Multiple genotypes of *Taenia solium* – ramifications for diagnosis, treatment and control

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Abstract

Mitochondrial DNA sequences of *Taenia solium* have fully been analyzed. Analysis of the full length of *cytochrome c oxidase subunit 1* (1620 bp) and *cytochrome b* (1068 bp) genes of *T. solium*, isolated from Asia (China, Thailand, Indonesia and India), from Latin America (Mexico, Ecuador, Bolivia, Peru and Brazil) and from Africa (Tanzania, Mozambique and Cameroon), has revealed that the two phylogenies obtained were similar to each other regardless of the genes examined. The isolates from Asia formed a single cluster, whereas those from Latin America combined with those from Africa to form an additional cluster. It was estimated that these two genotypes emerged approximately 4-8 x 10^5 years ago. These results together with recent study of the ancient of human taeniid cestodes, historical data of swine domestication, distribution of pigs and colonization suggest that *T. solium* was introduced recently into Latin America and Africa from different regions of Europe during the colonial age, which started 500 years ago, and that *T. solium* of another origin independently spread in Asian countries, perhaps from China. It is interesting to trace the reason why *T. solium* of European origin did not invade or spread into Asia during the colonial age. As it is easily expected that *T. solium* moves with the worm carriers, it is important to identify these two genotypes as well as other human taeniid species, *T. saginata* and *T. saginata asiatica* (*T. asiatica*), which can not be differentiated from each other morphologically. BESS T-base analysis for differentiation of these two genotypes of *T. solium*, *T. saginata* and *T. asiatica* has also been established. It is expected that BESS T-base analysis may differentiate African isolates from Latin American isolates. However, more samples should be analyzed for obtaining conclusive evidence for the latter. Serological analysis of cyst fluid of *T. solium* cysticerci obtained in China and Indonesia appears to differ from that in Mozambique and Ecuador. These differences are discussed in the light of possible differences in pathology of *T. solium* worldwide. As it has been speculated that the ancient *T. solium* emerged several million years ago in Africa, it is interesting to analyze more isolates from Africa. Such working hypothesis may be evaluated combined with symptomatology and serology when we get additional DNA data from such areas, since there are some varieties of manifestation of neurocysticercosis with or without subcutaneous cysticercosis and of antigens of cyst fluid.
of *T. solium* from Asia and from Africa and/or America. Transfer of techniques of molecular identification and sero- and immuno-diagnoses for researchers/technicians from endemic countries using their own materials from endemic countries should be promoted with the aim of better international cooperation for the control of cysticercosis.

Keywords: *Taenia solium*; Cestode zoonosis; Cysticercosis; Taeniosis; Mitochondrial DNA; DNA polymorphism; Control; Review

1. Introduction

*Taenia solium* cysticercosis is known to be highly endemic in Latin America, but over the past years more data are becoming available from Asia and Africa, which show that the prevalence of *T. solium* on these continents is as high as or even higher than in Latin America (Simanjuntak et al., 1997; Geerts et al., 2002; Ito et al., 2002a; Singh et al., 2002). In the European Union *T. solium* has been virtually eradicated, but the risk of reintroduction due to increased numbers of immigrants or refugees from endemic countries is real. Reintroduction of *T. solium* in the USA occurred already due to the high number of immigrants from Latin America (Schantz et al., 1992, 1998) and it is stressed that more than 1000 NCC patients/year are diagnosed in USA (White, 1997). As the life-cycle of *T. solium* is rather simple with humans as the only definitive hosts and pigs as the most important intermediate hosts, it is assumed that the parasite might be eradicable (Schantz et al., 1993; Craig et al., 1996).

Although genetic variation is well known in other cestodes such as *Echinococcus granulosus* (Pearson et al., 2002; Le et al., 2002) and is suspected in *T. saginata* (Wouters et al., 1987), not much is known about this phenomenon in *T. solium*. In this paper the available information on *T. solium* from the view points of molecular biological, serological and epidemiological studies is reviewed together with the possible impact on symptomatology, diagnosis and control.

