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HELMINTHOLOGY - ORIGINAL PAPER

Immunodiagnosis of human neurocysticercosis: comparative performance of serum diagnostic tests in Mexico

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Abstract

Immunodiagnosis has a supportive role in the diagnosis of neurocysticercosis (NCC). The aim of this study was to compare the validity of seven immunodiagnostic tests among serum samples from 58 patients with NCC, 26 patients with neurological diseases other than NCC, and 15 healthy controls. One test for viable parasite detection (HP10 antigen assay) and six for antibody detection were evaluated. For the entire sample, sensitivities ranged from 55.2% (NOVALISA) to 81.0% (enzyme-linked immunosorbent assay [ELISA] *Taenia solium* antibody), with the sensitivity of the latter test significantly higher than that of the in-house ELISA *Taenia crassiceps*, NOVALISA, enzyme-linked immunoelectrotransfer blot (EITB) CDC, and HP10. Overall, specificities were high, ranging from 85.4% (ELISA Ts) to 97.1% (NOVALISA), with no statistically significant differences. Detection of HP10 antigen was significantly associated with the presence of vesicular parasites. The simple and low-cost ELISA *Taenia solium* antibody Ab instead of EITB is recommended to support NCC diagnosis in both rural and hospital settings in Mexico.

Keywords ELISA · EITB · HP10 · Immunodiagnosis · Mexico · Neurocysticercosis

Introduction

Neurocysticercosis (NCC) is a zoonotic disease caused by the establishment of the larval form of *Taenia solium* in the central nervous system. NCC is still endemic in most countries in Latin America, Asia, and Sub-Saharan Africa where the disease is closely associated with poverty and deficient hygiene, including poor water quality and inadequate sanitary facilities

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(Torgerson et al. 2015). Moreover, the number of cases of NCC diagnosed in non-endemic countries is increasing mainly due to the entrance of immigrants from endemic regions (O'Neal and Flecker 2015).

Although great progress in the diagnosis, treatment, and prevention of NCC has been achieved, morbidity and mortality remain unacceptably high in endemic countries. One reason for this is the absence of a universally accepted and available means of diagnosis. The gold standard for diagnosis remains imaging studies, particularly computed tomography (CT) and magnetic resonance imaging (MRI) (Carpio et al. 2016). Unfortunately, the limited access of the population at greatest risk in endemic low- and middle-income countries to these expensive tools is a key limitation for diagnosis.

In this context, efforts have been made to identify and implement the use of immunodiagnostic tests with acceptable validity and cost, mainly to detect specific antibodies, which indicate a current or past infection but also to exposure to *Taenia solium*, and more recently to detect parasite antigens, which indicate the presence of viable cysticerci (Garcia et al. 2018a). The first immunodiagnostic test to detect specific antibody in patients with NCC, the complement fixation test, was identified in the 1950s (Nieto 1956), while the first test to detect specific antigen, an enzyme-linked immunosorbent assay (ELISA), was described in the 1980s (Estrada and Kuhn 1985). Since then, different techniques have been used for antibody detection, e.g., hemagglutination, radioimmunoassay, ELISA, dipstick ELISA, latex agglutination, and enzyme-linked immunoelectrotransfer blotting (EITB). Nowadays, ELISA using total extracts or vesicular fluid of cysticerci as antigen and EITB using lentil-lectin purified glycoprotein (LLGPs) are the assays most often used for antibody detection. Regarding antigen detection, ELISA is still the main technique used, and two assays, the in-house HP10 Ag-ELISA (Harrison et al. 1989) and the commercialized B158/B60 Ag-ELISA (Brandt et al. 1992), are available.

