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Immunological and molecular diagnosis of cysticercosis

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Cysticercosis, the infection with the larval stage of *Taenia solium*, is a cause of neurological symptoms including seizures, affecting the quality of life of patients and their families. Diagnosis focuses on brain imaging and serological tests are mostly used as confirmatory tools. Most cases, however, occur in poor endemic areas, where both kinds of diagnostic tools are poorly available. Development of point of care diagnostic tests is one of the most important priorities for cysticercosis researches today. The ideal point of care test would require detection of viable cysticercosis and hopefully identify cases with severe or progressive forms of neurocysticercosis, leading to referral of the patient for specialized medical attention. This manuscript describes the evolution of the serological diagnosis of cysticercosis over time, and the characteristics of the most common currently available tools, their advantages and disadvantages, and their potential use in future diagnostic tests.

Keywords: Cysticercosis, Neurocysticercosis, Immunodiagnosis, Antibody, Antigen, Serology, *Taenia solium*, Western blot

Introduction

The varied clinical presentations of neurocysticercosis (NCC) result from a series of factors, which include the number, stage, size, and location of parasites in the nervous system of the human host, factors which also influence case management and prognosis. In this context, the utility of immunodiagnosis as a tool on which to base clinical decisions by itself is quite limited. Diagnosis and characterization of human NCC should be based on a brain imaging examination to observe the characteristics of the lesions, accompanied by a serological test result to confirm the etiology. In the best possible scenario, the immunological test should not only be highly sensitive and highly specific for etiological confirmation but also be able to discriminate infections with living parasites from inactive infections, and correlate the characteristics of the infection with parasite load, for patient management and follow-up. A century of serological assay development for *Taenia solium* cysticercosis has provided some tests which fulfill several of the above requirements, albeit the ideal assay has yet to be developed.

This review will discuss most of the available immunodiagnostic tests for cysticercosis, focusing on the lentil lectin glycoprotein enzyme-linked immunoelectrotransfer blot (LLGP-EITB) assay as the test of choice, the advantages and disadvantages of antigen and antibody detection, as well as the importance of the selection of the type of sample to be processed. We will also stress the importance of evaluating new diagnostic assays with well-defined sets of sera reflecting the entire spectrum of NCC infection (active/inactive, intra/extraparenchymal, diverse numbers of lesions including viable or degenerating cysts for single-lesion cases, cases from diverse geographic areas).

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Antibody Detection Tests for Cysticercosis

Early in the twentieth century, the laboratory diagnosis of tissue parasites was limited to non-specific findings of increased white cell counts, strongly valuing the presence of increased eosinophil numbers.[1,2] The first serological assays for parasitic infections were complement precipitation and fixation techniques. In 1909, Weinberg used complement fixation with cystic fluid from cysticerci to demonstrate specific antibodies in the sera of a group of cysticercotic pigs.[3] This test became known as 'Weinberg's reaction' and was used until a few decades ago.[4–6] In 1911, Arthur Moses reported the use of an aqueous cysticercal extract to demonstrate the presence of antibodies in the serum of three patients with subcutaneous cysticercosis and in the cerebrospinal fluid (CSF) of a patient with cysticercosis encephalitis, thus demonstrating for the first

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time, the presence of anti-cysticercal antibodies in CSF.[1,7]

In the following decades, many attempts to develop better diagnostic tests focused on indirect, antibody detection assays. Antibody detection does not distinguish active from inactive infections, and is not useful to monitor changes over short periods;[8] however, its diagnostic efficacy is much higher than that of antigen detection assays.[9,10] These indirect assays include indirect hemagglutination, immunoelectrophoresis, double immunediffusion, precipitation, indirect immunofluorescence, and skin reaction, among others,[11–17] and are comprehensively described in Flisser *et al.*[18] By 1971, Engvall and Perlmann described the enzyme-linked immunosorbent assay (ELISA) technique.[19] The ELISA is a quite simple technique, is sensitive, quantitative, and can process many samples at the same time, thus it soon became the most frequently used antibody detection assay (ELISA-Ab). It was initially applied in 1978 for the diagnosis of NCC by Arambulo *et al.* in cases with high suspicion for NCC, reporting better sensitivity than the indirect hemagglutination, the test in use at that time.[20] Coker-Vann *et al.* then applied the ELISA technique to detect *T. solium* antibodies in epidemiological studies.[21] Many other laboratories adopted the ELISA-Ab with varied sensitivities according to the antigen and serum panels used. It was evident, however, that the ELISA performed better than the previous techniques. Initial assays used crude metacestode antigens.[21–24] Better results were obtained using cystic fluid as the antigens, but not with membrane or scolex antigens.[25]

