
Immunodiagnostic and molecular approaches for the detection of taeniid cestode infections

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This article summarizes the most recent advances in techniques and applications for the detection of taeniid cestode-infected persons or animals. In addition, the use of molecular approaches for strain identification and control of parasite transmission is discussed.

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Although neurocysticercosis (NCC) and echinococcosis as cystic (CE) and alveolar (AE) forms are not among the leading causes of parasite-induced mortality worldwide, these diseases cause considerable morbidity in humans and economic loss in terms of health and livestock costs [reviewed in 1–5]. Approximately 50,000 individuals die of NCC every year caused by *Taenia solium*: more than 25,000 CE cases have been surgically treated in western China alone since 1905. Human AE is among the most lethal of parasitic infections and 559 cases have been recorded in Western Europe for 1982-2000 [4,6].

Transmission of NCC, CE and AE occurs following environmental contamination with infective eggs released from definitive host mammals. Humans are the only definitive host of *T. solium*: therefore, it is theoretically possible to eradicate the disease in humans by means of diet and health education [7]. By contrast, predominant definitive hosts for *Echinococcus granulosus* and *Echinococcus multilocularis* are the dog and fox, respectively [5]. If the transmission of *E. granulosus* between dogs and domestic animals is interrupted, it might be possible to control or even eradicate *E. granulosus*, at least where significant wildlife reservoirs do not exist, as shown in Tasmania and New Zealand [5]. Because the principle definitive host of *E. multilocularis* is the fox, and intermediate hosts are small wild mammals, it is much more difficult to control the life cycle, and therefore AE is not considered to be an eradicable infection. Moreover, the number of urban foxes and stray dogs infected with *E. multilocularis* is increasing in Europe and elsewhere [4-6]. Owing to the great increase in immigrants, refugees and tourists, together with the worldwide importation of livestock and changes in livestock practices, it has become more difficult to control these parasitic diseases. Cestode zoonoses are now considered as emerging and re-emerging parasitic diseases [4].

**Cysticercosis**

A brief summary of the modern techniques for detection of NCC, CE and AE in humans and domestic animals, as well as for detection of the parasites in
definitive hosts, is given in Table 1. The incidence of NCC has been underestimated because of the lack of a reliable method to detect and/or confirm it. With the development of image analysis using computed tomography (CT) and magnetic resonance imaging (MRI), together with more-reliable serological methods, it is now much easier to detect NCC patients and porcine cysticercosis.

The most reliable serology to detect cysticercosis in humans and animals is to detect specific antibody against specific *T. solium* antigens. The original identification of specific bands of glycoproteins (GPs) recognized by serum antibodies on immunoblots [8] and the original purification by lentil-lectin affinity chromatography [9] have since been established as the gold standard for immunodiagnosis of human NCC [10]. However, because of the presence of nonspecific contaminants, it was impossible to establish an enzyme-linked immunosorbent assay (ELISA).

Recently, a simple method for the purification of *T. solium*-specific GPs has been reported using preparative isoelectric focusing; antigens prepared in this way are applicable not only for immunoblot, but also for ELISA. Both assays showing similar sensitivity and specificity [4,11]. This approach is useful to detect infection in humans [12] and in pigs, which are the main intermediate hosts [13], and even in dogs in certain endemic areas [14]. Although several groups have succeeded in producing recombinant antigens and synthetic peptides, the immunodiagnostic sensitivity of these recombinant or synthetic peptides is not as high as native GPs [15]. Although detection of circulating antigen by ELISA has been used in epidemiological surveys in humans [4], problems could occur when applied in pigs because of cross-reaction with other larval cestodes such as *Taenia saginata asiatica* (= *Taenia asiatica*) and/or *Taenia hydatigena* [13].

**Taeniasis**

Because humans are the only definitive hosts for *T. solium*, it is vital to detect worm carriers of *T. solium* in communities where NCC is endemic [16]. To accomplish this, the detection of coproantigens of taeniid cestodes has been
developed for both *T. solium* and *T. saginata* infections in humans, and *Echinococcus* spp. in carnivores. In this assay, tapeworm antigens are detected in fecal samples of the definitive host [17,18].

