WHO/FAO/OIE Guidelines for the surveillance, prevention and control of taeniosis/cysticercosis

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CHAPTER 4:
DETECTION AND DIAGNOSIS

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4. Introduction

In this Chapter, the diagnosis of taeniosis and cysticercosis in humans, and cysticercosis in pigs and cattle is reviewed. *Taenia solium*, *Taenia saginata* and Asian *Taenia saginata asiatica* are adressed. Classical coprological, morphological and meat inspection diagnostic methods, as well as immunodiagnostic and molecular methods, are critically reviewed.

4.1. Diagnosis of taeniosis

Several techniques are available for the diagnosis of taeniosis (adult worm intestinal infection): questioning, feces microscopy, the peri-anal adhesive tape test, the ELISA for coproantigen or circulating antibodies, the differentiation of somata based on morphology or iso-enzyme patterns and molecular tests such as the polymerase chain reaction (PCR). All have advantages and disadvantages, which are discussed in detail in this Chapter.

4.1.1. Diagnosis based on morphological criteria

The eggs of *T. solium, T. saginata* and *T. s. asiatica* cannot be distinguished morphologically. Up to now the only reliable technique to distinguish them is by PCR (see Annex 4.5). Although it is theoretically possible to distinguish the three adult taeniids on the basis of morphological characteristics of the scolex or the mature proglottids (see Table 4.1), the former is rarely available after treatment with modern anthelmintics, whereas the latter need to be fixed and stained in order to examine the ovarian lobes and the vaginal sphincter, a laborious procedure. The staining procedure is described *inter alia* by Morgan and Hawkins [378]. In order to improve the recovery of the scolex and the quality of the expelled proglottids, a purge with electrolyte-polyethyleneglycol salt (EPS), (a product which is commonly used to prepare patients undergoing colonoscopy), two hours before and two hours after niclosamide treatment can be applied [291]. However, even using this EPS regimen, the scolex is recovered in only about one third of patients. Since morphological abnormalities are not uncommon in taeniids, the presence or absence of hooks on the scolex does not automatically indicate that it is *T. solium or T. saginata/T. s. asiatica* [480].

In most cases, gravid proglottids of *T. saginata* and *T. solium* can be distinguished on the basis of the number of unilateral uterine branches (Table 4.1). However, some overlapping might occur [582]. Therefore, if the number of uterine branches falls between 11 and 16, it is advised to confirm the identity of the tapeworm using molecular tools or enzyme electrophoresis. Proglottids of *T. saginata* and *T. s. asiatica* can only be distinguished using molecular tools (see Annex 4.5). Counting of the uterine branches can be done by squashing a gravid proglottid (after putting it on filter paper to remove excessive fluid) between two glass slides or between the underside and the cover of a Petri dish. In order to facilitate the counting, dyes such as carmine or Chinese ink can be injected using a fine needle. Longitudinal histological sections stained with haematoxylin-eosin allow a more accurate counting of the branches [358].

4.1.2. Differentiation based on enzyme electrophoresis

Le Riche and Sewell [319, 320] described a simple technique to differentiate taeniid somata, based on glucose phosphate isomerase (E.C.5.3.1.9.) zymogrammes. The technique is faster and less labour intensive than staining tapeworm material. It can be applied on a routine basis when direct morphological

observations remain indecisive; however, it requires preferably fresh or frozen material. Typical preservative solutions for segments such as alcohol or formalin will destroy enzyme activity.

Table 4.1. Morphological differences between *Taenia solium*, *Taenia saginata* and *Taenia saginata asiatica* (adapted from [167, 339, 424])

	T. solium	*T. saginata*	*T. s. asiatica*
Scolex			
– rostellum	Present	Absent	Present
– hooks	22-32	Absent	Absent
Mature proglottids			
– number of testes	375-575	800-1200	324-1216
– ovary	3 lobes	2 lobes	2 lobes
– vaginal sphincter	Absent	Present	Present
Gravid proglottids			
– number of unilateral uterine branches	7-16	14-32	11-32
– branching pattern	Dendritic	Dichotomous	Dichotomous
– expulsion from host	Passively* (in groups)	Actively** (single)	Actively** (single)

*: *usually with feces*
**: *outside defecation*

4.1.3. Differentiation based on molecular techniques

In order to overcome limitations in the identification of *Taenia* species based on morphology or enzyme electrophoresis, various molecular approaches have been developed, including the use of DNA probes [118, 264, 471, 472], PCR, or PCR coupled to restriction fragment length polymorphism (RLFP) [245, 247, 284, 357, 479] and multiplex-PCR [620]. The use of DNA probes is time consuming and relatively insensitive; however, PCR with oligonucleotide primers derived from such species-specific probes provides a rapid and sensitive method [245]. PCR-RFLP and multiplex-PCR permit differential diagnosis of *T. saginata*, *T. s. asiatica* and *T. solium*, even when examination by morphology cannot be performed, because these methods do not rely on the availability of intact gravid proglottids. Molecular techniques can be applied on fresh, frozen or ethanol-preserved parasitic material. More details about these diagnostic techniques can be found in Annex 4.5.