The first studies using LLGPs-EITB to detect antibody reported very high sensitivity and specificity (Tsang et al. 1989; Wilson et al. 1991; Proaño-Narvaez et al. 2002), but with more extensive testing, some conflicting results have been reported (Kojic and White 2003; Cardona-Arias et al. 2017). As substantial differences exist between these tests regarding their cost (i.e., LLGPs-EITB is approximately 100 times costlier than ELISA) and their feasibility (i.e., necessary laboratory infrastructure and human skill are much greater for non-commercial EITB than that required for ELISA), studies making comparisons among tests are needed, but only a few have been reported. From them, the most extensive comparison among tests was reported by Michelet and colleagues using cerebrospinal fluid (CSF) of patients with NCC and patients affected by other neurological diseases (Michelet et al. 2011). In this study, no significant differences between LLGPs-EITB and ELISA were observed. However, serum is the preferred sample for testing and, unfortunately, the levels of background are much higher in serum than in CSF. A recent study evaluated LLGPs-EITB and two ELISAs (Garcia et al. 2018b); however, a criterion for inclusion was a positive LLGPs-EITB result, preventing a reliable comparison between tests.

In this study, we sought to evaluate the performance of different diagnostic kits and in-house tests using serum samples from patients with NCC, patients with other neurological conditions, and healthy controls in Mexico.

Material and methods

Patients

Fifty-eight patients with a definitive diagnosis of NCC were included (using the validated Carpio 2016 criteria), who sought care at the Instituto Nacional de Neurología y Neurocirugía (INNN) in Mexico City. Patients with NCC were classified according to the degenerative stage and locations of parasites in three groups: vesicular extraparenchymal cysts, vesicular parenchymal cysts, and calcified parenchymal cysts. The serum samples were part of a sera bank and patients gave their informed consent to use their samples in research studies and to publish the anonymized results. The present study complied with all Mexican and international laws and regulations for ethical clinical research practice and the study protocol was approved by the INNN. Informed consent was obtained from all individual participants included in the study and for their stored sera to be used for future research purposes.

A group of 26 patients with other neurological diagnoses and in whom NCC was ruled out was also included. Their neurological diagnoses were a follows: demyelinating diseases ($N = 17$, 65.4%), headache ($N = 3$, 11.5%), seizures ($N = 2$, 7.7%), tumors ($N = 2$, 7.7%), neuropathy ($N = 1$, 3.8%), neck pain ($N = 1$, 3.8%). A third group ($N = 15$) of apparently healthy individuals without any serious medical histories were also included. The age and sex distributions of the three groups are presented in Table 1.

Serological assays

Seven serological assays were evaluated; five of them (ELISA Tc, ELISA Ts, ELISA HP10, NOVALISA, EITB InDRE) were applied to all samples while two of them (EITB LDBIO, EITB CDC) were only applied to NCC patient samples.

Antibody detection

In-house ELISAs using *Taenia solium* (Ts) and *Taenia crassiceps* (Tc) antigen The antigens used for the in-house ELISAs were recovered from *T. solium* and *T. crassiceps* vesicular fluid as previously described in detail (Larralde et al. 1986; Fleury et al. 2003). Briefly, the procedure used was as

Table 1 Sex and age distribution of Mexican patients with neurocysticercosis and control participants

	Neurocysticercosis ($N = 58$)			Controls ($N = 41$)	
	Vesicular extraparenchymal ($N = 29$)	Vesicular parenchymal ($N = 8$)	Calcified parenchymal ($N = 21$)	Other neuropathologies ($N = 26$)	Healthy persons ($N = 15$)
Male sex (N, %)	13, 44.8%	3, 37.5%	9, 42.9%	9, 34.6%	9, 60.0%
Age (mean ± SD)	44.8 ± 14.5	34.5 ± 5.4	47.5 ± 9.6	36.6 ± 11.4	25.1 ± 1.3

