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Serodiagnosis of human cysticercosis for epidemiological studies in low-income countries is the ELISA “screening” strategy accurate.

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ABSTRACT

Background: Serodiagnosis of cysticercosis uses two serological tests, ELISA and Western blot, which are carried on sequentially. Only the samples positive in Elisa are confirmed with Western blot. The objective of this study was to assess the agreement between the ELISA and Western blot to evaluate false negative generate by ELISA alone. **Methods:** This is a retrospective study conducted on samples randomly drawn at random from a serum bank constituted during two cross-sectional surveys carried out in the departments of Dabou and Agboville, in the south of Côte d'Ivoire. These two sero-epidemiological studies were conducted on randomly selected asymptomatic subjects. The sera selected for this ancillary study were all tested using both native antigen Elisa and western blot methods. Western blot (EITB) was used as reference test. Sensitivity, specificity, false positive and false negative rates of the Elisa test were determined against EITB results. **Results:** A total of 594 sera (297 positives / 297 negatives in ELISA), were tested by Western blot. The sensitivity and specificity of the ELISA were 82.6% and 79.5%, respectively. The false negative and false positive rates of ELISA were 16.5% and 21.6%, respectively. A strong agreement was observed between these two tests with a Cohen's Kappa value equal to 0.62. **Conclusion:** These data reveal that the Elisa test alone is not sufficient for the serodiagnosis of cysticercosis. When used for diagnosis in symptomatic patient, ELISA alone is not sufficient to avoid the diagnostic. Elisa-negative patient who presents a critical clinical picture must be tested by Western blot.

Keywords: Sensitivity, specificity, ELISA, Western blot, human cysticercosis

Introduction

Caused by the larva of *Taenia solium* (*T. solium*), cysticercosis is a cosmopolitan parasitosis common in developing countries [1], with pig being the intermediate host and human the definitive [2]. However human cysticercosis occurs when human substitutes itself to a pig after ingestion of water or food contaminated with *T. solium* eggs, or sometimes after accidental self-infestation [1–3]. This tapeworm is therefore a public health problem with also repercussions on the agriculture sector [4]. The muscles, eyes and central nervous system are the most frequent localizations of the parasites [5,6]. Neurocysticercosis is the most severe form of the disease and is estimated to cause 30% of epileptic seizures in Africa [7]. The World Health Organization (WHO) estimates that *T. solium* affects more than 50 million people worldwide and causes more than 50.000 deaths each year [8,9]. In West Africa, people with epilepsy suffer from discrimination, unemployment, disability and marginalization [10]. Cysticercosis is therefore one of the major causes of family destabilization in Africa.

Given the impact of this parasitosis, WHO is supporting action plans to achieve its eradication. However, WHO faces the problem of a lack of reliable epidemiological data on this disease. Therefore, it recommends that researchers and national programs make recent and reliable data available in order to identify risk of outbreaks.

The ELISA test using glycosylated cysticercus fraction according to Tsang et al, is widely used as first line technic but is known to experiment cross-reactivity problems generating false positive results [11,12]. To circumvent this limit, ELISA positive samples are usually tested by western blot in order to confirm the result. However Western blot is a very expensive and time-consuming technic and due to the high sensitivity of the ELISA technic, ELISA negative serum is usually not retested.

The objective of this study was to assess the agreement between the ELISA test and the Western blot to evaluate false negative generate by ELISA alone.

In Abidjan, IgG serology methods using native glycosylated antigens have been implemented by the Parasitology unit of the Institute Pasteur in Côte d'Ivoire, in collaboration with the Pasteur institutes in Paris and in Madagascar. These serological tests were used to study populations at risk in Ivory Coast in order to clarify the epidemiological situation of human cysticercosis on Ivorian territory.

Material and methods

Type of study

This study reports on a retrospective analysis of samples drawn at random from a serum library. This serum bank was established during two cross-sectional surveys carried out in the villages of the departments of Dabou and Agboville, in the south of Côte d'Ivoire, as part of a study on the seroprevalence of human cysticercosis. These cross-sectional surveys took place between February and April 2017 for the Department of Dabou and between June and July for the Department of Agboville.

Sample size and choice

A total of 594 human sera were analyzed during this study, i.e., 297 positives and 297 negatives in ELISA. The 297 ELISA positive samples included in this study were the whole set of positive samples of the serum library, whereas negative samples were obtained by a systematic drawing using a sampling step of 1 over the bank.

Sample analysis

The anti-cysticercus antibody assay was performed using the glycosylated fraction of the *T. solium* cyst, purified according to the method of Tsang et al., 1989 [13]. All the sera were tested both by indirect ELISA (IgG assay), and by Western blot (IgG) techniques. The ELISA and Western blot techniques were performed on the serum samples according to the method previously described [14]. The western blot was considered as the gold standard during this study. Following Garcia et al, a serum was considered positive in western blot when at least two bands of molecular weight less than 50 kDa were detected.

Statistical analysis

All the results obtained (ELISA and Western Blot) were entered on Excel 2010 and analyzed with the XLSTAT 2014.5.03 software. The sensitivity and specificity of the ELISA test were calculated with their 95% confidence interval. The concordance (agreement without taking into account the effect due to chance) between the ELISA test and the reference test was calculated as follows: $(VP + VN) * 100 / (VP + VN + PF + FN)$ [15], where VP, VN, FP and FN respectively represent the number of true positive, true negative, false positive and the number of false negative. The Cohen's Kappa coefficient measuring the corrected agreement between the ELISA test and the Western blot was also determined using XLSTAT 2014 software version 5.03. This agreement was noted as fair, moderate, good and excellent when Cohen's kappa values ranged between (0.21 and 0.40), (0.41 and 0.60), (0.61 and 0.80) and (0.81 and 1), respectively [16].