4.1.4. Questioning of tapeworm carriers

Spontaneous expulsion of proglottids (independent of defecation) generally occurs with *T. saginata* and *T. s. asiatica*, and exceptionally this has been observed for *T. solium* [480]. Therefore, *T. saginata*/*T. s. asiatica* carriers are often aware of the presence of a worm, which is not necessarily the case for *T. solium* carriers, where the expulsion of proglottids is passive (together with feces). Furthermore, it has been observed that patients cannot always distinguish tapeworm proglottids from nematodes like *Enterobius vermicularis* leaving the body or the spontaneous expulsion of the large roundworm *Ascaris lumbricoides*, resulting in false positive answers. Therefore, questioning can be used as an auxiliary method for the diagnosis of *T. saginata*/*T. s. asiatica*, but is certainly not reliable in the case of *T. solium*.

4.1.5. Coprological examinations

4.1.5.1. Conventional fecal examinations

Coprology for taeniosis should include both macroscopic and microscopic examinations. In the case of *T. saginata* loose gravid proglottids may be found in the underwear or in bed. Both *T. solium* and *T. saginata*/*T. s. asiatica* proglottids can be found in the feces, but expulsion does not necessarily occur daily. Therefore, repeated macroscopic examinations are advised. Similarly the efficacy of microscopic examinations for the presence of *Taenia* spp. eggs increases when repeated [259]. Various concentration techniques (e.g. sedimentation and formalin-ether concentration methods) can be used, but it is generally

agreed that they lack sensitivity [417]. Anthelmintic treatment has been shown to detect many more tapeworm carriers than either coprological techniques or questioning [13, 108, 259]. Cleansing the intestine with a purge immediately before and again after treatment improves the recovery of parasite material, including the scolex, which facilitates species identification [211].

4.1.5.2. Peri-anal swabs

Taenia spp. eggs sticking to the skin in the peri-anal region can be detected by using adhesive tape ('Scotch' tape), also known as the method of Graham. For the detection of *T. saginata* eggs the peri-anal swab is considered to be more sensitive than a single coprological examination [417]. Although less data are available for *T. solium*, the eggs of this parasite are also frequently found using the 'Scotch' tape technique [108, 504].

4.1.5.3. Coproantigen detection

Taenia coproantigens are parasite specific products in the feces of the host that can be detected by a polyclonal antibody-based sandwich ELISA (see also Chapter 6 on Control). These antibodies are obtained from hyperimmune rabbit sera raised against *T. solium* adult worm somatic antigens [7]. The use of this test increases the detection of parasitologically proven intestinal *T. solium* cases by a factor of at least 2.6 times in comparison to microscopy [10]. Coproantigens are stable for weeks in unfixed fecal samples kept at room temperature and for years in frozen samples or in chemically-fixed samples (e.g. formalin) kept at room temperature. Coproantigens can be detected prior to patency and they are no longer detectable within a week of treatment. A major disadvantage of this test is that it is only genus specific, making it impossible to differentiate *T. solium* and *T. saginata* infections. However, the test shows no cross-reactions with other intestinal helminth infections including *Ascaris, Trichuris* and *Hymenolepis* spp. [13]. Coproantigen testing for *Taenia* has also been carried out using a dipstick ELISA format [10]. This field test proved faster, but less sensitive. than the micro-plate assay.

4.1.5.4. Copro-PCR

Methods have been developed to extract DNA of *Taenia* spp. from human feces, which can be used in a PCR for diagnosis of taeniosis [397, 617]. The high sensitivity and species-specific detection of the immature stages are definite advantages of this test. However, current DNA extraction methods are too expensive for use as a routine test.

4.1.6. Serological tests

The possibility of diagnosing *T. solium* taeniosis by the detection of species-specific circulating antibodies has been demonstrated [609]. This test uses excretory-secretory antigens derived from *T. solium* tapeworms in an EITB. Molecules ranging from 32.7 to 42.1 kDa are highly specific for *T. solium* taeniosis infections. No cross-reactions have been demonstrated in this test with sera obtained from individuals with other intestinal infections, including *T. saginata, Echinococcus* spp. and *H. nana*, and in patients infected with the metacestode stage of *T. solium*. The major advantages of this test are that it allows species specific diagnosis and avoids the handling of feces contaminated with *T. solium* eggs [14]. A drawback may be that antibodies persist after removal of the intestinal tapeworm.