follows: Immulon (Dynatech) flat-bottom plates sensitized with *T. solium* or *T. crassiceps* cysticerci vesicular fluid antigen at 1 μg/well in 100 μl of carbonate buffer pH = 9.6 (0.2 M) were incubated overnight at 4 °C. After washing four times for nonadherent proteins with phosphate-buffered saline (PBS) containing 0.3% Tween, plates were blocked with 200 μl/well of PBS containing 0.3% Tween, 1% bovine serum albumin (BSA) and incubated 1 h at 37 °C. Next, 100 μl/well of each serum controls negative, positive, and samples were diluted 1:1000 in blocking buffer and were incubated for 30 min at 37 °C. The presence of attached anti-cysticercus antibodies was detected by adding 100 μl/well of goat anti-human IgG (H+L) horseradish peroxidase (Invitrogen, Carlsbad, CA, USA) diluted 1:20,000 in blocking buffer and incubated for 30 min at 37 °C. Next, the reaction was revealed with 100 μl/well of tetramethylbenzidine (Invitrogen, Carlsbad, CA, USA) incubated for 5 min at room temperature. The reaction was stopped with 100 μl/well of 0.2 M sulfuric acid (Baker, Mexico). Optical density readings at 450 nm were done in an Opsys MR microplate reader (Dynex Technology, VA, USA). Samples were run in duplicate. A sample was considered positive if the mean optical density at 450 nm was greater than the cutoff value (corresponding to the mean of 5 negative sera samples + 2 standard deviations). The cutoff value was estimated for each plate using five samples from confirmed neurological patients without NCC (i.e., patients with negative CT scan and MRI). A group of five additional samples from neurological patients confirmed as NCC positive controls from the INNN was included.

NOVALISA assay

The commercial NOVALISA test (NovaTec Immundiagnostica GmbH, Dietzenbach, Germany) based on microtiter strip wells was precoated with *T. solium* antigens, incubated with control positive, negative, cutoff, and serum samples, diluted 1:100 in dilution buffer, and were incubated for 1 h at 37 °C, after washing the wells three times. Horseradish peroxidase labeled protein A conjugated was added 100 μl/well and incubated 30 min at room temperature in the dark. The reaction was revealed for 15 min at room temperature in the dark with 100 μl/well of tetramethylbenzidine, after which the reaction was stopped using 100 μl/well of stop solution. Optical density was read at 450/620 nm in an Opsys MR microplate reader (Dynex Technology, VA, USA).

The test was considered positive or negative if the absorbance value was higher or lower than 10% of the cutoff value, respectively. The interpretation of the results in NovaTec units (NTU: patient [mean] absorbance value × 10 / cutoff) was as follows: cutoff, 10 NTU; gray zone, 9–11 NTU; negative, < 9 NTU; and positive, > 11 NTU.

Immunoblotting

Cysticercosis western blot immunoglobulin G (IgG) test The western blot assay was performed with the strips containing purified cysticercus antigen and reagents provided with the kit according to the manufacturer's instructions (LDBIO Diagnostics, Lyon, France). Briefly, the strips were incubated with 25-μl sera diluted in 1.2 ml of sample buffer and were incubated for 90 min at room temperature. After washing three times with washing buffer, the strips were incubated with 1.2 ml of anti-human IgG alkaline phosphatase conjugate for 60 min at room temperature. The reaction was revealed for 30 min with 1.2 ml of nitro-blue tetrazolium chloride/5-bromo-4-chloro-3′-indolyphosphate p-toluidine salt (NBT/BCIP) substrate. The reaction was stopped by washing the strips three times with distilled water. The strips were dried and glued to paper for reading and storage. Positive and negative controls were tested in each assay. For the result validation, protein bands on the specimen sample strip were compared with the protein bands on the test positive control strip.

Lentil-lectin affinity-purified *T. solium* cysts glycoprotein antigens (LL-GP *T. solium* cysts antigen) The enzyme-linked immunoelectrotransfer blot (EITB) assay developed at the CDC (EITB CDC) was carried out as described by Tsang et al. (1989), using prepared glycoprotein antigen strips and storage at − 60 °C and covered with a sheet of filter paper saturated with PBS until used. Each strip was incubated with the serum diluted 1:100 in PBS containing 0.3% *v*/*v* tween and nonfat milk (5% *w*/*v*) and incubated overnight at 4 °C. The strips were washed four times for 5 min each with PBS Tween 0.3%. Antibody binding was visualized by incubating with the HRP-Goat Anti-Human IgG (H+L) Conjugate (ZyMax™

Grade) (Invitrogen, Carlsbad, Ca, USA) diluted 1: 5000 and incubated for 1 h at room temperature, and the enzymatic activity was developed with DAB (3,3′-diaminobenzidine) in PBS for 1 h until specific bands appeared. The strips were then washed with distilled water. For the result validation, we identified the protein bands pattern on each strip and compared it with the positive control.