All the subjects sampled during the field studies were asymptomatic. No data was available to estimate the timeline of the contamination or the concordance between serological results and clinical signs.

Ethics of the study

Informed consent was signed by all participants included in the field studies conducted.

Results

Results obtained for the two tests (ELISA and Western blot) are presented in Table 1. The Western blot was considered as the reference test to estimate true and false results by ELISA, i.e., ELISA true positives 233 (78.4%), true negatives, 248 (83.5%), false negatives 49 (16.5%) and false positives 64 (21.6%).

Table 1. Contingency table of ELISA and Western blot results

		Western blot		
		Positive	Negative	Total
ELISA-IgG	Positive	233	64	297
	Negative	49	248	297
Total		282	312	594

The ELISA test showed good sensitivity and specificity results with 82.6% (77.7 - 86.6) and 79.5% (74.6 - 83.6), respectively (table 2). The ELISA's index of agreement with the western blot was 81% with a Cohen's Kappa value of 0.62.

Table 2. Performance evaluation (sensitivity and specificity) of the IgG-ELISA test compared of the western blot

	Se (%)	Sp (%)	Agreement (%)	Kappa value
	IC (95%)	IC (95%)		
ELISA-IgG	82.6%	79.5	81	0.62
	(77.7 - 86.6)	(74.6 - 83.6)		

Se: Sensitivity ; **Sp:** Specificity ; and **CI:** Confidence interval

Profiles of the sera negative in ELISA but positive in Western Blot (false negative) were as follows: 20 (40.81%) samples were positive with specific bands at high intensity and the 29 (59.18%) remaining were positive but with specific bands at very low intensities. Regarding the 64 false positive sera, serological analysis with the western blot showed that 40 (62.50%) of these samples were not positive for any protein band while 37.5% (24/64) slightly reacted with the antigenic proteins with a predominance of 50 Kda at 14.06% (Table 3).

Table 3. Distribution of false positive samples in ELISA according to the results of Western blot and according to the size of the protein bands.

kDa : kilodalton, **CI** : Confidence Interval

Number of sera	Number of bands observed	Protein size in kDa	Percent (%)	CI (95%)
1	1	18 kDa	1.56	0.04 – 8.4
4	1	39 kDa	6.25	1.02 – 21.49
3	1	45 kDa	4.69	0.98 – 13.09
1	1	40 kDa	1.56	0.04 – 8.4
9	1	50 kDa	14.06	6.64 – 25.02
1	2	50 kDa et 70 kDa	1.56	0.04 – 8.4
3	2	50 kDa et 90 kDa	4.69	0.98 – 13.09
1	2	50 kDa et 65 kDa	1.56	0.04 – 8.4
1	1	60 kDa	1.56	0.04 – 8.4
40	None		62.50	49.51 – 74.3

Discussion

ELISA tests using crude glycosylated *T. solium* antigens for the detection of antibodies during human cysticercosis have been evaluated in the literature [11,17–19]. However, differences in sensitivity and specificity have been reported [11,20–22].

The sensitivity and specificity of the ELISA test obtained from this study were 82.6% and 79.5% respectively. These values were lower than the values of 96.6% and 97.4% obtained [22]. However, during this study authors arbitrary set the threshold of positivity at an optical density equal to 0.4. Our results were also lower than those reported by Machado et al., 2007 [17], who obtained ELISA (IgG) sensitivity and specificity values of 92.5% and 84.5%; or of 92.5% and 93.3% when using respectively crude phase or detergent phase of the antigenic fractions of *T. solium* metacystodes. The sensitivity and specificity obtained from our work were also lower than those obtained by Gabriela et al., 2017 [19], which varied from 95% to 100% for the sensitivity and from 92.3% to 97.4% for the specificity. However, the sensitivity and specificity obtained during our study were higher than those obtained by Diaz et al (65% and 63%, respectively) [23]. All these discrepancies could be explained by difference in the purification process of the antigens as well as differences in the protocols used for the ELISA and western blot tests. They can also be due to difference in the sera used to perform the studies. Indeed, antibody titers are/could be much higher during acute disease than for asymptomatic people which could explain better performance of the tests. Serology should then perform better for diagnostic (ill patients) than for epidemiology studies (asymptomatic people).

In this study, ELISA false positive rate was 21.6%, which could be due to cross reactions with other parasitic diseases including *Echinococcus granulosus* and *Hymenolepis nana* [12,23,24]. For these sera considered as negative in Western blot, 37.5% harbored at least one band on the strip. The presence of only one of bands could be due to an old infection as bands of 18, 39, 40, 45 or 50 kDa can be found during inactive cysticercosis [22]. At the other hand 16.5% of ELISA negative samples were found to be positive using Western blot. These false negative samples in ELISA could be also explained by a small number of antibodies in the sera, inducing an optical density below the positivity threshold. These false negatives are then the missing part of the positive subjects. This result has important implications both in diagnostic and in epidemiology as the positivity rates in the population would be underestimated.

Conclusion

This study showed a good value for sensitivity and specificity for ELISA, with a strong agreement between the two tests. Despite underestimation of the prevalence, this confirms that ELISA alone can be used for epidemiological studies. However Western blot should be preferred for diagnostic.

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