4.2. Diagnosis of *Taenia solium* cysticercosis in humans

4.2.1. Parasitological diagnosis

The diagnosis of *T. solium* cysticercosis (extraintestinal metacestode stage) is made parasitologically by demonstrating the scolex with the hooks or fragments of the bladder wall in biopsy or autopsy material. In some parts of Asia, especially, where subcutaneous cysticercosis is rather frequent [459], it is easy to obtain biopsy material for further histopathological confirmation. With less invasive techniques such as fine needle cytology, the diagnosis of cysticercosis can often be made [31, 298].

4.2.2. Imaging

Various imaging techniques, such as radiography, CT-scanning, magnetic resonance imaging, etc., are available for the diagnosis of human cysticercosis and are described in Chapter 2.

4.2.3. Serological diagnosis

Immunological methods for the diagnosis of human cysticercosis can be used for the detection of individual cases or for epidemiological surveys. In the former, sensitivity is more important than specificity, since the diagnosis is usually made on an individual who has symptomatology suggestive for cysticercosis. For epidemiological purposes, the specificity of the test is an important factor.

Immunodiagnostic techniques include detection methods for specific antibodies and for circulating parasite antigen in serum or CSF.

4.2.3.1. Antibody detection methods

Infection with *T. solium* results in a specific antibody response, mainly of the IgG class [69]. Different techniques have been described to detect antibodies to *T. solium,* such as the complement fixation test, haemagglutination, radioimmunoassay, ELISA, dipstick-ELISA, latex agglutination and immunoblot techniques [134]. Antigens used in these tests are either cyst fluid or crude homogenates of *T. solium* cysticerci or crude preparations of the related parasite *T. crassiceps*, which can be maintained in laboratory rodents [410]. These unpurified antigens have moderate sensitivities and relatively poor specificities [171, 172, 504].

Research on the antigenic properties of cyst fluid and surface-associated glycoproteins, and improved protein purification techniques have resulted in much more reliable serological tools [248, 286, 410, 567]. The most specific test developed is the EITB, an immunoblot of seven cysticercus glycoproteins, purified by lentil lectin-purified chromatography, which gives close to 100% specificity and a sensitivity varying from around 70% to 90% [567]. However, a sensitivity of only 28% has been found in cases with single, enhancing parenchymal cysts in the brain [616]. In developing countries, ELISA is preferred because of its better availability, its simplicity and its lower cost compared to immunoblot [483]. Purification of glycoproteins from cyst fluid by single step preparative isoelectric focusing was shown to produce very specific antigens, which are applicable both in immunoblot and ELISA [286]. The specificity and sensitivity of this ELISA were reported to match those of the immunoblot [288].

Since the preparation of purified antigens relies on the availability of parasite material and may be subject to the quality of this material, attempts were made to produce recombinant antigens and synthetic peptides. Different authors synthesised 10, 7-10 and 14 kDa recombinant polypeptides that can be used in immunoblot and ELISA [81, 487]. While the specificity of these antigens is reported to be high, the sensitivity is generally lower than with the native antigens. The use of synthetic peptides in ELISA is another promising option [171].

4.2.3.2. Antigen detection methods

Several assays have been developed to detect parasite antigens, but only the monoclonal antibody-based ELISA directed at defined parasite antigens may ensure reproducibility and specificity [62, 134, 265]. Antigen detection may be done on serum as well as on CSF [79, 214, 217]. Because of the localisation of the cysts in the brain, antigen detection in CSF may be more appropriate for diagnosis than in serum; however, sampling of CSF is more cumbersome than blood sampling.

Ag-ELISA's detect only cases of active cysticercosis, i.e. the presence of living cysticerci [154, 205, 211, 395]. The sensitivity of the Ag-ELISA is very high, even in light infections. In one study, in patients with a single viable cyst or only enhancing lesions, the sensitivity was only 65% [217]. The Ag-ELISA is very specific, and no cross-reactions have been observed in sera from patients with confirmed infections with *Schistosoma*, hydatid cysts, *Ascaris*, *Trichuris*, filaria, *Entamoeba*, *Plasmodium* and *Trypanosoma* [154].

4.2.3.3. Immunodiagnosis in epidemiological studies

In humans, no clinical features are specific for cysticercosis, even asymptomatic brain lesions are not uncommon, and imaging methods are not appropriate for epidemiological studies. Therefore, definition of cases is often based solely on immunodiagnostic methods [178]. In surveys on cysticercosis, immunodiagnosis is useful in estimating the prevalence and identifying the risk factors associated with transmission of *T. solium*; a high seroprevalence in a community indicates a 'hot spot' where preventive and control measures should be applied [220, 548]. Immunodiagnostic tools also offer the possibility of surveillance of the infection during and after control programs [217, 495, 575].