Unfortunately, a series of factors affected the diagnostic capacity of these early techniques: the sensitivity and specificity of each technique, the difficulties in defining appropriate reference sera sets, and the use of crude or minimally purified antigens, leading to non-specific reactions mainly with echinococcosis, schistosomiasis, angiostrongyliasis, sparganosis, and fasciolasis.[22,26–29] Case definitions and reference serum batteries were greatly improved with the advent of computed tomography (CT) in 1977 and magnetic resonance imaging in 1986. Cases of NCC could then be differentiated in terms of number of lesions, stage, and location (intraparenchymal or extraparenchymal NCC), variables, which strongly influence the host's humoral immune response.[30]

Antigen characterization became then the objective of researchers looking for improved serological tests. One of the more studied antigens was antigen B, described by Flisser *et al.* in 1980 as the antigen more frequently recognized by sera from NCC-infected patients,[31] producing a strong antibody response.[32–34] Use of antigen B in an antibody detecting ELISA in serum[35] as well as in CSF, did not demonstrate much advantage over other antigen sources.[36,37] Grogl *et al.* in 1985 characterized a series of 37 antigenic proteins from the total metacestode extract as suitable candidates for immunodiagnosis,[38] using for the first time the EITB technique, originally used for immunodiagnosis of schistosomiasis.[38,39] A series of other antigens were then purified using chromatographic techniques and were reported to perform with high sensitivity in cysticercosis immunodiagnosis.[27,28,40–43]

In 1989, the EITB (also known as western blot or immunoblot) using the LLGP fraction was developed and quickly became the assay of choice for serodiagnosis. The LLGP-EITB combines the specificity of using antigens previously purified by chromatography plus the resolution capacity of polyacrylamide gel electrophoresis with sodium dodecyl sulfate coupled with the sensitivity of enzyme-based immunodetection. Seven antigenic glycoproteins (GP) were isolated from a total metacestode homogenate and then purified using lentil-lectin chromatography, namely GP50, GP42-39, GP24, GP21, GP18, GP14, and GP13, where the numbers referred to their molecular mass in kilodaltons (Fig. 1). The presence of any one of the seven antibody bands defines a positive test, with an initial sensitivity and specificity reported to be 98 and 100%, respectively. No cross-reactions were found in 376 sera from 18 heterologous infections.[44] Further comparative testing demonstrated the superiority of the LLGP-EITB over ELISA for the diagnosis of human[10,45–48] and porcine cysticercosis.[49]

Antibody-detecting techniques in general do not have the capacity to distinguish between exposure, inactive infection and active infection, have a low positive predictive value in cases with viable cysticercosis (due to positive antibody reactions in individuals with calcified cysticerci and a high background of seroprevalence in the general population in endemic areas), and have low sensitivity in cases with a single brain lesion.[50,51] Even though the LLGP-EITB is currently considered the test of choice for serodiagnosis of cysticercosis, it has its own drawbacks, which include the source of antigen (the method requires fresh cysts from infected pigs), and is also a complicated procedure. Thus, more recent research efforts have focused on the characterization and synthesis or production of recombinant forms of the seven LLGP diagnostic antigens to produce simpler and more reproducible assays.

Molecular studies showed that the seven LLGP diagnostic antigens comprise three protein families: GP50, T24/T42, and the 8 kDa family.[52–54] GP50 is the largest of the LLGP antigens.[53] Although no defined cross-reactions have been reported to any of the LLGPs, a 'bogus' band can appear slightly above GP50 and generates a problem of interpretation when reading the strips,[44] even in patients without evidence

