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Infection with versus Exposure to *Taenia solium*: What Do Serological Test Results Tell Us?

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Abstract. *Taenia solium* cysticercosis is an endemic zoonosis in many developing countries. Serological tests are the most appropriate diagnostic tools to understand the transmission dynamics of the parasite, but the performances of these methods in such a setting are not known. A south Ecuadorian human population living in an endemic area was tested using three common serological tests. Because none of them is a gold standard, a Bayesian Latent Class analysis was used to estimate the test characteristics. Two definitions of a case were considered to differentiate between prevalence of current infection and prior exposure to the parasite. Differences between the performances of the same test in function of the definition of a case were observed. This study shows that test results and prior information should be interpreted carefully in a Bayesian analysis framework, particularly when the latter is based on clinical studies.

INTRODUCTION

Cysticercosis (CC) is a zoonosis caused by the larval stage of *Taenia solium*, which locates in the muscles and/or central nervous system causing, in the latter case, neurocysticercosis (NCC).[1] The presence of the parasite in the muscles is often asymptomatic. In contrast, NCC may cause important neurological disorders, such as epilepsy, and even death. Diagnosis of CC requires the use of imaging and serological techniques.[2] Based on these tools, hospital-based studies have been conducted to determine the NCC prevalence and diagnostic performances of serological tests among epileptic patients. Additionally, epidemiological studies are useful to understand the transmission dynamics of the parasite.[3,4] However, it is known that the sensitivity and specificity of a test may vary when applied on symptomatic versus asymptomatic individuals. The use of estimates of the sensitivity and specificity of a diagnostic tool based on a selected population of symptomatic patients would, therefore, not be completely appropriate in the framework of an epidemiological study.[5] Estimations of the performances of a test for its use among an entire exposed population are then required. However, such studies on CC face two main constraints: (1) for ethical and practical reasons, imaging techniques such as magnetic resonance imaging (MRI) and computed tomography (CT) scan are not applicable in the field, and (2) epidemiological studies should not only focus on NCC but all CC cases to understand the transmission dynamics of the parasite, and gold-standard methods (diagnostic methods having a sensitivity and specificity of 100%) are lacking for this purpose. Under these conditions, estimating the prevalence of CC and the sensitivity and specificity of a test is only possible using a Bayesian approach. Bayesian statistical modeling is based on the integration of the results of multiple diagnostic tests together with prior knowledge on the characteristics (sensitivity and/or specificity) of these tests.[6] The aim of the present study was to estimate the performances of three serological diagnostic tests and the prevalence of CC using a Bayesian approach in a south Ecuadorian community where *T. solium* is endemic. Two different definitions of a case have been considered to make a distinction between prevalence of infection (proportion of individuals currently infected with living cysticerci) and prevalence of prior exposure to *T. solium* (proportion of individuals who have been in contact with the parasite during the precedent year, with or without development of cysticerci and with or without [cured individuals] current infection with living cysticerci).[3,7]

MATERIALS AND METHODS

The sampling protocol of the present study has been described elsewhere.[4] Briefly, a community-based study was conducted between September and November 2007 in the parish of Cazaderos, situated in the southern Andean province of Loja, where CC is endemic.[8]

A total of 791 human serum samples were collected and tested using three serological methods, namely the enzyme-linked immunosorbent assay (Ag-ELISA) for the detection of circulating antigens of the metacestode of *T. solium*,[9] the enzyme-linked immunoelectrotransfer blot assay (EITB) for the detection of antibodies directed against seven specific *T. solium* glycoprotein antigens,[10] and the ELISA for the detection of antibodies directed against crude cyst-fluid extracts (Ab-ELISA).[11] The results of the three tests applied on 791 individuals are shown in Table 1.

Bayesian analysis. Because none of the serological tests included in this study is a gold standard, a Bayesian analysis was used to estimate the prevalence and characteristics of the tests. Two definitions of a case have been considered to make a distinction between prevalence of infection (an individual who is currently infected by living cysts[7]) and prevalence of recent exposure to the parasite (an individual who has been in contact with the parasite during the precedent year, with or without development of cysticerci and with or without [cured individuals] current infection with living cysticerci[3,7]). A multinomial Bayesian model adapted from Berkvens and others[6] was used (Supplemental Appendix S1, available at www.ajtmh.org). Prior information on the test characteristics was extracted from the available literature according to the two above-mentioned definitions of a case and adapted by experts of the Institute of Tropical Medicine of Antwerp (Belgium) to be expressed as conditional probabilities (Tables 2