Antibody detection tends to overestimate the prevalence of cysticercosis because a transient antibody reaction may occur following exposure to *T. solium* eggs, without establishment of cysticerci, or with self-cure [213]. Antigen detection assays in epidemiological studies, however, measure active cysticercosis, not merely exposure [134].

4.2.3.4. Immunodiagnosis of neurocysticercosis

Serological tests may also be used for the diagnosis of neurocysticercosis. They can be applied both on serum and on CSF [89, 401, 630].

Serological tests can be very useful for confirmation of imaging techniques, for differential diagnosis of other 'cyst forming conditions', including echinococcosis, brain tumours and tuberculosis [76, 122]. The general opinion is that consistent diagnostic criteria of neurocysticercosis should be based on combined neuro-imaging studies, serological tests, clinical presentation and exposure history.

Antibody detection is particularly useful for the identification of neurocysticercosis as the etiological agent of epilepsy since dead cysts are more often responsible for epileptic seizures than living cysts [394]. Ag-ELISA only detects active cysticercosis, which may be an advantage when a decision has to be taken on whether or not anti-parasitic treatment should be started, according to the consensus guidelines proposed by Garcia *et al.* [205]. Patients with only calcified cysts, who don't need anthelmintic treatment, are consistently negative in the Ag-ELISA [205].

Antigen detection has also proven to be an efficient tool for the follow-up of neurocysticercosis patients after treatment since circulating antigen disappears within one to three months from the serum of cured patients, which is not the case if the patients are not cured [205, 394].

4.2.3.5. Limitations of immunodiagnosis

Immunodiagnosis contributes to a better understanding of the prevalence and the epidemiology of the infection, and to the diagnosis of neurocysticercosis and the follow-up of treatment. However, because of the polymorphic clinical manifestations of neurocysticercosis, immunodiagnosis cannot replace neuro-imaging for the clinical management of neurocysticercosis. Anthelmintic treatment of epileptic patients that have a positive antibody or antigen serology, without a CT-scan or MRI examination, is considered to be very hazardous.

Finally, efforts should be made to make cheap, reliable and standardised immunodiagnostic tools more widely available.

4.3. Diagnosis of porcine cysticercosis

4.3.1. Porcine cysticercosis due to *Taenia solium*

4.3.1.1. Tongue inspection

In many endemic countries tongue inspection is carried out by the local population in order to identify pigs infected with *T. solium* cysticercosis. If carried out correctly (both palpation and visual inspection

throughout the base) by experienced people, it is generally agreed that the specificity of this technique is 100% [135, 238]. The sensitivity of the technique, however, depends very much on the degree of infection of the animals. Although in heavily infected animals tongue inspection might detect up to 70% of the cysticercotic pigs, in lightly infected animals the sensitivity is much lower. Several studies have shown that in experimentally or naturally infected pigs harbouring less than 100 cysts, none of the animals could be detected by tongue inspection [135, 395]. In moderately to heavily infected animals (>100 cysts) the sensitivity is lower than 50% [434, 438]. In Zambia, using Bayesian analysis the overall sensitivity of tongue inspection was estimated at 21% (CI: 14-26%) [135].

4.3.1.2. Meat inspection

The procedures for the detection of *T. solium* cysticercosis during conventional meat inspection vary widely from one country to another (see also Chapter 5). In some countries, visual inspection only is carried out on one or several so-called predilection sites, such as the heart, diaphragm, masseter muscles, tongue, neck, shoulder and intercostal and abdominal muscles. In other countries, regulations also require incisions in some of these muscles. It is obvious that the efficacy of meat inspection will depend not only on the thoroughness of the inspection methods, but also on the degree of infection of the pigs. Given the fact that, in rural areas of Africa and South America, lightly infected animals have been shown to occur more frequently than previously believed [135, 511, 512], meat inspection in these areas will seriously underestimate the real prevalence of porcine cysticercosis. Using a Bayesian approach, the overall sensitivity of meat inspection in Zambia (only visual examination of masseter muscles, triceps brachii, tongue and heart) was estimated at 22.1% (CI: 15-27%) [135].

4.3.1.3. Serological techniques

Immunodiagnosis in pigs is used in prevalence surveys, community-based surveys and in intervention studies. In endemic areas, pigs can also be used as sentinels to measure the environmental contamination of *T. solium* eggs.

Most of the techniques developed for the diagnosis of cysticercosis in humans have been adapted for analysing pig sera, including the EITB [569], Ab-ELISA using isoelectric focusing-purified glycoproteins [286, 499], and Ag-ELISA [135].

