).

As the majority of Indonesian people are Muslim, *T. saginata* is the only species not expected to be common. Therefore, nowhere in Indonesia do the three human taenid species occur sympatrically. This is due to the barriers of religion and social culture in each respective area (Wandra et al., 2006b, 2007).

**In Thailand**

To date, there has only been a report demonstrating *T. asiatica* from Thailand (Anantaphruti et al., 2007a). *T. saginata* was confirmed from Chiang Mai, Thailand, with molecular tools (Bowles and McManus, 1994; Morakote et al., 2000; Anantaphruti et al., 2007b). In Kanchanaburi, close to the border to Myanmar, three human taenid species were found sympatrically. An interesting finding was a dual infection with *T. solium* and *T. asiatica* in one carrier, who harbored two *T. solium* and one *T. asiatica* (Anantaphruti et al., 2007a).
In China

Taeniasis and cysticercosis are rather common diseases in China (Chen et al., 2005; Ikejima et al., 2005). Three human taeniid species have been reported to occur sympatrically in Yunnan (Eom et al., 2002; Yamasaki et al., 2004a) and in Sichuan Province (Li et al., 2006). More information will be available from China as discussed previously (Ito et al., 2003b).

PERSPECTIVES

In Asia-Pacific regions, it is now very clear that three human taeniid species are sympatrically occurring in several areas. Therefore, we have to re-examine T. saginata in these regions (Ito et al., 2003a). To do epidemiological studies in such areas, real-time molecular identification is the best for identification and treatment of worm carriers. Such endemic areas are rather remote, and multiplex PCR is not easily applicable. Therefore, a new molecular tools using LAMP method is expected to be available for such fieldwork in sympatric areas for the three species (Nkauawa and Sako, unpublished).

As Asian people, including immigrants and refugees from Asia-Pacific regions, are moving out of Asia-Pacific and working mainly in Europe, America, and Africa. Simultaneously, people from Africa, Americas, Australia, New Zealand, and Europe are traveling to Asia-Pacific, we have to re-evaluate all specimens of T. saginata to determine whether they are real T. saginata or T. asiatica. There is a similar situation in Japan, because there is no challenge to re-evaluate T. saginata cases treated in Japan if they are real T. saginata or T. asiatica.

CONCLUSION

1) Three human Taenia species are occurring in Asia-Pacific regions. Taeniasis may be detected by several tools, including classical morphology, copro-ELISA, and copro-DNA; 2) Molecular identification of these human Taenia species is feasible; 3) Serology using purified glycoproteins or recombinant antigens of T. solium is highly useful for detection of cysticercosis of T. solium in humans, pigs, and dogs. Serology for detection of taeniasis is not yet so sensitive; 4) Confirmation of NCC is based on neuroimaging, serology, histopathology, and molecular identification, although histopathology and molecular identification are options after surgery; 5) T. saginata in the world as well as in Asia-Pacific regions should be re-evaluated by molecular tools.

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