An in-house LL-GP *T. solium* cysts antigen developed and used by the Mexican Diagnostic and Epidemiologic Reference Institute (Instituto de Diagnóstico y Referencia Epidemiológicos [InDRE]) following the technique described by Tsang et al. (1989) was also evaluated. The methodology and interpretation of results were similar to that as described above.

Criteria and EITB band interpretation The LL-GP *T. solium* cysts antigen includes the recognition of seven glycoprotein bands identified as GP50, GP42-39, GP24, GP21, GP18, GP14, and GP13, with the number indicative of molecular weight in thousands. As reported by Tsang et al. (1989), since all of the bands are specific for cysticercosis, the recognition of at least one band is considered to be positive for cysticercosis infection or exposure.

Antigen detection: HP10 antigen assay

HP10 antigen was detected by Ag-ELISA as described previously (Fleury et al. 2003). All samples were run in duplicate. Briefly, plates (Nunc, Rochester, NY, USA) were coated with monoclonal antibody (MoAb) HP10 (100 μl at 10 μg/ml in 0.07 M sodium chloride buffered with 0.1 M borate, pH = 8.2) and left overnight at 4 °C, washed four times with 200 μl/well of wash solution (0.9% *w*/*v* sodium chloride containing 0.05% *v*/*v* Tween 20) and then blocked using 200 μl of PBS containing bovine serum albumin (Roche, Mexico) (1.0% *w*/*v* and 0.05% *v*/*v* Tween 20) and left 60 min at room temperature. Next, undiluted serum samples (100 μl/well) were added and incubated for 45 min at 37 °C. Bound HP10 parasite antigen was detected using biotinylated MoAb HP10 (2 μg/ml in diluent) and incubated for 45 min at 37 °C, with horseradish peroxidase-conjugated streptavidin (Zymed, San Francisco, CA, USA) (1:4000 in diluent, 45 min at 37 °C) and tetramethylbenzidine (Invitrogen) as substrate. The color reaction was allowed to proceed for 5 min at room temperature in the dark and was stopped by adding 100 μl 0.2 M sulfuric acid (Baker, Mexico). Optical densities (450 nm) were determined in an ELISA processor (Opsys MR Dynex Technology, VA, USA). As for the detection of antibodies, a sample was considered positive if the mean optical density at 450 nm was greater than the cutoff value (corresponding to the mean of 5 negative sera samples + 2 standard deviations). The cutoff value was estimated for each plate using five samples from confirmed neurological patients without NCC (i.e., patients with negative CT scan and MRI).

Statistical analysis

Sensitivity and specificity with exact 95% confidence intervals (CIs) were computed for all assays, first looking at the overall samples of NCC and controls, and then by subgroups (vesicular extraparenchymal NCC, vesicular parenchymal NCC, calcified NCC, neurological controls, healthy controls). McNemar's test was used to compare the sensitivity and specificity between each test for the overall sample.

Inter-assay reliability (using Cohen's Kappa coefficient) along with 95% CIs was calculated between all assays, first overall then by subgroup. The mean number of bands for EITB assay was compared by computing Spearman's rank correlation coefficients and tested using the Kruskal-Wallis Test.

Comparisons of the number of bands of EITB were made using non-parametric analysis of variance (Kruskal-Wallis test) and a post hoc test (Dunn's multiple comparisons test).

Results

Sensitivity and specificity of the tests

As shown in Table 2, the sensitivity of the different tests for all forms of NCC ranged from 55.2% (NOVALISA) to 81.0% (ELISA Ts).

The sensitivity was significantly higher for ELISA Ts compared with ELISA Tc, ELISA HP10, NOVALISA, and EITB CDC. Sensitivity was also significantly higher for both EITB InDRE and EITB LDBIO compared with both ELISA HP10 and NOVALISA. Overall, the specificities of the different tests were high, ranging from 85.4% (ELISA Ts) to 97.1% (NOVALISA), with no statistically significant differences.

