Molecular and serological diagnosis of taeniasis and cysticercosis in Asia and the Pacific

A. Ito and Colleagues

Asahikawa Medical College

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Neurocysticercosis of *Taenia solium* is one of the most lethal parasitic diseases worldwide and on the list of neglected infectious diseases WHO 2005
Distribution of Taeniasis/Cysticercosis of *T. solium*

50,000,000 people  50,000 die annually

(MMWR 1993, 42: 1-25)
Clinical Manifestations of NCC

No specific symptom

The majority of NCC cases asymptomatic: no treatment
<table>
<thead>
<tr>
<th>Clinical pictures</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epileptic Seizures</td>
<td>62</td>
</tr>
<tr>
<td>Intracr. hypertension</td>
<td>34</td>
</tr>
<tr>
<td>Meningitis</td>
<td>29</td>
</tr>
<tr>
<td>Mental disorders</td>
<td>11</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>2</td>
</tr>
<tr>
<td>Spinal</td>
<td>0.5</td>
</tr>
<tr>
<td>Combined</td>
<td>37</td>
</tr>
</tbody>
</table>

No symptoms specific to NCC of *T. solium*
Epilepsy due to *T. solium*

Main cause of late-onset epilepsy

Developing countries

Endemic areas for *Taenia solium*
Epileptic Seizures

Most common symptom of NCC

70% - 90%

By courtesy of Dr. O.M. Takayanagui
Most of the symptoms of NCC become evident when the parasite has just been damaged by the host immune responses or by treatment with metacestocidal drug such as praziquantel through mass screenings.

Treatment of NCC patients based on crucial diagnosis

Chemotherapy vs Surgery
No symptoms specific to NCC of *T. solium*

How to suspect NCC?

1) Neurologic disorders: no symptoms specific to NCC

2) Neuroimaging: not always typical

3) History of traveling to and/or living in *T. solium* endemic areas

Then,

4) Serology!
Cysticercosis

* T. solium: not indigenous in Japan

Gold standard for cysticercosis is immunoblot using specific antigens for identification of patients (CDC, USA and AMC, Japan)

Differential Serodiagnosis of Cysticercosis by Immunoblot
Comparison of native GPs and recombinant chimeric antigens (Sako Y et al. 2000. JCM 38)

Similar sensitivity in ELISA and IB between these two antigens

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Healthy Persons</th>
<th>Cysticercosis Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native GPs</td>
<td>65/70</td>
<td>92.9%</td>
</tr>
<tr>
<td>Ag1V1/Ag2</td>
<td>66/70</td>
<td>94.3</td>
</tr>
</tbody>
</table>

healthy persons

•
cysticercosis patients
How to diagnose NCC?

Neuroimaging: not always typical

Serology: sensitivity not always 100%

Surgery suspected brain tumor

Histopathology

Molecular identification

Molecular identification of metacestode in the histopathological specimens

No antibody Response : ID?
Solitary NCC? Calcified NCC?
With or without hooklets?
Are the causative agents really cysticerci of *T. solium*?
Other zoonotic taeniid species?

The majority of such cysts may be expected to be due to *T. solium*. However, molecular confirmation is essential nowadays.
Two genotypes of *T. solium* worldwide

Case 1

53-year-old, Japanese woman; a solitary cyst in the left frontal lobe; repeated travel histories to India and Southeast Asian countries from 1993 to 2001.

Such cases may retrospectively be analyzed as to where they were exposed to eggs based on subtyping of mtDNA.

This case is suspected to have been exposed to *T. solium* eggs in Bali, Indonesia.

PCR-amplification of *cox1*

PCR: 94C, 30sec; 58C, 30sec; 72C, 90sec; 35 cycles
A long-standing puzzle has been that adult taeniid tapeworms expelled from people in Asia-Pacific seem to be *T. saginata*, the beef tapeworm, although these people eat pork rather than beef (from Ito et al. 2003. Lancet 362, 1918-1920).
How many human *Taenia* species are distributed in Asia-Pacific?