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TABLE 1

Results of the three serological tests applied on 791 individuals of Cazaderos

Ag-ELISA	EITB	Ab-ELISA	Number
1	1	1	11
1	1	0	2
1	0	1	5
1	0	0	5
0	1	1	90
0	1	0	96
0	0	1	206
0	0	0	376

0 = negative test result; 1 = positive test result; Ag-ELISA = enzyme-linked immunosorbent assay for the detection of circulating antigens of the metacestode of *T. solium*; EITB = enzyme-linked immunoelectrotransfer blot assay for the detection of antibodies directed against seven specific *T. solium* glycoprotein antigens; Ab-ELISA = enzyme-linked immunosorbent assay for the detection of antibodies directed against crude cyst-fluid extracts of *T. solium*; number = number of individuals for each result category.

and 3).[9–12] The models allow for estimation of the credibility intervals for differences between the prevalence estimates of infection and exposure, between the characteristics of the same test estimated for each prevalence definition, and between the characteristics of the tests (2×2) for the detection of infected and exposed individuals. A credibility interval with both limits having the same sign (zero not included in the interval) can be interpreted as the equivalent of a significant result in a frequentist approach.

The analysis was conducted in WinBUGS and R.[13,14] Criteria assessing the fit between prior information and test results were evaluated (i.e., the Bayesian *P* value [Bayesp], the Deviance Information Criterion [DIC], and the number of parameters effectively estimated by the model [pD]).[6,13]

Ethical clearance. The protocol of this study was approved by the Ethical Committee of the Central University of Ecuador and the Ethical Committee of the University Teaching Hospital of Antwerp, Belgium. Informed consent was obtained from all adult participants and the parents or legal guardians of minors.

RESULTS

The estimates of the prevalence of infection/exposure and the characteristics of the three serological tests for the detection of infected/exposed individuals are shown in Table 4. The prevalence of infection by living cysts was estimated at 1% (95% confidence interval [CI] = 0–3%), whereas the prevalence of exposure to *T. solium* within the precedent year was 23% (95% CI = 19–27%).

A statistical difference was detected between the prevalence of infection and exposure and between the sensitivity of the Ag-ELISA, the sensitivity of the Ab-ELISA, and the specificity of the EITB, depending on the case definition.

A statistical difference was detected between the specificities of Ag-ELISA and EITB, the specificities of Ag-ELISA and Ab-ELISA, and the specificities of EITB and Ag-ELISA for the detection of infected individuals. A statistical difference was detected between the sensitivities of Ag-ELISA and EITB, the sensitivities of Ag-ELISA and Ab-ELISA, the sensitivities of EITB and Ab-ELISA, the specificities of Ag-ELISA and Ab-ELISA, and the specificities of EITB and Ab-ELISA for the detection of exposed individuals.

TABLE 2

Prior information for the detection of infected individuals (uniform distributions)*

Conditional probabilities	Prior information
Sensitivity of the Ag-ELISA for the detection of infected individuals (th1[2] in the model in Supplemental Appendix S1)	0.8–1
Specificity of the Ag-ELISA for the detection of infected individuals (th1[3] in the model in Supplemental Appendix S1)	0.9–1
Probability to have a positive result for the EITB if the individual is infected and positive for the Ag-ELISA (th1[4] in the model in Supplemental Appendix S1)	0.95–1
Probability to have a positive result for the Ab-ELISA if the individual is infected and positive for the Ag-ELISA and the EITB (th1[8] in the model in Supplemental Appendix S1)	0.9–1

*The other probabilities are not constrained and left as uniform distributions (0–1).
Ag-ELISA = enzyme-linked immunosorbent assay for the detection of circulating antigens of the metacestode of *T. solium*; EITB = enzyme-linked immunoelectrotransfer blot assay for the detection of antibodies directed against seven specific *T. solium* glycoprotein antigens; Ab-ELISA = enzyme-linked immunosorbent assay for the detection of antibodies directed against crude cyst-fluid extracts of *T. solium*.