When evaluating subgroups of patients (Table 3), sensitivities of the tests were highest in the group of patients with vesicular extraparenchymal NCC (ranging from 82.7% [NOVALISA] to 100.0% [ELISA Ts and EITB LDBIO]), followed by patients with vesicular parenchymal NCC (ranging from 37.5% [NOVALISA] to 87.5% [ELISA Ts and EITB InDRE]), and lowest among patients with calcified NCC (ranging from 9.5% [HP10] to 52.4% [ELISA Ts]). When stratifying sensitivities by number of cysts, sensitivity was lower for single vs. multiple cysts for ELISA HP10 (37.5% vs. 64.3%), EITB CDC (35.7% vs. 76.2%), EITB InDRE (56.3% vs. 78.6%), and EITB LDBIO (50.0% vs. 80.0%), but relatively similar for the other assays. Specificity was high for both healthy persons and patients with other neurological pathologies.

Table 2 NCC immunodiagnostic in Mexico: sensitivity and specificity of the different tests and *P* values for comparison among all tests

		ELISA Ts Ab	ELISA Tc Ab	ELISA HP10	NOVALISA	EITB InDRE	EITB CDC
Sensitivity							
ELISA Ts Ab	81.0% [68.6–90.1]						
ELISA Tc Ab	69.0% [55.5–80.5]	0.039					
ELISA HP10	56.9% [43.2–69.8]	< 0.001	0.092				
NOVALISA	55.2% [41.5–68.3]	< 0.001	0.057	1.000			
EITB InDRE	72.4% [59.1–83.3]	0.125	0.754	0.023	0.031		
EITB CDC	66.1% [52.2–78.2]	0.008	0.727	0.227	0.210	0.289	
EITB LDBIO	71.4% [56.7–83.4]	0.543	0.688	0.008	0.013	1.000	0.375
Specificity							
ELISA Ts Ab	85.4% [70.8–94.4]						
ELISA Tc Ab	95.1% [83.5–99.4]	0.125					
ELISA HP10	94.9% [82.7–99.4]	0.289	1.000				
NOVALISA	97.1% [84.7–99.9]	0.063	1.000	1.000			
EITB InDRE	95.1% [83.5–99.4]	0.289	1.000	1.000	1.000		
EITB CDC	NC	NC	NC	NC	NC	NC	
EITB LDBIO	NC	NC	NC	NC	NC	NC	NC

NC, not computed (because of effective sample size of ≤ 1). *Ts*, *Taenia solium*; *Tc*, *Taenia crassiceps*

The presence of the HP10-Ag was significantly associated ($P < 0.001$) with vesicular parasites (83.8% positivity) compared with its presence in only 9.5% calcified parasites.

Comparison of band distribution between western blots

Comparison of band distribution in the three western blot was done in patients who were positive for these tests. The mean band number was significantly higher in InDRE (5.4 ± 2.1) than in LDBIO (3.9 ± 1.1) and CDC (2.5 ± 0.9), $P = 0.008$ and $P < 0.001$, respectively. The difference was also significant between mean number of bands of CDC and LDBIO ($P < 0.001$). Significant positive correlations in the number of bands were found between CDC and InDRE ($R = 0.48$, $P = 0.004$) and between CDC and LDBIO ($R = 0.61$, $P < 0.001$). For CDC and InDRE EITB, the number of bands was significantly higher in the vesicular extraparenchymal group (2.8 ± 0.8 for CDC and 6.4 ± 1.4 for InDRE) than in the vesicular parenchymal group (1.7 ± 0.6 for CDC, $P = 0.04$, and 3.9 ± 2.2 for INDRE, $P = 0.002$) and in the calcified group (1.6 ± 0.5 for CDC, $P = 0.001$, and 4.4 ± 1.9 for INDRE, $P = 0.01$). Although the number of bands using LDBIO was also higher in the extraparenchymal group (4.2 ± 0.9) vs. parenchymal (4.0 ± 1.4) and calcified groups (3.3 ± 1.2), these differences were not statistically significant.