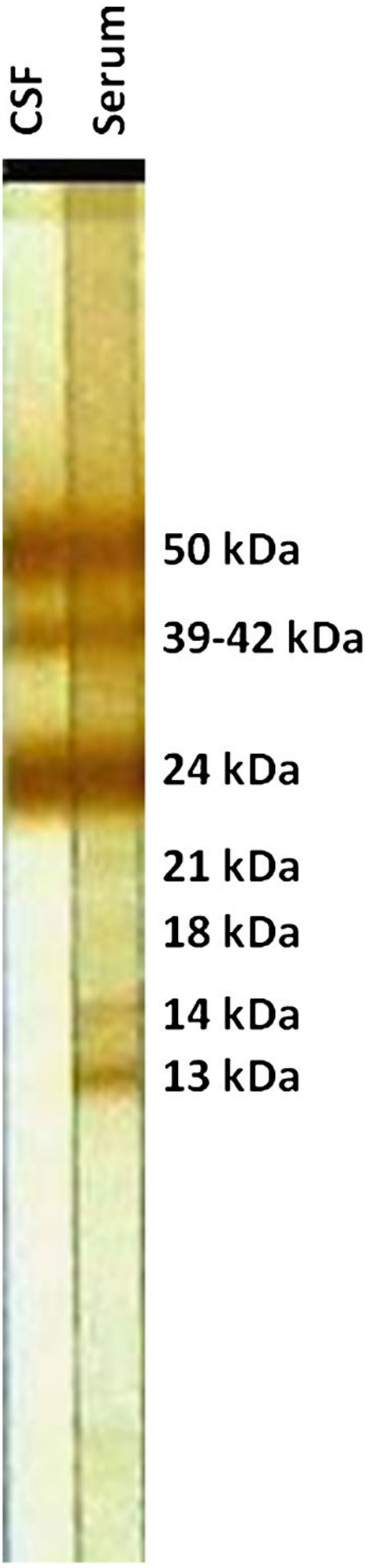

Figure 1 Lentil lectin glycoprotein enzyme-linked immunoelectrotransfer blot (LLGP-EITB) showing the molecular weights of the seven diagnostic bands, and the difference between antibody levels in paired cerebrospinal fluid (CSF) and serum samples from the same individual.

of exposure to *T. solium*, thus its presence as a single reactive band should be taken with caution.[55,56] GP50 and GP39–42 are the more immunodominant antigens, inducing vigorous IgG-response,[57] both are membrane proteins. Studies have demonstrated that GP24 is a monomeric form and GP42–39 is a homodimeric form of the same protein.[53,58] Both have already been produced as recombinants (rGP50 and rT24H) in an eukaryotic expression system, with good diagnostic performances in EITB and in ELISA,[53,54,59] as well as in a novel proprietary technique, the QuickELISA.[60] In general, rT24H performs slightly better than rGP50, but neither antigen alone reaches the sensitivity and specificity of the combined native LLGP set.

The remaining LLGPs, of lower molecular weights, correspond to a complex group of 8 kDa peptides, which can be found alone or in oligomeric structures, which have molecular masses as large as 42 kDa.[61] Similar antigens have been reported in other taeniid cestodes, such as *T. hydatigena*, *T. multiceps*, and *Echinococcus granulosus*.[62–65] These small peptides have been described as excretory/secretory (E/S) products and have been associated with immune evasion functions.[61,66] In some cases, the immunogenic activity of synthetic and native forms correlate well and thus do not seem to depend on secondary structure resulting from post-translational modifications such as a glycosylation;[67,68] in some cases, there seems to be a component of the immune response that depends on conformational epitopes.[69] The presence of low molecular bands in the LLGP-EITB is rarely seen in absence of reactivity to the higher LLGP antigens and seems to be associated with more severe infections. The use of these smaller 8 kDa LLGP peptides as serodiagnostic tools has been proposed because of their capacity to discriminate between active and inactive infections,[70,71] and their availability as synthetic peptide. TsRS1 and Ts18var1 are two peptides in this family, with reasonable sensitivity and specificity in ELISA, which greatly improve when used in EITB format. They however, show lower diagnostic utility when compared to the native forms to detect single lesion cases.[68] Other diagnostic candidates in the 8 kDa family have been expressed and produced as recombinant proteins including Ts8B1, Ts8B2, and Ts8B3. Among these, the Ts8B2 antigen was better able to discriminate between cases of active and inactive NCC,[72] although some cross-reactions with echinococcosis and schistosomiasis were observed.[73]

Splitting the Ts8B2 in smaller synthetic peptides greatly affected the antigen performance.[69] Yang *et al.* described a 10 kDa antigen from cyst fluid, also belonging to the 8 kDa family, reacting mainly to IgG4 and IgG1.[74] This 10 kDa antigen was also produced in recombinant form and showed a good performance to differentiate active from inactive NCC.[71]

Other native antigens under research include parasite proteases. In 2005, Baig *et al.* described a protease from the *T. solium* metacestode with L-cathepsin activity and able to degrade IgG (suggesting a role in immune evasion).[75] A second similar protease was also identified by a different group soon after. This protease, produced in recombinant form, had antigenic activity recognized by sera from patients with NCC.[76] Two other protease fractions highly abundant in cystic fluid have been isolated and evaluated in ELISA and EITB with promising results, and in dot blot form, with lower sensitivity, albeit higher specificity.[77,78]

Synthetic peptide production is appealing for its ease of production and inherent reproducibility. However, to date no synthetic peptide has performed at the level of native antigens for diagnostic purposes.[61,67,79–81] A possible alternative is to use more than one synthetic peptide in the same assay,[82] as in the multiantigen print immunoassay, in which several recombinant or synthetic antigens are printed at different positions along a single strip and thus obviating the need for electrophoretic separation.[83] Recombinant proteins perform better than synthetic peptides, most likely because part of the response is directed towards conformational epitopes.[69] Recombinants to several of the seven LLGP described by Tsang *et al.*, have shown better results than related synthetic peptides in EITB as well as in ELISA.[53,54,60,84] A recently reported recombinant protein, Tsolp27, promisingly detected all cases, but specimens from only 13 cases were tested (Table 1).[85]