Although the monoclonal antibodies (mAbs) or polyclonal antibodies employed to detect coproantigens were genus specific, the use of *Taenia* coproantigen ELISA has increased the ability to detect tapeworm carriers by threefold. Immunoblot detection of serum antibodies against specific 24/26 kDa antigen [19] of *T. solium* adult worms has been established in taeniasis cases, although residual antibodies from past exposure might result in false positives. On the basis of such data (especially coproantigen positivity), it is possible to identify and treat worm carriers using anthelmintics [16]. When such immunodiagnostic techniques are used, it is important to check the expelled tapeworm by morphology and/or DNA analysis for confirmation of the species [4,12,14,16]. Copro PCR for *T. solium* taeniasis is currently under development by several groups.

Although *T. saginata* has less medical importance, recent reports from Asia have revealed that there is a third form called Asian *Taenia* [20]. It was generally noticed that adult taeniid tapeworms expelled from local people in parts of Southeast Asia appeared to be *T. saginata*, although they used to eat pork [20]. The debate on whether it should be a new species, *T. asiatica* [21] or a subspecies, *T. saginata asiatica* [22] is still ongoing [23-27]. We expect that it is a sub-species of *T. saginata* [27]. From the view point of epidemiology, it is crucial to differentiate *T. saginata* and *T. saginata asiatica* in Asia. The latter is endemic in remote areas where local people eat uncooked minced pork with fresh blood and viscera. Therefore such endemic areas may simpatric become endemic for taeniasis/cysticercosis of *T. solium*.

A multiplex PCR for use on faeces as well as parasite specimens (proglottids, eggs or cysts) which can differentiate *T. saginata, T. saginata asiatica, T. solium* (Asian type) and *T. solium* (African/American type) has been developed based on the species-specific sequences of mitochondrial DNA of these cestodes [4,28,29]. This will also be useful for molecular epidemiological studies.
CE and AE Serology

It is a matter of some debate whether image diagnosis [by CT, MRI, X-ray or ultrasonography (US)] or serology for detection of specific antibodies is the more useful, reliable or practical approach. There is no doubt that a combination of image and serological analyses is essential for differential diagnosis of echinococcosis (either CE or AE) [5,30,31] and cysticercosis [4,31]. It is now recommended that image diagnosis for detection of a hepatic abnormality is the first choice not only in highly endemic areas but also in developed countries where AE is rare and should be followed by serological confirmation using specific antigens [4,5,30,31].

Major efforts have been undertaken to establish more-reliable serology with higher specificity and sensitivity and application for detection of the early stage of these diseases [1,3-5,31–33]. There are several candidate antigens (Table 1). Production of both recombinant antigens and synthetic peptides is underway in several laboratories. Serology using crude *Echinococcus* antigens, as opposed to purified antigens, is not as reliable for identification of AE or CE, although their high sensitivity could be used for mass serological screening [5,31,33]. It has been revealed that all candidate protein antigens for serodiagnosis of AE identified and characterized independently in Germany (EM10), Australia (EM4), Switzerland (EmII/3 or EmII/3-10) and Japan (Em18) are the same protein family and fragments of EM10 [34,35]. Em18 is the smallest product of degradation of EM10 (from 349K to 508K of EM10) by cysteine proteinase [32]. Although EM10 has been shown to have functional similarity to the human ERM (ezrin, radixin and moesin) family of molecules [35], the Em18 has the lowest homology with ERM and expected to be more specific and sensitive. Both Em2plus-ELISA and Em18-ELISA have been confirmed to be highly useful for monitoring of clinical prognosis [4,5,36].

Immunodiagnosis of human CE is currently best facilitated by use of native or recombinant antigen B derived originally from hydatid cyst fluid [37-39]. ELISA and especially immunoblot for antibody recognition of the 8/12 kDa small subunit of antigen B is highly genus specific, though a degree of cross-reactivity occurs with human AE cases. Antigen B has been shown to comprise
three subunits (8/12, 16 and 20/24 kDa) of which the 8/12kDa molecule itself comprises three forms AgB8/1, AgB8/2 and AgB8/3 appears to be most useful for serodiagnosis of human CE, and the AgB8/1 motif has been detected in *E. multilocularis* [37,39]. The 8 kDa subunit has been cloned from both *E. granulosus* and *E. multilocularis* [37,39]. Post-treatment (usually with albendazole) surveillance of CE by serological tests still remains to be developed for practical clinical use. Antigen B also appears to have same value in testing sheep and domestic livestock for CE infection [40].