Concordance among diagnostic tools

Levels of agreement among the different tests are shown in Table 4. Most comparisons (16 of 21, 76.2%) had a substantial level of agreement (Kappa coefficient between 0.60 and 0.79), while the others (5 of 21, 23.8%) had a moderate level of agreement (Kappa coefficient between 0.40 and 0.59). Most of these involved the NOVALISA test. Kappa coefficients for subgroups are presented in the Supplementary Table. Concordance was highly variable, ranging from null to almost perfect. Kappa could not be calculated in 20 of the 104 (19.2%) comparisons as the value of one of the tests was similar for all the samples tested.

Discussion

Diagnosis of NCC is still a challenge particularly in endemic countries where imaging studies, which are the gold standard tool for diagnosis, are not available for a large proportion of the population due to their cost to the patient or to their

Table 3 NCC immunodiagnostic in Mexico: sensitivity and specificity of the different tests by subgroup

	ELISA Ts Ab*	ELISA Tc Ab*	ELISA HP10*	NOVALISA*	EITB InDRE*	EITB CDC*	EITB LDBIO*
Sensitivity							
All NCC cases ($N = 58$)	47, 81% [68.6–90.1]	40, 69% [55.5–80.5]	33, 56.9% [43.2–69.8]	32, 55.2% [41.5–68.3]	42, 72.4% [59.1–83.3]	37ˇ, 66.1% [52.2–78.2]	35 , 71.4% [56.7–83.4]
Vesicular extraparenchymal ($N = 29$)	29, 100.0% [88.1–100.0]	28, 96.6% [82.2–99.9]	26, 89.7% [72.7–97.8]	24, 82.8% [64.2–94.2]	26, 89.7% [72.7–97.8]	27, 93.1% [77.2–99.2]	21‡, 100% [83.9–100.0]
Vesicular parenchymal ($N = 8$)	7, 87.5% [47.4–99.7]	5, 62.5% [24.5–91.5]	5, 62.5% [24.5–91.5]	3, 37.5% [8.5–75.5]	7, 87.5% [47.4–99.7]	3°, 50.0% [11.8–88.2]	6, 75.0% [34.9–96.8]
Calcified parasite ($N = 21$)	11, 52.4% [29.8–74.3]	7, 33.3% [14.6–57.0]	2, 9.5% [1.2–30.4]	5, 23.8% [8.2–47.2]	9, 42.9% [21.8–66.0]	7, 33.3% [14.6–57.0]	8+, 40.0% [19.1–64.0]
Single cyst($N = 16$)	12, 75.0%[47.6–92.7]	10, 62.5%[35.4–84.8]	6, 37.5%[15.2–64.6]	8, 50.0%[24.7–75.4]	9, 56.3%[30.0–80.0]	5, 35.7%[12.8–64.9]	7, 50.0%[23.0–77.0]
Multiple cysts ($N = 42$)	35, 83.3%[68.6–93.0]	30, 71.4%[55.4–84.3]	27, 64.3%[48.0–78.5]	24, 57.1% [41–72.3]	33, 78.6%[63.2–89.7]	32, 76.2%[60.1–88.0]	28, 80.0%[63.1–91.6]
Specificity							
All controls ($N = 41$)	6, 85.4% [70.8–94.4]	2, 95.1% [83.5–99.4]	2, 94.9% [82.7–99.4]	1, 97.1% [84.7–99.9]	2, 95.1% [83.5–99.4]	NC	NC
Other neurological pathologies ($N = 26$)	1, 96.2% [80.4–99.9]	1, 96.2% [80.4–99.9]	1, 96.1% [80.4–100.0]	0†, 100% [82.4–100.0]	2, 92.3% [74.9–99.1]	NC	NC
Healthy persons ($N = 15$)	5, 66.7% [38.3–88.2]	1, 93.3% [68.1–99.8]	1, 93.3% [68.1–99.8]	1, 93.3% [68.1–99.8]	0, 100.0% [78.2–100.0]	NC	NC