Other attempts to develop immunodiagnostic tests include the lymphocyte transformation test (LTT) described by Prasad *et al.*, in 2008, who found 94% of sensitivity and 96% of specificity. This assay requires lymphocyte separation, long incubation time, and a radioactive developer. The authors proposed their use in patients with a single brain lesion but further evaluations are still missing.[86] LTT seems to offer a good alternative to evaluate a host's exposure to a given antigen but will likely not differentiate active from inactive cases because the assay is based on the presence of memory T cells.[87]

Phage display peptide selection was reported as early as in 1999.[88] Almost 10 years later, Hell *et al.* produced a synthetic peptide against a scolex antigen with this technology.[89] Initial promising results have been reported for two other peptides produced with this technique.[90,91] As proposed by Esquivel-Velazquez *et al.*, in 2011, new tools like phage display peptide selection, production of synthetic, and recombinant antigens, could permit us to shorten the path to identify specific antigens capable to distinguish not only the stages of the parasite, but also exposure from viable and non-viable infection.[92] In this way, a good alternative to distinguish exposure from infection could be the use of oncospheral antigens, which to date have mostly been used as vaccine candidates. The 8 kDa antigens seem to be promising candidates to distinguish viable from non-viable NCC; however, the sensitivity of these assays needs improving.[70,71,73,93]

Advantages and disadvantages of antibody detection in cysticercosis

Sound use of serological assays goes beyond the choice of a test and greatly depends on appropriate interpretation of results in the context of a given patient or a given population. Antibody detection with a sensitive and specific assay is the best alternative to diagnose whether somebody has been infected with cysticerci.[9,10] Antibody detection however does not discriminate between active and inactive infections and thus its clinical utility is restricted to etiological confirmation (although strong antibody reactions suggest severe infections[94–96] and, unlike total IgG,[8] IgG4 detection can be associated with active infection[97,98] as well as provide a good monitoring marker[99,100] for cure).

In field conditions, antibody seroprevalence overestimates the actual prevalence of infection because persons with antibodies from exposure and from past infections are also detected. Even more, there is evidence that almost 40% of the positive results in an endemic area are produced by transient antibodies, which become undetectable within 1 year.[101] Detection of parasite specific antibodies in asymptomatic individuals has limited clinical use. Antibody prevalence, however, can provide valuable information on exposure to the parasite, transmission dynamics, risk factors, and incidence calculations, thus it should still be considered a tool for control programs.[47,102–107]

In summary, a positive antibody test associated with a suggestive brain image strongly supports the diagnosis of NCC, while in endemic regions where no CT or magnetic resonance imaging is available, a positive antibody test should be mainly used to refer patients with neurological symptoms to a more equipped center for imaging diagnosis and etiological case management.

Appropriate samples for antibody detection

Selection of a particular type of sample depends on the available test and antigen. In a study of Sahu

et al., serum performed better than CSF when using E/S antigens but there were no differences when using somatic antigens.[108] In general, the evidence suggests that with properly specific assays, serum performs better than CSF for antibody detection.[10,46,48,50,109,110] Some authors note that the simultaneous use of both samples can provide important information on the infection status of the patient.[111]

Tests with less specificity and/or less purified antigens give better results with CSF due to the lower frequency of cross-reacting antibodies in CSF. However, a lumbar puncture to obtain CSF is, however, painful and invasive, and poorly accepted by patients. It is important to note that CSF taken from the ventricles (during surgery or through a shunt) or cisterns (cisternal puncture) can differ from samples collected by lumbar puncture in terms of protein concentration. A gradient along the neuroaxis has been described.[112,113] Also, there may be intrathecally produced antigens in the CSF, particularly during antiparasitic treatment.[114–119]

Venipuncture is the preferred collection method for clinical laboratory studies; however, blood can be also collected by finger prick on filter paper. This procedure is quite advantageous for sample storage and transport and is quite well accepted by the population and can be of great use in field studies or whenever venipuncture is not possible. The LLGP-EITB