The benefits of immunodiagnosis in pigs are: (i) tests offer diagnosis on live animals; (ii) blood sampling followed by serological testing is more sensitive than the tongue examination; and (iii) the tests are relatively inexpensive and easy to perform on large numbers of serum samples [134].

However, there are some problems related to sero-diagnosis in pigs: (i) it has been reported that the sensitivity of the available techniques is low in pigs with low levels of cyst burdens [512], although other authors [394] were able to detect pigs harbouring one single cyst using an Ag-ELISA; (ii) when measuring antibodies, antigen exposure is measured rather than actual infection; as in humans, the problem of transient antibodies may have to be considered to also apply to pigs, (i.e. a transient antibody response to a *T. solium* infection, without the establishment of a patent infection); (iii) interpretation of sero-positive results in young pigs may be complicated by maternal antibodies, transferred by colostrum from a sero-positive sow to its piglet, which may persist for up to seven months; this has to be considered in pig prevalence studies [243]; and (iv) cross-reactions with *Cysticercus tenuicollis* are the rule rather than the exception in most antibody and antigen detection tests [134]. *C. tenuicollis* infection in pigs is uncommon in Africa and most regions of Latin America, but very common in the People's Republic of China and Vietnam. Ab-ELISA using isoelectric focusing-purified glycoproteins was claimed not to cross react with *C. tenuicollis* antigens [499].

Using Bayesian analysis on Zambian village pigs, the overall sensitivity and specificity of Ag-ELISA were estimated at 87% (CI: 62-98%) and 95% (CI: 90-99%), respectively [135]. For Ab-ELISA (using crude somatic *Taenia crassiceps* antigen) the sensitivity was only 36% (CI: 26-41%) and the specificity 92% (CI: 85-99%).

4.3.2. Porcine cysticercosis due to *Taenia saginata asiatica*

4.3.2.1. Parasitological diagnosis

Pigs are also the most important intermediate hosts of *T. s. asiatica* [164]. Contrary to the cysticerci of *T. solium*, which are mainly found in the muscles and only rarely in the organs, the predilection site of the cysts of *T. s. asiatica* is the liver (at the surface and/or in the parenchyma). Besides the liver, cysts are sometimes found in the lungs or attached to the omentum or the serosa of the colon [83, 153]. The cysticerci of *T. s. asiatica* are much smaller than those of *T. solium* and possess a rostellum and rudimentary hooklets. Just like the cysticerci of *T. saginata* the bladder surface of *T. s. asiatica* shows wartlike processes. The cysts surrounded by host tissue capsules have a diameter of about 2 mm when they are living or somewhat larger when they are degenerating [150]. In naturally infected animals, the cysts of *T. s. asiatica* are often degenerated and have to be distinguished from the liver lesions caused by *Ascaris suum* larvae (the so called white spots).

4.3.2.2. Serological diagnosis

Similarly to immunodiagnosis in *T. solium* infections, both antibody and antigen detecting assays may be used for the diagnosis of *T. s. asiatica.* Excretory/secretory products of *T. s. asiatica* metacestodes and crude somatic antigen of *T. crassiceps* have been used as coating antigens in Ab-ELISA; and monoclonal antibodies raised against excretory/secretory products of *T. saginata* have been used in a sandwich-ELISA to detect circulating parasite antigen [163, 225]. While antibodies can be detected from three weeks post-infection onwards and may persist for at least nine weeks post-infection, circulating antigens may be detected as early as one week post-infection but disappear soon after the cysticerci degenerate [163, 225]. Infection with as few as five *T. s. asiatica* viable cysticerci can be detected by Ag-ELISA [163].

4.4. Diagnosis of *Taenia saginata* cysticercosis in cattle

4.4.1. Meat inspection

In many countries the 'knife and eye' method is used whereby the so-called predilection sites (heart, tongue, masseter muscles, oesophagus and diaphragm) are visually examined and/or incised to detect cysticercosis (see also Chapter 5). However, several studies have shown that, except for the heart, none of the other muscles should be considered as real predilection sites [310, 345]. Detailed dissection of carcasses of lightly infected animals has proven that, in 51% to 56% of them, cysticerci are not present in these presumed predilection sites [361, 590]. Therefore, it is not surprising that routine meat inspection (if carried out properly) detects only the more heavily infected animals and underestimates the real prevalence of bovine cysticercosis by at least a factor of three to ten [158]. Another disadvantage of current meat inspection techniques is the fact that they are labour intensive and very subjective. It depends very much on the skills and motivation of the meat inspector as to whether or not cysticerci will be detected [158]. Living cysticerci, which are usually present in small numbers, are especially difficult to detect. Once the cysticerci die and become caseous or calcified, they have to be differentiated from other lesions. Immunohistological staining of sections of these lesions using monoclonal antibodies allows confirmation of the parasitic nature of degenerated lesions [398]. PCR can also be used to demonstrate parasitic DNA, which might still be present in this kind of lesions (Geysen *et al.,* unpublished results).