**Detection of copro-DNA**

Detection of coproantigens of *Echinococcus* spp. in the definitive host has been developed and improved, and these techniques are now available in several laboratories [17,18]. Because there is a good correlation between real infection prevalence confirmed by purgation or necropsy to detect adult *E. granulosus* or *E. multilocularis* worms and copropositive prevalence rates, detection of coproantigens is reasonably expected to be useful for primary screening [17,18,41,42]. However, current coproantigen tests are primarily genus specific but not species specific. Therefore, it is important to establish species-specific techniques to differentiate *E. granulosus* and *E. multilocularis*, especially in dogs where both AE and CE are co-endemic, for example, in western China [3,4,33]. Because the risk of infection from dogs may be higher than from foxes in China [33], detection of copro-DNA is required for species-specific confirmation. Such PCR techniques for the detection of worm carriers and differentiation of *E. multilocularis* are now available [43-45]. Similar species-specific DNA techniques for detection of *E. granulosus* eggs has also been established and a novel copro-PCR test has now been developed [46].

In order to avoid exaggerated public anxiety – especially when domestic pets are concerned – it has to be stressed that any definitive diagnosis of *E. multilocularis* in dogs and cats has to be based on specific criteria: (i) the morphological detection of adult worms expelled after purging with arecoline (not after dosing with praziquantel) of animals positive with coproantigen tests as used to be applied for detection of dogs infected with *E. granulosus*, or
(ii) the detection of species specific DNA in fecal samples, or from eggs or adult worms present in the faeces.

**Strain variation**

Identification of strain-specific nucleotide sequences for several genes of *E. granulosus* has enabled up to nine genotypes (strains) to be characterized [4,5,47]. The horse-dog strain of *E. granulosus* (G4) has been recommended to be elevated to species status *Echinococcus equinus* [47]. Although the dog–sheep strain (G1) appears to be responsible for the majority of zoonotic CE infections, recent studies also indicate human infections with camel, cattle and pig genotypes [4,5]. There appears to be much less genetic heterogeneity in *E. multilocularis* and *T. solium*. However, microsatellite and mitochondrial DNA analyses have identified polymorphisms within the *E. multilocularis* endemic region of Hokkaido [48], and between isolates from Asia and Africa/Latin America (complexes) of *T. solium* [27-29]. As these zoonotic cestodes travel the world with the tapeworm carriers, it is crucial to differentiate such genotypes of *T. solium* and other zoonotic cestodes.

**Vaccination of animal stock**

Recombinant oncosphere subunit peptide vaccine candidates against several taeniid cestodes including *Taenia ovis*, *T. saginata*, *T. solium* and *E. granulosus*, and recently *E. multilocularis* have now been produced or identified by Lightowlers *et al.* [49], and Gauci *et al.* [50] and Siles-Lucas *et al.*[51], respectively. The EG95 vaccine against *E. granulosus* is 95% protective in Phase 2 trials for ovine CE [49]. Vaccination of intermediate hosts (e.g. sheep, yak) against *E. granulosus* might be the only option in some remote areas. The homologue of EG95 has now been identified in *E. multilocularis* (EM95) [49,50]. Another vaccine candidate E14t, a recombinant *E. multilocularis* 14-3-3 protein, has also been identified [51]. It has been shown that EM95 and E14t provide 78-83% and 97% protection in rodents against experimental egg challenge with *E. multilocularis*, respectively [50,51].
contrast, vaccination of pigs against *T. solium* could be more difficult for the control of NCC in highly endemic areas such as Irian Jaya, Indonesia [4,12,14,16,25]. Sustainable health education and targeted or mass treatment of human tapeworm carriers with integrated vaccine use and oxfendazole use in pigs could be the way for future control of NCC [52].

**Summary**

It is important to establish reliable strategies and methods to identify infected humans and animals for the surveillance, prevention and control of cysticercosis and echinococcosis. The use of modern techniques for the screening and identification of infected persons and animals provides a sound scientific basis for the prevention and control of these zoonotic cestodiasis. Strategies for the control of these three important zoonotic cestodiasis are complicated and necessary in the long term. Modern developments in detection, diagnostic, vaccine and chemotherapeutic methodologies will increase the likelihood of effective intervention and surveillance.