Table 1 Antigens used to diagnose NCC by antibody capture

Test	Type of antigen	Sensitivity (1 lesion)	Sensitivity (>2 lesions)	Specificity	Cross-reactions	CSF better than serum	References
LLGP-EITB	Native semi-purified from whole cyst	~50%	98%	100%	No	No	[10,44,48,50, 51,109,179]
rGP50-EITB	Recombinant from 50 kDa	N/E	90%*	100%	N/E	N/E	[53]
rGP50-ELISA	Recombinant from 50 kDa	N/E	95%*	94%*	No	Yes	[59]
rGP50-QuickELISA	Recombinant from 50 kDa	42%*	94%*	99%*	No	N/E	[60]
rGP50-MAPIA	Recombinant from 50 kDa	53%	93%	100%	No	N/E	[83]
rT24-EITB	Recombinant from 24 kDa	63%*	94%*	98%	No	N/E	[54]
rT24-QuickELISA	Recombinant from 24 kDa	47%*	96%*	99%*	No	N/E	[60]
rT24-MAPIA	Recombinant from 24 kDa	60%	97%	99%	*S. mansoni*	N/E	[83]
rT24-MICT	Recombinant from 24 kDa	N/E	94%	99%	No	N/E	[180]
TsRS1-EITB	Synthetic from 8 kDa	85%*	96%*	98%	N/E	N/E	[68]
TsRS1-ELISA	Synthetic from 8 kDa	50%*	77%*	93%	N/E	N/E	[68]
TsRS1-FASTELISA	Synthetic from 8 kDa	N/E	100%*	100%	N/E	N/E	[61]
Ts18var1-EITB	Synthetic from 8 kDa	35%*	97%*	100%	N/E	N/E	[68]
Ts18var1-ELISA	Synthetic from 8 kDa	44%*	95%*	85%	N/E	N/E	[68]
Ts18var1-ELISA	Synthetic from 8 kDa	N/E	90%*	90%*	No	Yes	[59]
Ts18var1-FASTELISA	Synthetic from 8 kDa	N/E	97%*	100%	N/E	N/E	[61]
Ts18var1-QuickELISA	Synthetic from 8 kDa	16%*	90%*	96%*	No	N/E	[60]
Ts8B2-ELISA	Recombinant from 8 kDa	N/E	97%	93%	Echinoccocosis Schistosomiasis	No	[72,73]
10 kDa-EITB	Native 10 kDa from fluid cyst	N/E	85%*	N/E	Echinoccocosis	N/E	[74]
10 kDa-EITB	Recombinant from 10 kDa	N/E	97%*	98%	Echinoccocosis	N/E	[71]
53/25 kDa EITB	Cathepsine L-like 53/25 kDa	78%*	96%*	98%*	Echinococcosis	N/E	[77]
53/25 kDa ELISA	Cathepsine L-like 53/25 kDa	84%*	98%*	93%*	Echinococcosis *T. saginata*	N/E	[77]
53/25 kDa dotELISA	Cathepsine L-like 53/25 kDa	29.4%*	84.8%*	100%*	Echinococcosis	N/E	[78]

Notes: NCC, neurocysticercosis; LLGP-EITB, lentil lectin glycoprotein enzyme-linked immunoelectrotransfer blot; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; MAPIA, multiantigen print immunoassay; MICT, Magnetic Immuno-Chromatographic Test; N/E, not evaluated.
*Calculated using sera with previously positive serology.

works well in blood samples eluted from filter paper,[120,121] with a high agreement with paired serum samples,[122,123] although some antibody activity is lost along the process. Placing the piece of filter paper in a liquid buffer preserves the amount of recovered antibodies.[124]

Detection of antibodies in other biological fluids has not been extensively explored. Two reports showed low sensitivity in urine.[125,126] Saliva gave promising results[45,127] for diagnosis or even for IgG4 monitoring,[100] but no further experiments have been reported. Tears have also been used for diagnosis of ophthalmic cysticercosis (IgA response) with 100% sensitivity and 92% specificity, although this was tested on only a few cases.[128]

Antigen Detection Tests for Cysticercosis

Direct immunodiagnosis (detection of products of the infective agent in the host) has the advantage of demonstrating active infection and in most cases, the antigen levels are associated with the infective burden and thus the severity of the infection, so this type of test can be used to determine therapeutic decisions and guide the prognosis of the patients. Cure is frequently associated with negative antigen results, and on the other hand, relapses, reinfections, or complications result in increases in circulating antigen levels. Unfortunately, in most cases, the sensitivity of antigen detection assays is inferior to that of indirect, antibody-detecting assays.

The initial reports on finding *T. solium* antigens in the CSF of patients with NCC used ELISA assays with rabbit polyclonal antisera raised against crude cysticercal extracts. Their results were promising, particularly in terms of specificity (likely resulting from the use of CSF instead of serum, as detailed above).[129,130] As expected, circulating antigen cannot be demonstrated in the CSF of all NCC patients. Also, only a fraction of all antigens present in the cyst fluid can be detected in the patient's CSF.[131,132] Circulating antigen can also be detected in serum, as initially demonstrated for *T. saginata* cysticercosis in cattle[133] and later in human samples.[134]