2. The origin of the genus *Taenia*
The occurrence of *T. solium* and *T. saginata* has traditionally been linked to the domestication of the obligatory intermediate hosts, pigs and cattle, respectively and coincidental colonisation of humans by cestodes typical of companion carnivores (dogs) not more than 10,000 years ago (Baer, 1940; Cameron, 1956; Epstein & Bichrad, 1984; Bradley et al., 1996). However, recent research indicates that the occurrence of *Taenia* tapeworms in humans predates the development of agriculture, animal husbandry and domestication of cattle and swine (Hoberg et al., 2000, 2001). Phylogenetic and divergence data analyses indicate that (i) *T. saginata* + *T. asiatica* and *T. solium* represent the result of two independent host shifts to hominids; (ii) the immediate ancestors of these tapeworms used carnivores as definitive hosts and bovids as intermediate hosts and (iii) host switching occurred in sub-Saharan Africa prior to domestication of ungulates (Hoberg et al., 2001). Dietary and behavioural shifts from herbivorous to scavenging and carnivorous habits were at the origin of host switching by tapeworms to hominids from hyaenids and felids. This ‘hominids first’ hypothesis is in contrast with the long held belief linking the origins of human *Taenia* spp. to animal domestication but provides a better explanation of how tapeworms circulating among carnivores and antelope in Africa could have colonized a novel definitive host.

3. Multiple genotypes of *Taenia solium*

3.1. Two genotypes of *Taenia solium* worldwide

Nakao et al. (2000, 2002a, 2002b) have analyzed the full length of mitochondrial DNA of taeniid cestodes, mainly *T. solium* and *E. multilocularis* (Ito et al., 2002a). Mitochondrial DNA sequences of a total of 17 cestode species were deposited in GenBank by Nakao in 1998. Okamoto et al. (2001) analyzed the partial sequence of *cytochrome c oxidase subunit 1 (Cox1)* gene of *T. solium* obtained from various areas in Asia and America and found two genotypes, Asian and American types. Similar results using 391 bps of *Cox1* gene were reported by Hancock et al. (2001), but they failed in finding the basic genetic differences between Asia and American isolates. Later, Nakao et al. (2002a) did similar work based on analysis of full length of *Cox1* gene (1620 bps) and *cytochrome b (Cytb)* gene (1068 bps) and some other genes using additional samples including African isolates and confirmed Okamoto’s two genotypes, and these authors revealed that *T. solium* from
Africa form another cluster with that from America.

Recent work by Hoberg et al. (2000, 2001) indicated that *T. solium* was very closely related to *T. hyaenaei* and emerged in Africa several million years ago (MYA) then spread out to Eurasia. As there are no fossil records of tapeworms, it is impossible to estimate directly the speed of molecular evolution in tapeworms. Using a pair model for mice and rats which diverged 9-12 MYA (Jaeger et al., 1986), the dates of divergence in the 2 genotypes of *T. solium* were estimated to be 0.4-0.8 MYA in *Cox1* gene and 0.8-1.3 MYA in *Cytb* gene. Based on historical data of swine domestication, distribution of pigs and colonization, it was speculated that *T. solium* was introduced recently into Latin America and Africa from different regions of Europe during the colonial age, which started 500 years ago, and that *T. solium* of another origin independently spread in Asian countries (Nakao et al., 2002a, Ito et al., 2002b).

3.2. *T. solium* of European origin

From European countries, *T. solium* cysticercosis has been eradicated except a few areas where sporadic human cases are reported (Overbosch et al., 2002). However, it is unclear if these cases are due to the European *T. solium* or due to foreign *T. solium* reintroduced by immigrants or travelers from other areas. We expect that some isolates of European origin might remain in Spain/Portugal and Russia or the northern part of Mongolia where *T. solium* cysticercosis still exists.