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Table 1. Differential diagnosis of NCC, CE and AE in humans and detection of adult *Taenia* spp. in humans and *Echinococcus* spp. in carnivores

<table>
<thead>
<tr>
<th>Disease</th>
<th>Method and comments</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCC</td>
<td>1: Image analysis: CT, MRI (brain), CT, X-ray (subcutaneous tissues)</td>
<td>2-4</td>
</tr>
<tr>
<td></td>
<td>2: Serology: Detection of serum antibodies to glycoproteins</td>
<td>3,4,8-12</td>
</tr>
<tr>
<td></td>
<td>(GP-Immunoblot 14-52 especially 24/26 kDa, GP-ELISA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3: Confirmation of species specific DNA and morphology using resected specimens</td>
<td>27-29</td>
</tr>
<tr>
<td>CE</td>
<td>1: Image analysis: CT, US (liver), CT, X-ray (lung), CT, MRI (brain)</td>
<td>4,5</td>
</tr>
<tr>
<td></td>
<td>2: Serology: Detection of serum antibodies to antigen B\textsubscript{c} subunits</td>
<td>30,37-40</td>
</tr>
<tr>
<td></td>
<td>(immunoblot especially 8-12 kDa, AgB-ELISA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3: Confirmation of species specific DNA and pathology using resected cysts</td>
<td>4,5</td>
</tr>
<tr>
<td>AE</td>
<td>1: Image analysis: CT, US (liver), CT, X-ray (lung), CT, MRI (brain)</td>
<td>4,5</td>
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<tr>
<td></td>
<td>2: Serology: Detection of serum antibodies to Alkaline phosphatase\textsuperscript{c}</td>
<td>30-35</td>
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<tr>
<td></td>
<td>(Alkaline phosphatase-ELISA), Em\textsuperscript{2c} (Em2-ELISA), II/3\textsuperscript{10}</td>
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<tr>
<td></td>
<td>(II/3\textsuperscript{10}-ELISA), Em2 plus II/3\textsuperscript{10} (Em2\textsuperscript{plus}-ELISA), Em18</td>
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<tr>
<td></td>
<td>(Em18-Immunoblot, Em18-ELISA), EM10 (EM10-ELISA), EM4 (EM4-ELISA)</td>
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<tr>
<td></td>
<td>3: Confirmation of species specific DNA and pathology using resected cysts</td>
<td>4,5</td>
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<tr>
<td>Taeniasis</td>
<td>1: Detection of coproantigens</td>
<td>4,16,17</td>
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<tr>
<td></td>
<td>2: Detection of copro-DNA</td>
<td>19,29</td>
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<tr>
<td></td>
<td>3: Detection of serum antibodies to adult stage specific antigens</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>4: Confirmation of species specific DNA from proglottids or eggs</td>
<td>16,23,24,</td>
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<td></td>
<td>and proglottis morphology using the worms from copro- or sero-positive persons after anthelminthic treatment</td>
<td></td>
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<tr>
<td>Echinococcosis</td>
<td>1: Detection of genus-specific copro-antigens for screening</td>
<td>4,5,17,18,41</td>
</tr>
</tbody>
</table>
 Confirmation of species specific DNA from proglottids or eggs and proglottis morphology using the worms from copro-antigen positive animals especially from dogs and cats after anthelminthic treatment

Abbreviations: NCC, neurocysticercosis; GP-ELISA, glycoprotein-ELISA; CT, computed tomography; MRI, magnetic resonance imaging; US, ultrasonography; CE, cystic echinococcosis; AE, alveolar echinococcosis

There are many asymptomatic infections without demonstrable cysts. Improved sensitivity of CSF (cerebrospinal fluid) over serum antibodies has been debated but examination of serum samples for detection of specific antibodies is in most cases sufficient and more acceptable.

These marker candidate antigens are now considered to be genus specific but expression of such antigens is highly related to the pathogenicity of typically multi-vesicular AE (Em2, Em18) or to that of cyst type for CE. Antibodies to antigen B are usually predominant in advanced CE cases. Routine serodiagnosis for differentiation of these three taeniid larval cestodiasises at Asahikawa Medical College may be undertaken as follows: AE: test antibody positivity to recombinant Em18, CE: test antibody positivity to recombinant Antigen B, but should be negative to recombinant Em18\(^4\)\(^3\)\(^1\). NCC: antibody positivity to glycoproteins purified by preparative isoelectric focusing\(^1\)\(^1\)\(^1\) or recombinant chimeric protein\(^1\)\(^0\) for ELISA and immunoblot\(^3\)\(^1\).

This is very important to confirm the direct evidence of the species and check the quality of copro-antigen test.