Monoclonal antibody (MoAb)-based antigen detection greatly improved the performance of these assays. The initial tests for *T. solium* antigen detection originated from assays developed against *T. saginata* and performed well thanks to an unexpected interspecies cross-reaction. In 1989, Harrison *et al.* developed a MoAb against a repetitive epitope from excretory/secretory glycoprotein products of the *T. saginata* metacestode, HP10. In an ELISA format, HP10 detected circulating antigen in cattle with 200 or more live cysts, with levels detectable in cattle serum as early as 4–5 weeks post-infection. No cross-reactions other than the above described with *T. solium* were reported.[133] The sensitivity of the HP10 ELISA in CSF of confirmed NCC cases was 72%.[36] A similar method was pursued by Brandt *et al.* in 1992. They found eight MoAbs of IgM isotype, which when used in combination, had a lower detection limit of 88 live cysts in infected cattle, and also were able to detect antigens as late as 5 weeks post-infection.[134] These MoAbs were also directed against repetitive glycoprotein epitopes.[135] Further studies generated IgG MoAbs, which improved the antigen assay performance, reaching 92% sensitivity and 98.7% specificity in sera from cysticercosis-infected cattle. They also showed that the target antigen was thermostable. Heat treatment of samples prior to testing gave better results, in particular fewer non-specific reactions (Fig. 2).[136]

MoAbs against *T. solium* were first described in 1991.[137] The initial report concludes that the antigen detection test performed well for diagnosis of *T. solium* cysticercosis, but was not 100% sensitive; the test worked better on CSF than on serum, and antigen levels dropped to undetectable levels after successful treatment.[137] Another anti-*T. solium* MoAb targeted cyst fluid (1F11, IgG1 isotype) and had a diagnostic sensitivity of 82%, mainly missing cases with fewer lesions or only calcifications.[138] This same group also developed MoAb 4F8. A 4F8-based ELISA was used by them to demonstrate that patients with subcutaneous nodules had higher levels of circulating antigen, likely because subcutaneous cysticercosis is found in patients with higher parasite burdens.[139]

Interesting and very promising tools are nanobodies (Nbs), single domain antibodies that are produced in immunized camelids. These molecules are, highly stable and soluble, devoid of light chains, and capable of binding to antigens with high affinity and specificity. Their small size (12–15 kDa) allows detection of hidden epitopes and expression in various microorganisms.[140] Nbs directed against an 8 kDa antigen of the metacestode have been developed and proven specific for *T. solium*, without cross-reactions with *T. hydatigena*, *T. saginata*, *T. crassiceps*, and *Trichinella spiralis* (Table 2).[141] Further work is needed to determine the utility of these reagents for antigen detection in *T. solium* cysticercosis.

Advantages and disadvantages of antigen detection in cysticercosis

Currently available antigen capture assays do not reach the same sensitivity and specificity as antibody-detecting assays, so they are not the best option for initial diagnosis of clinical cases. They, however, provide an exceptional tool for clinical management and follow-up of confirmed cases. As described above, the levels of circulating antigen correlate with