The question remains why *T. solium* of European origin did not invade or spread in Asia during the colonization age. There must have been ample opportunities due to the intensive and longstanding exchange which took place between the British people and India or between the Dutch and Indonesia. It may be rational to speculate that the Asian genotype of *T. solium* emerged in the southern part of China where domestication of pigs started independently from Europe (Clutton-Brock, 1987). The Chinese character for the meaning of “house” consists of “pigs are kept under roof” and such life style is typical in the southern part of China and southeast Asian countries until now.

3.3. Molecular identification of *T. saginata*, *T. asiatica* and two genotypes of *T. solium*

*Taenia solium* and *T. saginata* are well-known human cestodes with worldwide
distribution and medical as well as economic importance, respectively. Recently, another species or subspecies of *T. saginata* was discovered in Asia and supported with detailed experimental data (Fan, 1988). Whether this Asian *T. saginata* has to be considered as *T. saginata asiatica* (Fan, 1988; Fan et al., 1995; Zarlenga et al., 1994; Bowles & McManus, 1994) or as a separate species *T. asiatica* (Eom & Rim, 1993; Hoberg et al., 2000) remains a matter of debate. Brief historical comments on this problem have been summarized by Simanjuntak et al. (1997). As mentioned above, mitochondrial DNA analysis of *T. solium* worldwide revealed that *T. solium* could be divided into two genotypes, Asian and America/Africa types (Okamoto et al., 2001, Nakao et al., 2002a, Ito et al., 2002b). These all make the morphological identification of these taeniid tapeworms more complicated and more difficult. In order to overcome these problems, molecular approaches such as restriction fragment length polymorphism (RFLP) (Bowles & McManus, 1994), single-strand conformation polymorphism (Gasser et al., 1999) and PCR (Gasser et al., 1995, Gonzalez et al., 2000) have been developed. Using the databases of all these taeniid species and some other cestodes (Nakao et al., 2002a), Base Excision Sequence Scanning Thymine-Base (BESS T-base) Reader system was introduced for differentiation of all these human taeniid species and two genotypes of *T. solium* (Yamasaki et al., 2002a, 2002b). Multiplex PCR analysis for differentiation of these all human taeniid cestodes is now available (Yamasaki et al., in prep.).

### 3.4. Differentiation of the African from the American genotype of *T. solium*

Since there are some critical differences of DNA sequences between isolates from Africa and America (Yamasaki et al., 2002a, 2002b), it is expected to be able to differentiate the African genotype from the American genotype. However, more specimens from different regions of Africa including not only pigs, dogs or patients, but also from wild animals especially carnivore species such as hyena are needed in order to validate this hypothesis (Hoberg et al., 2001). Further analysis of DNA of *T. solium* worldwide is important to obtain genetic maps of *T. solium* worldwide.

### 4. Impact of the genotype on symptomatology, diagnosis and control

#### 4.1. Antigenic differences of cyst fluid of *T. solium*
When cyst fluids of *T. solium* obtained from Asia was compared with those from Africa and America by immunoblots, there appeared to exist some differences under reducing condition. Glycosilation is more abundant in cyst fluid of *T. solium* from African and American origin so far we have examined (Ito et al. 2002b). Recently, we checked another sample from India and found it corresponded also to the Asian type (Oomen, Rajishekhar, Ito, Murrell, unpublished). Up to now, however, there are no indications that the origin of the cyst fluid might have an impact on its diagnostic efficacy.

4.2. Neurocysticercosis with or without subcutaneous cysticerci

Several authors have mentioned that most of NCC patients in Latin America have no evidence of subcutaneous cysticercosis (SCC), and this differs from those patients in Asia who usually manifest with both NCC and SCC (Feng et al, 1979; Simanjuntak et al., 1997). In Africa, it is somewhat more complicated, since subcutaneous cysticerci are quite rare in some regions whereas in other regions they are common in NCC patients similar to Asia (Dumas et al., 1990; Cruz et al., 1994; Boa et al., 1995; Vílhena et al., 1999; Nguekam et al., 2002). Up to now it has not been demonstrated that *T. solium* strains, which have a preference for the brain, are genetically different from those which have a wider range of predilection sites. However, as *T. solium* may travel around the world with human tapeworm carriers, more research work is required on molecular and immunodiagnostic aspects in order to better understand its epidemiology.