TABLE 3

Prior information for the detection of exposed individuals (uniform distributions)*

Conditional probabilities	Prior information
Specificity of the Ag-ELISA for the detection of exposed individuals (th2[3] in the model in Supplemental Appendix S1)	0.9–1
Probability to have a positive result for the EITB if the individual is exposed and positive for the Ag-ELISA (th2[4] in the model in Supplemental Appendix S1)	0.95–1
Probability to have a positive result for the EITB if the individual is exposed and negative for the Ag-ELISA (th2[5] in the model in Supplemental Appendix S1)	0.95–1
Probability to have a negative result for the EITB if the individual is not exposed and negative for the Ag-ELISA (th2[6] in the model in Supplemental Appendix S1)	0.95–1
Probability to have a positive result for the Ab-ELISA if the individual is exposed and positive for the Ag-ELISA and the EITB (th2[8] in the model in Supplemental Appendix S1)	0.9–1
Probability to have a negative result for the Ab-ELISA if the individual is exposed and negative for the Ag-ELISA and the EITB (th2[12] in the model in Supplemental Appendix S1)	0.5–1

*The other probabilities are not constrained and left as uniform distributions (0–1).
Ag-ELISA = enzyme-linked immunosorbent assay for the detection of circulating antigens of the metacestode of *T. solium*; EITB = enzyme-linked immunoelectrotransfer blot assay for the detection of antibodies directed against seven specific *T. solium* glycoprotein antigens; Ab-ELISA = enzyme-linked immunosorbent assay for the detection of antibodies directed against crude cyst-fluid extracts of *T. solium*.

TABLE 4

Estimates of the prevalence of infection/exposure to the parasite and the characteristics of the three serological tests for the detection of infected/exposed individuals

		Ag-ELISA		EITB		Ab-ELISA	
	Prevalence	Se	Sp	Se	Sp	Se	Sp
Infection	0.01	0.90	0.98	0.93	0.76	0.89	0.61
CI lower 95% limit	0.00	0.80	0.97	0.82	0.72	0.79	0.58
CI upper 95% limit	0.03	0.99	0.99	0.99	0.79	0.97	0.65
Exposure	0.23	0.05	0.98	0.97	0.97	0.51	0.64
CI lower 95% limit	0.19	0.01	0.96	0.95	0.94	0.41	0.60
CI upper 95% limit	0.27	0.10	0.99	1.00	0.99	0.60	0.68

CI = credibility interval; Se = sensitivity; Sp = specificity; Ag-ELISA = enzyme-linked immunosorbent assay for the detection of circulating antigens of the metacestode of *T. solium*; EITB = enzyme-linked immunoelectrotransfer blot assay for the detection of antibodies directed against seven specific *T. solium* glycoprotein antigens; Ab-ELISA = enzyme-linked immunosorbent assay for the detection of antibodies directed against crude cyst-fluid extracts of *T. solium*.

DISCUSSION

This study gives an estimation of the prevalence of infection and exposure and the characteristics of three diagnostic tests to detect individuals infected with *T. solium* cysts and individuals exposed to the parasite inside an entire population living in an endemic area. The estimated prevalence of infection was statistically lower than the estimated prevalence of exposure to the parasite. As expected, antibody detection by EITB showed a high sensitivity and specificity to detect exposed individuals. One could expect identical results for the Ab-ELISA because it also detects antibodies, but both sensitivity and specificity of this test were statistically lower than the same estimates for the EITB. The use of crude cyst-fluid extracts could explain the lack of specificity of this test to detect exposure, because cross-reactions with many other parasites are suspected.[15] However, the low sensitivity of the technique could be caused by the complex conformation of the crude antigens, which stay inaccessible for antibodies in contrast to the EITB-purified glycoproteins. The three tests had a relatively high sensitivity to detect infection, but the specificity of both EITB and Ab-ELISA were statistically lower than the specificity of the Ag-ELISA. This can be explained by the high number of false positives for these two tests detecting antibodies, because they were not able to discriminate between exposure and infection. Indeed, antibodies can be present in currently infected individuals but also in individuals who were infected and cured and individuals who were exposed to the parasite without developing the disease.[3] Additionally, the Ag-ELISA was insensitive to detect exposure to the parasite, because it only detects individuals infected with living cysts excreting antigens.[7]

In conclusion, this study shows the importance of the definition of a case (infection or exposure) when attempting to estimate the prevalence of a disease and the characteristics of the diagnostic tests. It underlines the necessity of using a multiple-testing approach when no gold standard is available but highlights the need of interpreting expert opinion and published information carefully to use it properly in a Bayesian analysis, particularly when this information is based on clinical studies. Moreover, these findings point out the low performances of the Ab-ELISA using crude cyst-fluid extracts and cast doubt on the suitability of the use of crude antigens for diagnosing CC. Prior information on the test characteristics was based on the available literature according to the two definitions of a case and expert opinion. However, ongoing cohort studies on NCC using imaging as a gold-standard technique suggest a variation in the performances of the serological methods as a function of the level of infection (unpublished results). When available, different prior information scenarios should be included in the present Bayesian statistical model, and the results should be compared.

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Note: Supplemental appendix is available online at www.ajtmh.org

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