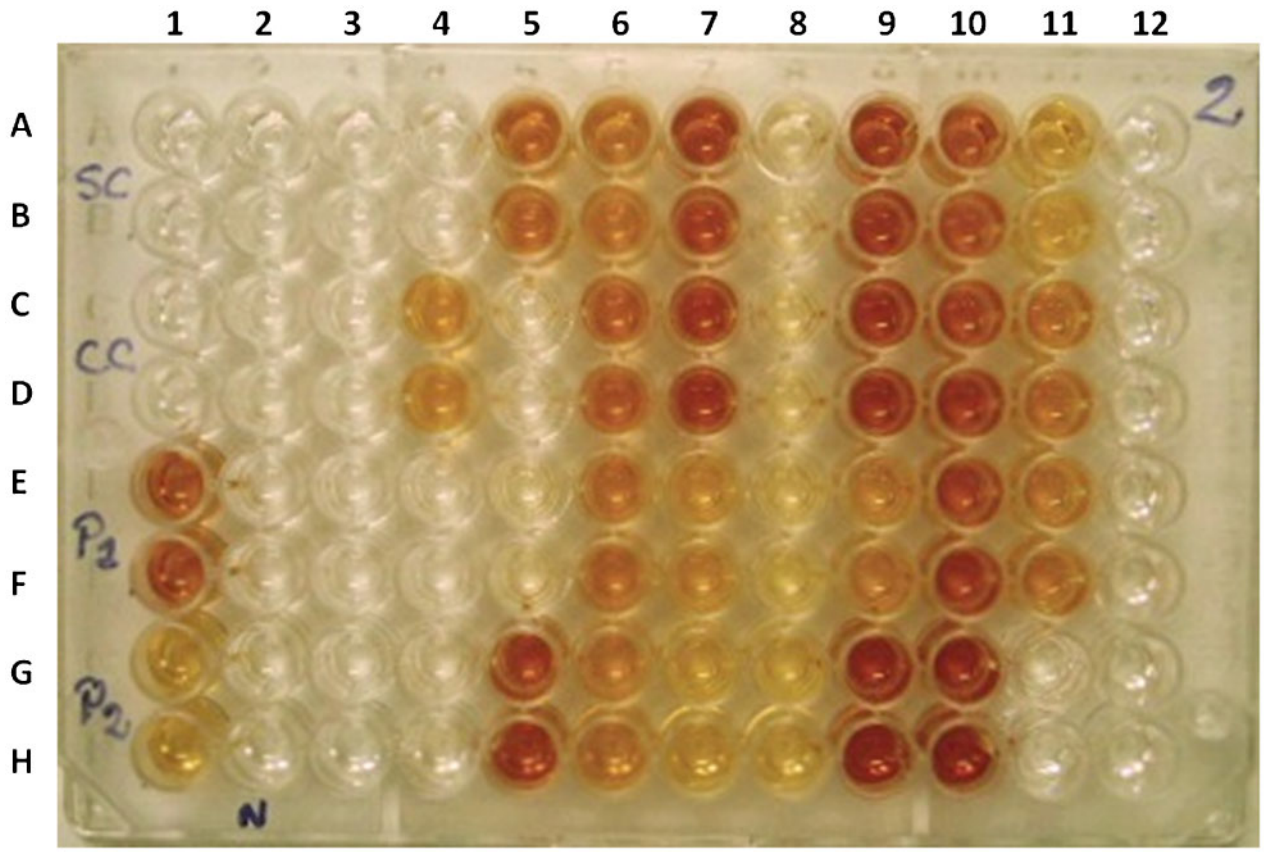

Figure 2 Antigen detection enzyme-linked immunosorbent assay (ELISA) using MoAbs B158 and B60 showing serial samples of different cases.

the severity and type of NCC infection. In intraparenchymal brain cysticercosis, the higher the number of viable cysts, the higher the antigen level, both in humans[142,143] and in pigs.[144] In cases of extraparenchymal NCC, which carry a poorer prognosis, the levels of antigen are much higher, particularly in patients with basal subarachnoid NCC[10,145–147] and also correlate with the volume of the lesions except when hydrocephalus is present. (unpublished data, the Cysticercosis Working Group in Peru 2012) Very high antigen levels should thus raise the suspicion of extraparenchymal NCC or massive intraparenchymal NCC. Since the levels of antigen drop quickly in cured NCC patients, serum antigen monitoring is of great help in the follow-up of clinical cases.[142,145–149] Detectable levels of circulating antigen do not always correlate with neurological symptoms. A study in India followed 42 antigen-positive individuals for up to 5 years, and none of them developed neurological symptoms associated with NCC.[150] It follows that the isolated finding of circulating antigen without neurological symptoms or compatible neuroimage should not be interpreted as an indication for therapeutic intervention.

The usefulness of population-based antigen detection surveys is yet unclear. From the available data, the expected proportion of asymptomatic individuals with viable brain cysticerci and thus positive antigen levels should be quite small. Its use would likely be more productive if focused in patients with compatible symptoms such as seizures or intracranial hypertension.[151,152] In field studies, one would expect

Table 2 Antibodies used to diagnose NCC by antigen capture.

Test	Type of antibody	Sensitivity (1 lesion)	Sensitivity (>2 lesions)	Specificity	Cross-reactions	CSF better than serum	References
HP10-ELISA	MoAbs against *T. saginata* larval antigens from fluid and tegument of cyst	N/E	75%**	100%**	*T. solium* *T. hydatigena*	No	[36,133]
B158/B60-ELISA	MoAbs against *T. saginata* excretory/secretory larval antigens	N/E	N/E	N/E	*T. solium* *T. hydatigena*	Yes	[10,134,136,181]

Notes: NCC, neurocysticercosis; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; N/E, not evaluated.
**Calculated using only CSF.

to find a higher prevalence of antibody-positive individuals than antigen-positive individuals, since antibody responses occur in non-viable infections and individuals with exposure only, and for an unknown time after resolution. However, population-based studies show discordant results. In a community in Mexico, 1% of all people were positive to circulating antigen versus 4.8% seropositive to antibodies.[151] In Ecuador, 2.25% of 800 participants in a rural community were positive for antigen, while the antibody seroprevalence measured in a subset of 100 randomly selected samples was 40%.[153] On the other hand, a study in South Africa found more antigen-positive than antibody-positive cases,[154] and another study in Burundi found higher prevalence of antibodies in patients with epilepsy than in controls but no differences in the frequencies of antigen-positive cases between these groups.[155]

Appropriate samples for antigen detection

Compared to serum, CSF had better sensitivity (78% versus 68%) and specificity (73% versus 60%) for antigen detection, when MoAbs are used as capture antibodies.[10,137] Assays using polyclonal rabbit antisera against specific purified antigens did not show differences in serum versus CSF.[156] Also no advantage was demonstrated when using saliva.[157]