4.3. Conclusion

What is the relevance of the occurrence of different genotypes of *T. solium*? Is there any effect on the clinical manifestations of cysticercosis and is there any impact on diagnosis or for control? It is still difficult for us to answer these questions at this moment. Based on the currently available information, there seems not to be any impact. It is obvious, however, that more data need to be collected before reliable conclusions can be drawn.

5. Regional reference centers for taeniosis/cysticercosis

In order to stimulate the collaboration between different regions and countries and to
improve the exchange of parasite materials and reference sera, the establishment of an international and several regional reference centers should be encouraged. Technical transfer and training using own materials from endemic countries is the most important prerequisite and no one does recommend purchasing commercially available kits for epidemiological survey or identification of patients in endemic countries but encourage those who have responsibility for the control of taeniosis/cysticercosis in endemic countries to use their own materials and prepare antigens of high quality and apply for serodiagnosis or seroepidemiology using such home-made antigens and techniques and ideas.

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References


Hancock, K., Broughel, D.E., Moura, I.N.S., Khan, A., Pieniazek, N.J., Gonzalez, A.E.,
cytochrome oxidase I, internal transcribed spacer 1, and Ts14 diagnostic antigen
sequences of *Taenia solium* isolates from South and Central America, India, and Asia.
Int. J. Parasitol. 1601-1607
hypothesis for species of the genus *Taenia* (Eucestoda: Taeniidae). J. Parasitol. 86, 89-
98.
Ito, A., Sako, Y., Nakao, M., Nakaya, K.; 2002a. Neurocysticercosis in Asia:
Cestode Zoonoses: Echinoccosis and Cysticercosis – An Emergent and Global
DNA of *Taenia solium*: from basic to applied science. In: Singh, G, Prabhakar, S. (Eds.)
*Taenia solium* Cysticercosis. CABI Press, Oxson, pp. 47-55.
data compared with the molecular clock. Comptes rendus de l’Academie des Sciences
302, 917-922.
Trends Parasitol. 18, 206-213.
Nakao, M., Yokoyama, N., Sako, Y., Fukunaga, M., Ito, A.; 2002b. The complete mitochondrial DNA sequence of the cestode *Echinococcus multilocularis*
(Cyclophyllidea: Taeniidae). Mitochondrion 1, in press.
Nguekam, Zoli, A.P., Zogo, P.O., Kamga, A.C.T., Speybroeck, N., Dorny, P., Brandt, J.,
Losson, B., Geerts, S.; 2002. A seroepidemiological survey of human cysticercosis in

Okamoto, M., Nakao, M., Sako, Y., Ito, A.; 2001. Molecular variation of *Taenia solium* in

Neurocysticercosis in Europe. In: Craig, P., Pawlowski, Z. (eds.) Cestode Zoonoses:
Echinococcosis and Cysticercosis – An Emergent and Global Problem. IOS Press,
Amsterdam, pp. 33-40.

Molecular taxonomy and strain analysis in *Echinococcus*. In: Craig, P., Pawlowski, Z.
(Eds.) Cestode Zoonoses: Echinococcosis and Cysticercosis – An Emergent and Global

Schantz, P.M., Moore, A.C., Munoz, J.L., Hartman, B.J., Schaffer, J.A., Aron, A.M.,

Schantz, P.M., Cruz, M., Sarti, E., Pawlowski, Z.; 1993. Potential eradicability of taeniasis

Schantz, P.M., Wilkins, P.P., Tsang, V.C.W.; 1998. Immigrants, imaging, and
immunoblots: the emergence of neurocysticercosis as a significant public health


taeniasis and cysticercosis in Asia. In: Singh, G, Prabhakar, S. (Eds.) *Taenia solium*
Cysticercosis. CABI Press, Oxson, pp. 111-127.

Vilhaena, M., Santos, M., Torgal, J.; 1999. Seroprevalence of human cysticercosis in