Note: cysticerci in the liver of cattle can be either *T. saginata* or *T. s. asiatica;* the latter are much smaller [153, 164]. Data on the prevalence of *T. s. asiatica* cysticercosis in cattle in Asia are lacking.

4.4.2. Serological techniques

Immunodiagnosis of bovine cysticercosis has been utilized in epidemiological studies and for individual and herd diagnosis. Both antibody and antigen detecting tests have been developed, the former to measure exposure to the parasite, the latter to detect active infections. The majority of techniques in general use for the detection of immune responses have been employed, with ELISA being the most popular test. Various antigens have been used in Ab-ELISA, from crude somatic extracts, excretory/secretory products, to

recombinant antigens [466] and synthetic peptides [169]. For Ag-ELISA, monoclonal antibodies produced against cyst fluid or excretory/secretory products of the metacestode are used [62, 265]. In slaughterhouse cattle, antigen detection is three to 10 times as sensitive as meat inspection [137, 400] and is therefore an interesting tool for estimating prevalence and studying risk factors for infection at the farm level. However, there is generally little agreement between cattle found positive at meat inspection and those found positive by antigen detection ELISA [137, 400]. One possible reason is that Ag-ELISA detects only live cysts, while lesions left by dead cysts are more noticeable at meat inspection. Another problem in immunodiagnosis in cattle is that of low sensitivity in light infections [62, 533, 572, 592], which are the rule rather than the exception, not only in industrialized countries but also in the tropics. The sensitivity of Ag-ELISA is near 100% when 50 or more viable cysticerci are present in the carcass, but is less than 50% in lighter infections [572]. Therefore, greatest value of immunodiagnosis lies in its application as a screening test in a cattle herd, rather than as a diagnostic test at the individual animal level.

Annex 4.5: Application of molecular techniques for identification of human *taenia* spp.

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4.5.1. Methods for identification

The most accurate methods for identifying the *Taenia* species infecting humans are based on molecular and immunological diagnostic approaches combined with comparative morphology [284]. This review covers the use of deoxyribonucleic acid (DNA)-based techniques. Immunological and morphological approaches are described in Chapter 2.

4.5.1.1. Molecular identification of specimens collected from patients or domestic animals

Parasite samples should be freshly obtained, rinsed several times in physiological saline solution and frozen or fixed in ethanol without delay. DNA analysis on formalin-fixed samples is difficult and thus formalin fixation/storage is not recommended. Fixing samples in 75% ethanol is the method of choice due to the convenience for transportation and the ease of storage.

Adult proglottids expelled from human carriers, whole worms expelled after chemotherapy and eggs are all amenable to molecular identification. Fecal samples collected in endemic areas are useful for DNA detection of worm carriers; fecal samples frozen or fixed in ethanol are suitable for the copro-DNA test [397, 617]. It is best that frozen fecal samples be examined as soon as possible after collection and within ten years as a maximum [617]. Metacestodes, collected from domestic animals after necropsy, should be washed in saline and then fixed in ethanol for DNA analysis.

DNA from samples is prepared by proteinase K digestion, phenol/chloroform extraction and ethanol precipitation. Alternatively, a range of commercial kits, such as the DNeasy Tissue Kit (Qiagen, Hilden, Germany) is available for isolation of DNA.

4.5.1.2. Principles of methods

A number of techniques has been employed for DNA identification of *Taenia* species [60, 364, 284].

4.5.1.3. Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) of nuclear ribosomal DNA (rDNA) or other genomic regions, including mitochondrial DNA, can be used to detect differences between taxa. The DNA is digested by restriction enzymes, the resulting fragments are electrophoretically separated on an agarose gel, transferred to a nitrocellulose or nylon filter and hybridized with a specific DNA probe that has been radioactively or otherwise labelled in a Southern blot approach (RFLP-SB) [60, 471, 625].

The rDNA RFLP technique has been linked with the RFLP-PCR or PCR-linked RFLP to provide a greatly simplified and less time-consuming procedure, without loss of resolution or accuracy [60, 626].

During the PCR, a fragment of DNA, defined by oligonucleotide primers at either end, is amplified several million fold using a thermostable Taq polymerase.