Urine samples are easy to collect, non-invasive and thus are easily provided by patients or villagers. Immunodiagnostic tests in urine have been developed for a variety of infectious diseases,[158–161] including cysticercosis. The initial study found detectable antigen levels in five out of eight confirmed NCC cases, in an agglutination assay using rabbit antisera to a *T. solium* total metacestode extract.[162] We have found 92% sensitivity in patients with two or more viable cysts, and 62.5% in single lesion cases using the MoAbs described by Van Kerckhoven *et al.*,[136] as well as a strong correlation between serum and urine levels of antigen.[163] Mwape *et al.*[164] tested paired urine and serum samples collected in Zambia and Ecuador. A very high agreement (90%) was found between samples of the same individual, but lower specificity when urine samples were tested. The Zambian samples had more non-specific reactions. Urine can also be concentrated to take advantage of the large sample volumes, which can be obtained. Lyophilization avoids the need for further refrigeration or freezing and also results in a slight increase in sensitivity. It, however, also decreases specificity by 2–4%. Other concentration methods were less efficient.[163]

Single-Lesional NCC

A single brain-enhancing lesion is a very frequent presentation of NCC in the Indian subcontinent, where it is also a major contributor to seizures in younger patients.[165–167] In Latin America, the proportion of NCC patients with a single-enhancing lesion varies from 3.5 to 34%,[168] likely reflecting different definitions of a single-enhancing lesion. More importantly, other infections, tumors, vascular lesions, and other etiologies need to be considered in the differential diagnosis of single brain lesions,[168–170] and thus serology could be of great help in saving unnecessary invasive procedures or treatments.[171]

In general, all immunodiagnostic tests show low sensitivity for the diagnosis of single-lesional NCC, even worse when the lesion has entered in an involutive, degenerative process of resolution following antiparasitic treatment or by natural evolution ('single-enhancing lesion' or single cysticercal granuloma'). A proportion of these cases could also correspond to early lesions, caused by cysticerci, that resolved soon after infection, before full establishment and thus not provoking a strong immune response.[172] Most seronegative NCC cases in ELISA[111] or in the LLGP-EITB[48,50,51] correspond to cases with a single lesion, although the estimates of sensitivity of the assays in this type of NCC vary greatly. Prabhakaran *et al.* reported an increase in sensitivity following the use of urea to expose the tertiary structure of the antigenic glycoproteins, detecting 46% of previously negative cases in EITB.[173]

DNA-Based Technology

The greatest contribution of DNA-based technology has been in the genotyping of the genus *Taenia*, which has served to determine the phylogeny and taxonomy of its species and to understand the level of genetic diversity in the genus.[174] Another important contribution of molecular biology is the identification and production of antigenic molecules used as vaccines candidates or as candidates for serological tests.

Direct use of molecular techniques for NCC diagnosis was first reported in 2006 and demonstrated *T. solium* DNA in the CSF of 29 of 30 consecutive patients, using a PCR with primers against pTsol9, specific for *T. solium*.[175] Another study, using primers against HDP2, based on a non-coding sequence of *T. saginata*, which cross-reacts with *T. solium*, also found parasite DNA in human CSF, as well as reported higher sensitivity by type of NCC (10/14 extraparenchymal NCC cases compared to 4/24 of intraparenchymal, degenerating NCC).[176] A recent study compared antigen and antibody capture techniques with a pTsol9 PCR. In 150 CSFs of patients with different types of NCC, PCR had the best sensitivity, although its specificity was only 80% using negative controls from Mexico. Unexpectedly, 28/31 patients with only calcified

Table 3 Challenges for new serological tests for NCC diagnosis

Test	Challenges
Antibody test	Identify antigens or epitopes to maintain high sensitivity and specificity compared to native antigens. Increase the sensitivity for single brain lesion. Identify antigens capable to differentiate exposure from infection. Identify antigens capable to differentiate viable and non-viable cysticercosis. Produce these antigens or epitopes in recombinant or synthetic way to have an easier and reproducible source of antigen. Use these antigens or epitopes to develop a point of care test, to have a primary tool in field settings.
Antigen test	Produce MoAbs or nanobodies against *T. solium* metacestodes in order to increase sensitivity and specificity. Standardize a qualitative assay with a better reproducibility and repeatability. Produce a test capable of differentiating viable and non-viable cysticercosis, with a high PPV for extraparenchymal NCC. Assay needs to perform well in urine samples to avoid invasive and risky sampling methods. Develop a point of care test, to have a primary tool in field settings.

Note: NCC, neurocysticercosis.

NCC were PCR-positive (compared to 19 antibody-positive and seven antigen-positive by EITB and ELISA-HP10, respectively).[177] Only one study using PCR in porcine cysticercosis has been published, with poor performance: sensitivities of 23% and 32% in heavily infected animals, which improved to 64% using a nested PCR. On the other hand, these PCR assays were 100% specific.[178]

Conclusion

A variety of laboratory methods are available to support the diagnosis of NCC. The LLGP-EITB remains the optimal assay for clinical diagnosis, while antigen detection is useful to monitor patients after anthelminthic treatment (Table 3). Despite initial reports, molecular methods have not yet proven useful to diagnose NCC in clinical settings. More research is necessary to evaluate their real potential.

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