*Number of positive samples, sensitivity (first 4 rows), or specificity (3 last rows), [95% CI]

ˇDone on 56 samples; Done on 47 samples; † Done on 19 samples; ‡ Done on 21 samples; °Done on 6 samples; + Done on 18 samples

Ts, *Taenia solium*; *Tc*, *Taenia crassiceps*; *Ab*, antibodies

NC, not computed (because of effective sample size of ≤ 1)

Table 4 NCC immunodiagnostic: inter-assay levels of agreement

	ELISA Ts Ab	ELISA Tc Ab	ELISA HP10	NOVALISA	EITB InDRE	EITB CDC
ELISA Tc Ab	*K: 0.74* *[0.61–0.87]*					
ELISA HP10	**K: 0.52** **[0.36–0.67]**	*K: 0.64* *[0.48–0.79]*				
NOVALISA	**K: 0.54** **[0.39–0.69]**	*K: 0.67* *[0.51–0.82]*	*K: 0.62* *[0.46–0.79]*			
EITB InDRE	*K: 0.70* *[0.56–0.84]*	*K: 0.71* *[0.57–0.85]*	*K: 0.64* *[0.49–0.79]*	**K: 0.56** **[0.39–0.72]**		
EITB CDC	*K: 0.66* *[0.46–0.87]*	*K: 0.68* *[0.48–0.89]*	*K: 0.60* *[0.39–0.81]*	**K: 0.42** **[0.19–0.66]**	*K: 0.68* *[0.47–0.88]*	
EITB LDBIO	*K: 0.63* *[0.38–0.88]*	*K: 0.71* *[0.50–0.93]*	*K: 0.66* *[0.46–0.86]*	**K: 0.42** **[0.19–0.66]**	*K: 0.74* *[0.53–0.95]*	*K: 0.75* *[0.55–0.95]*

Cohen's Kappa coefficients (95% CI) between all tests. Boldface signifies moderate inter-assay reliability (K = 0.40–0.59), and italic signifies substantial inter-assay reliability (K = 0.60–0.79)

complete absence from the healthcare infrastructure. In rural areas, serological immunodiagnostic tests could be of great value as a screening tool; for example, to identify which patients must have imaging studies done. In hospital settings, these tests could be useful to establish a diagnosis in patients with unclear findings from imaging studies.

For immunodiagnosis, serum or CSF samples are commonly used. Although the specificity is higher in CSF than in serum, lumbar puncture to obtain CSF can only be done in a hospital setting and in patients without intracranial hypertension. Thus, the only realistic policy, particularly in rural setting, is the testing of serum samples. However, imaging studies are essential before the administration of treatment because the therapeutic regimen depends on the number, localization, and degenerative stage of the parasites, as well as the intensity of the host inflammatory reaction.

Currently, the two commonly used serological tests are the LLGPs-EITB and ELISA, using native cysticercal antigens as the antigen source. Commercial and in-house tests are used in endemic and non-endemic countries and the objective of this study was to compare the most widely used tests using a collection of appropriately characterized control and NCC serum samples.

We observed the highest sensitivity among patients with vesicular extraparenchymal parasites, with sensitivities ranging from 82.7 to 100.0%. The sensitivity was lower in the group with parenchymal vesicular parasites (37.5 to 87.5%), and even lower in the group with calcified parasites (9.5 to 52.4%). The in-house HP10 assay had the lowest sensitivity for calcified parasites; however, this finding was expected since the presence of HP10 antigen is evidence of viable cysticerci, as reported previously (Fleury et al. 2007). This confirmatory result is particularly relevant in the context of treatment, as HP10 antigen detection should be considered a good indicator of treatment success particularly for extraparenchymal parasites (Fleury et al. 2007; Bobes et al. 2006).