Ribosomal RNA genes are organised into rDNA units with the very highly conserved coding regions separated by relatively poorly conserved non-coding spacer regions. Internal transcribed spacer 1 (ITS1) has been used successfully as one target sequence for PCR amplification [60]. Primers were designed based on highly conserved regions at the 3' end of the 18S rRNA gene (forward primer BD1) and within the 5.8S rRNA gene (reverse primer 4S). The PCR product, which spans ITS1 of the rDNA repeat unit and includes most of the 5.8S gene, can be amplified from various *Taenia* species and digested with one of a number of 4-base cutting restriction enzymes. Characteristic RFLP patterns are produced when samples of the various species are analyzed by agarose gel electrophoresis. Other markers including mitochondrial (mt) cytochrome c oxidase subunit 1 (*cox 1)* [60, 557], mt 12S rDNA [480] and other target DNA sequences [246] have also been used with success in PCR-RFLP analysis.

4.5.1.4. Comparison of PCR-amplified DNA sequences

The nucleotide sequences of fragments of targeted genes are determined using pairs of conserved PCR primers. The variable segment between the primers is PCR-amplified for a particular *Taenia* sample and then directly sequenced [60]. The sequences obtained can then be directly compared with sequences already published for *T. solium, T. saginata* and the Asian ('Taiwan') *Taenia, T. s. asiatica* and the identity of a particular sample thus determined. The mt *cox1*, NADH dehydrogenase subunit 1 (*nad1*), cytochrome b (*cob*) and 12S rDNA genes, and nuclear 28S rDNA and ITS1/ITS2 rDNA genes have proven valuable markers amenable to this approach [60, 149, 222, 246, 284, 285, 322, 399, 588, 591].

4.5.1.5. Random amplified polymorphic DNA-PCR (RAPD-PCR)

This is a technique by which genomic DNA is amplified by PCR using a single oligonucleotide primer of arbitrary nucleotide sequence [149, 351, 576]. This technique is relatively simple, requiring only small amounts of DNA (approximately 25 ng) and it is rapid. However, reliable results are only obtained under carefully controlled conditions, especially with regard to the quantity and quality of template DNA. Therefore, it is recommended that RAPD-PCR should be used simultaneously with one or other of the DNA techniques available.

4.5.1.6. Single-strand conformation polymorphism (SSCP)

This is a mutation scanning method with the potential to discriminate DNA sequences differing by a single nucleotide. The method is based on the principle that the electrophoretic mobility of a single-stranded DNA molecule in a non-denaturing gel is dependent on its size and structure. A mutation or base change at a particular site in the primary sequence can modify the conformation of the molecule that alters its electrophoretic mobility. SSCP has been used for the direct visual display of sequence variation in PCR-amplified fragments of the mt *cox1* and *nad1* genes of different *Taenia* species [222]. Although, the technique has to be very carefully controlled, it has the advantage that there is no need for DNA sequencing or restriction analysis, and large numbers of samples can be analyzed in a short period.

4.5.1.7. Multiplex-PCR

Multiplex-PCR uses primers of interspecies-conserved and species-specific sequences for the simultaneous differential diagnosis of *Taenia* taxa. This is an easy and time saving technique that does not require DNA sequencing. The approach uses a combination of different primer pairs in the same amplification reaction with the aim of producing different specific PCR products that can be distinguished after electrophoresis on an agarose gel [246, 617, 618]; for example, a multiplex-PCR based on mt *cox1* as target gene has been developed. Using mixed species- or genotype-specific primer sets, diagnostic 827 bp and 269 bp-products are amplified from *T. saginata* and *T. s. asiatica,* respectively. In *T. solium*, 720- and 984 bp-products are amplified from the American/African genotype and Asian genotype, respectively.

4.5.2. Comments on the taxonomy and population structure of the human *Taenia* spp.

Phylogenies derived from sequence comparisons of complete PCR-amplified *cox1* and *cob* genes obtained from a number of geographically distributed samples of *T. solium* provide molecular evidence for two genotypes; one is restricted to Asia and the other occurs in both Africa and America [385]. Whether the two genetic forms of *T. solium* differ in important characteristics such as infectivity or the pathology they cause remains to be determined. Minor sequence differences for the mt *cob* gene have recently been reported between isolates of *T. saginata* [322]. Minor sequence variation in the mt *cob* and nuclear rDNA ITS2 genes have also been shown between isolates of Asian *Taenia* [321].