Now we have three in Asia-Pacific: *Taenia solium*, *Taenia saginata* and *Taenia asiatica*.

Historically we recognized only two in the world: *T. solium* and *T. saginata*. 
Multiplex PCR for differentiation of three human taeniid species

Lane 11: taeniid egg sample from Yunnan, China
(mixture of *T. saginata* and *T. asiatica*)

Three countries where we have confirmed three species are briefly introduced.

1) Indonesia where three species are distributed basically separately due to the barriers of the religion (Wandra et al. 2006 Trans R Soc Trop Med Hyg 100)

2) Thailand where three species are sympatrically occurring (Anantaphruti et al. 2007 Emrg Infect Dis 13)

3) China where three species are sympatrically occurring (Li et al. 2006 Acta Trop 100)
Taeniasis/Cysticercosis in Indonesia

North Sumatra
T. asiatica
T. solium ?

Bali
T. saginata
T. solium

Papua
T. solium
Immunooblots of NCC suspected patients’ sera from Bali 2005

A: GPs prepared by isoelectric focusing

B: recombinant Ag

Blood vs CSF

no difference (unpublished)

*: not NCC but malignant tumor (Feb 2006)

Taenia solium taeniasis/cysticercosis: highly endemic

Neurocysticercosis (NCC) in Irian Jaya, Indonesia

Current situation of Taenia solium taeniasis/cysticercosis in Indonesia

Pigs and dogs have free access to human feces
The dog was suspected to be infected with *T. solium* cysticerci by highly specific antibody-ELISA and IB (Ito A et al. 2002. J Helminthol 76).

Traditional tongue examination for porcine cysticercosis
Taeniasis of *T. asiatica* is still endemic in Samosir island, north Sumatra!

In Indonesia, there is no area where three human taeniid species are sympatrically occurring (Wandra et al. Parasitol Int 2006: 55 Supplement; SEAJTMPH 2007: 38; Trop Med Health in press).
Three human *Taenia* species are sympatrically occurring in some areas in Thailand

Anantaphruti MT et al. 2007. *Emerging Infectious Diseases* 13, 1413-1416
Three worms were expelled from one patient in Thailand 2004 (Anantaphruti MT et al. 2007 EID 13, 1413-1416)

2 Taenia solium

Taenia saginata or Taenia asiatica
Tibetan people in the western part of Sichuan, China

(Li TY et al. 2006. Acta Tropica 100, 223-231)

Three species are sympatrically occurring!
*T. asiatica* has been confirmed from Taiwan, China, Korea, Indonesia, Philippines, Vietnam, Thailand through our collaboration projects.
How to use viable eggs of *Taenia* species

Metacestodes of *Taenia saginata* in the peritoneal cavity of NOD/Shi-scid mouse after 23 weeks of infection with *in vitro* hatched oncospheres
Huge number of metacestodes of *Taenia asiatica* after 19 weeks of inoculation with in vitro hatched oncospheres in a NOD/Shi-*scid* mouse

from Nakaya K et al. Parasitol Int 2006; 55Supplement
“A long-standing puzzle that adult taeniid tapeworms expelled from people in Asia-Pacific seem to be *T. saginata*, the beef tapeworm, although these people eat pork rather than beef” has been resolved not to be *T. saginata* but to be *T. asiatica*.

We have to re-evaluate all *T. saginata* worms in the world when the patients were from Asia-Pacific or having visiting or living history in Asia-Pacific.
Summary

1. Three human *Taenia* species are occurring in Asia-Pacific. Taeniasis may be detected by several tools.

2. Molecular identification of these human *Taenia* species is feasible.

3. Serology is highly useful for detection of NCC.

4. Confirmation of NCC is based on neuroimaging, serology, histopathology and molecular identification.

5. *T. saginata* in the world as well as in Asia-Pacific should be re-evaluated by molecular tools (Ito et al. 2003. Lancet 362, 1918-20).