Specificities, among all of the controls evaluated, were high and ranged from 85.4 to 97.1%, without any statistically significant differences among the tests evaluated. These results are consistent with previous studies (Proaño-Narvaez et al. 2002; Fleury et al. 2007; Carod et al. 2012; Gabriël et al. 2012; Zea-Vera et al. 2013), although significantly lower specificity was reported in a Mexican study 25 years ago (Ramos-Kuri et al. 1992). As the detection of anti-cysticercal antibodies, regardless of detection method used, does not distinguish between NCC, cysticercosis outside of the central nervous system, and parasite exposure rather than infection, it is possible that the improvement in specificity in our study might be related to decreased infectious pressure in Mexico, at least in some geographic areas.

The level of reliability between the different tests was substantial (Kappa coefficients between 0.60 and 0.80) in 76.2% of the comparisons made, which can be considered a good result. The lowest reliability was with the commercial NOVALISA (Kappa of 0.42 with EITB CDC and EITB LDBIO) and this could be related to its poor sensitivity (Table 2). It is important to note that our results are consistent with another study comparing different ELISAs for the diagnosis of NCC (Bobes et al. 2006), which found that NOVALISA had a high specificity and low sensitivity. It is interesting to note that some differences in sensitivity between EITB CDC and EITB InDRE were observed despite a Kappa coefficient was 0.66 showing a good reliability between these tests. The reasons for the differences between the two tests are not obvious, but it is possible that some variability between the antigens used in the two EITB are involved, as they do not come from the same source (Vega et al. 2003; Maravilla et al. 2008). Indeed, in the EITB InDRE, the proteins used are from "Mexican" cysticerci, while the origin of the glycoprotein

antigens used in the EITB CDC is not clear. Slight differences between the antigens used perhaps explain the higher sensitivity of EITB InDRE in our study in which Mexican samples were evaluated.

Another point that merits comment is the results obtained using the ELISA with *T. crassiceps* antigens. Although its sensitivity was significantly lower with respect to the assay using *T. solium* antigens, its sensitivity was similar to that of the three EITB assays and its specificity was similar to the EITB InDRE. These results are relevant since *T. crassiceps* can be produced under experimental conditions in mice, providing an accessible source of antigens when *T. solium* material is not available (Fleury et al. 2003; Arruda et al. 2005; Suzuki et al. 2007).

Surprisingly, particularly because of its reputation and prohibitive cost, the EITB CDC assay had a significantly lower sensitivity than the ELISA Ts Ab. Our results are consistent with a systematic review and meta-analysis recently published (Cardona-Arias et al. 2017). Therefore, we would recommend the ELISA Ts Ab for the initial screening of patients in both hospital and rural settings.

Since imaging is still necessary before the administration of specific treatment, it is important in rural settings to use a low-cost and easy-to-perform test with a high sensitivity to detect viable parasites (i.e., those that require specific treatment), although specificity might not be optimal. In this study, the test that best fits this profile is the in-house ELISA-Ab using vesicular fluid of *T. solium*. Recently, a panel of experts stated that the best test to use in rural setting should be the detection of antigen, using a rapid diagnostic technology (Donadeu et al. 2017). Although our results are basically in accordance with this proposal, they also highlight the risk of not detecting patients with viable parenchymal cysts. This finding was reported previously in another study by our group (Fleury et al. 2007) but has not been found by other research groups (Gabriël et al. 2012; Zea-Vera et al. 2013; Parkhouse et al. 2018). We are aware that the low number of patients with vesicular parenchymal parasites included in our study precludes a definitive conclusion; however, the evidence reported herein supports that this is a topic that needs to be studied further. Finally, we must stress that this study was limited to Mexican patients, and so similar conclusions in other endemic countries must await the appropriate studies. Clearly, false positives will result from cross-reacting antibodies to prevailing pathogens and these will vary from country to country.

Authors' contributions This study was designed, supervised, and coordinated by RMEP, TG, ES, and AF. MH, OGA, GD, JLRA, AML, RGR, and AT carried out the clinical assessment and the immunoassays; MLR and AF carried out the analysis and interpretation of these data. JLRA, RMEP, ES, and AF drafted the manuscript. All authors critically revised the manuscript for intellectual content, read, and approved the final manuscript. ES and AF are guarantors of the paper.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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