There has been considerable discussion over a number of years regarding the taxonomic position of Asian *Taenia* and whether it should be regarded as a genotype, strain, sub-species or sister species of *T. saginata* [364]. Early DNA-based studies by Zarlenga and his group [624, 626] showed that Asian (Taiwan) *Taenia* is genetically, and hence phylogenetically, more similar to *T. saginata* than to *T. solium.* Bowles and McManus (60) compared mt *cox1* (~366 base pairs) and nuclear 28S rDNA D1 region (~300 base pairs) sequences for *T. saginata, T. solium,* Asian *Taenia* and a number of other recognized species within the genus *Taenia.* The sequence comparisons indicated that the Asian *Taenia* is much more closely related to *T. saginata* than the recognized taeniid species are to each other. The 28S rDNA sequences were identical and there were only nine nucleotide differences in the *cox1* sequences. Importantly, the sequence information clearly showed also that both *T. saginata* and Asian *Taenia* are distantly related to *T. solium.* These molecular genetic studies supported those of Zarlenga and his team [624, 625, 626] and provided evidence that classification of Asian *Taenia* as a subspecies or strain of *T. saginata* was more appropriate than its designation as a separate species. Wang and Bao (591) came to the same conclusions after DNA diagnosis of *Taenia* samples from four areas of Yunnan and Guizhou provinces, the People's Republic of China. Using *cox1* as a marker, they identified the *Taenia* prevalent in Lanping, Dali and Duyun as *T. saginata asiatica,* while that isolated in Congjiang was the typical *T. saginata.*

Molecular phylogenetic analysis [273, 447] using previously published mt *cox1* and nuclear 28S rDNA D1 region sequence information, not surprisingly, showed that *T. saginata* and Asian *Taenia* are very closely related. Eom *et al.,* (149) undertook phylogenetic analysis based on nuclear rDNA ITS2 sequence obtained for *T. saginata,* Asian *Taenia* and *T. solium.* The comparison showed distinct features for Asian *Taenia* and *T. saginata* with many insertion/deletion (indel) regions which they suggested argued against subspecies status for the two taxa. It is noteworthy, however, that some of the indels were not present in all three Asian *Taenia* isolates examined, whereas others were present in *T. saginata* and also in some of the Asian *Taenia* isolates. More sequence information for the ITS2 region from additional *T. saginata* and Asian *Taenia* isolates may help to further clarify the phylogenetic position of Asian *Taenia.* It is noteworthy, however, that multiple rDNA repeats or rDNA genes are present in *T. saginata* [626]. If a similar situation, as seems likely, occurs in the other *Taenia* taxa, this will complicate future phylogenetic analysis that targets the rDNA repeat unit.

Hoberg *et al.,* (272) and Ito *et al.,* (285) have advocated that Asian *Taenia* and *T. saginata* should be considered as sister species, distantly related to *T. solium.* It is noteworthy that the morphological phylogenies produced by Hoberg *et al.,* (272, 273) again show a very close relationship between Asian *Taenia* and *T. saginata.* No hybrids between Asian *Taenia* and *T. saginata* have yet been identified in sympatric zones such as the People's Republic of China where both taxa occur [149, 617]. Though this argues against subspecies status and supports independent species status for the two forms, the number of isolates that have been examined from sympatric endemic areas is limited, and definitive cross-breeding experiments have not yet been done [285]. The available molecular genetic data do not support independent species status for Asian *Taenia* and *T. saginata.* What is in agreement is that both taxa are closely related to each other but distantly related to *T. solium.* This is important in public health terms as it predicts that cysticercosis in humans attributable to Asian *Taenia* does not occur, because cysticercosis is unknown in *T. saginata.*

4.5.3. Selected laboratories experienced in using DNA techniques for identification of *Taenia* taxa

- Dr Minoru Nakao, Department of Parasitology, Asahikawa Medical College, Japan
- Dr Hiroshi Yamasaki, Department of Parasitology, Asahikawa Medical College, Japan
- Dr Munehiro Okamoto, Department of Laboratory Animal Science, School of Veterinary Medicine, Tottori University, Japan
- Dr Keeseon S. Eom, Department of Parasitology and Medical Research Institute, Chungbuk National University College of Medicine, Chongju, Chungbuk, South Korea
- Professor D.P. McManus, Molecular Parasitology Laboratory, Queensland Institute of Medical Research, Brisbane, Australia
- Dr Robin Gasser, Department of Veterinary Science, University of Melbourne, Victoria, Australia
- Dr.Kathy Hancock, CDC, Atlanta, United States of America
- Professor Ana Flisser, Direccion de Investigacion, Hospital General Dr Manuel Gea Gonzalez, SSA, 11400 Mexico, DF, Mexico
- Dr Thanh Hoa Le, Department of Immunology, Institute of Bio-technology of Vietnam, Hanoi, Vietnam.
- Dr C.M. Nunes, Department of Animal Health and Production, Universidade Estadual Paulista Julio de Mesquita Filho, Curso de Medicina Veterinaria, UNESP, campus Aracatuba, Rua Clovis Pestana, Jd. D. Amelia, Aracatuba, Brazil
- Dr R. Rodriguez-Hidalgo, Laboratorio de Immunodiagnostico e Investigacion, Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito, Ecuador
- Dr L.M. Gonzalez, Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III, Centro Nacional de Microbiologia, Madrid, Spain.