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## Field evaluation of urine antigen detection for diagnosis of *Taenia solium* cysticercosis

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### ABSTRACT

(Neuro)cysticercosis is an important zoonotic disease caused by infection with *Taenia solium* metacestode larvae. Existing immunodiagnostic techniques detect antibodies and circulating antigens (Ag) in serum and cerebrospinal fluid (CSF). Blood/CSF collection is an invasive procedure associated with blood-borne infections and is often not well accepted by communities. Detection of circulating Ag in urine has been suggested as an alternative, however this has been evaluated in clinical settings only. The aim of the present study was to evaluate the performance of a urine Ag-ELISA under field conditions. Paired serum and urine samples were obtained from participants in endemic areas of Ecuador ( $n = 748$ ) and Zambia ( $n = 690$ ) and were subjected to a monoclonal antibody-based Ag-ELISA. Calculation of positive and negative agreement indices (AI) showed better agreement in the negative direction both for Ecuadorian and Zambian samples (AI of 93.1 and 86.8, respectively). Using a Bayesian approach to determine the test characteristics, similar sensitivities were obtained for serum and urine Ag detection, whereas a decreased specificity was determined for the urine Ag-ELISA with a lower specificity (78.6%) for Zambian samples than for Ecuadorian samples (88.4%). This study indicates a higher specificity for the serum test under field conditions and promotes further research to improve the urine test.

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### 1. Introduction

Cysticercosis is a zoonotic parasitic infection caused by the metacestode larval stage of *Taenia solium*.<sup>1</sup> The infection has a propensity to occur in the brain resulting in neurocysticercosis (NCC), an important cause of human

morbidity and mortality.<sup>2</sup> Cysticercosis is most prevalent in many Latin American, African and Asian countries.<sup>3</sup>

Diagnosis of (neuro)cysticercosis depends primarily on imaging techniques and on immunodiagnostic methods that detect either antibodies or circulating antigens (Ag) in serum or cerebrospinal fluid (CSF).<sup>4</sup> Imaging techniques are expensive and are often not available in endemic areas. Detection of antibodies in serum may indicate exposure to infection but not necessarily the presence of established viable infection.<sup>5</sup> Moreover, antibodies may persist

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long after the parasite has been eliminated, resulting in false positivity and unnecessary antiparasitic therapy. In contrast, Ag positivity indicates an infection with viable cysticerci. A monoclonal antibody (mAb)-based ELISA (B158/B60 Ag-ELISA)<sup>6</sup> was estimated to have a sensitivity of 90% and a specificity of 98% for the detection of infection in serum.<sup>7</sup>

However, CSF or blood collection is an invasive procedure and is associated with the risk of acquiring blood-borne infections such as hepatitis B and HIV if the method is not carried out under stringent conditions. Urine collection is non-invasive, relatively easily accepted by the community and very convenient if multiple samplings are required per day. It can thus be of help both in clinical and epidemiological settings.

Tests to detect antigenuria have been developed for several other parasitic diseases such as leishmaniasis,<sup>8</sup> malaria,<sup>9</sup> schistosomiasis,<sup>10</sup> Chagas' disease,<sup>11</sup> filariasis<sup>12</sup> and cystic echinococcosis.<sup>13</sup> These have proven to be a good alternative to the common tests that utilise serum as a specimen. They all have been reported to work well in clinical settings; however, their performance in field conditions is not well described. The same holds for the urine Ag-ELISA for the diagnosis of human NCC, which has so far only been evaluated in clinical settings.<sup>5,14</sup>

The aim of this study was to evaluate the performance of the urine B158/B60 Ag-ELISA for the detection of human cysticercosis under field conditions.

## 2. Materials and methods

### 2.1. Study areas and participants

Community-based studies were carried out in *T. solium*-endemic rural areas in the parish of Cazaderos, situated in the southern Andean province of Loja, Ecuador,<sup>15</sup> and in the Eastern Province of Zambia.<sup>16,17</sup> Paired urine and blood samples were collected between September and November 2007 from 748 participants in Ecuador and between July and August 2009 from 690 participants in Zambia (K.E. Mwape et al., unpublished data). Samples were collected from all people from the community who volunteered to participate in the study, including both sexes and ages of 1–98 years. Participation was requested of individuals of all ages and permission to collect urine and blood samples was obtained after informed consent. For individuals below the age of 18 years, permission was sought from their parents or guardians.

### 2.2. Urine and blood sample collection and storage

All participants were provided with a disposable plastic container in which to place the urine. Upon submission of a urine sample, 5 ml of blood (2 ml from children) was then collected in plain blood tubes. The urine was aliquoted in duplicates of 1.8 ml vials and was stored at  $-20^{\circ}\text{C}$  until use. Blood was allowed to clot and was left to stand overnight at  $4^{\circ}\text{C}$ , after which it was centrifuged at  $3000 \times g$  for 15 min. The obtained serum was aliquoted in 1.8 ml vials and was stored at  $-20^{\circ}\text{C}$  until use.

### 2.3. Detection of cysticercal antigens in urine and serum

The B158/B60 Ag-ELISA to detect cysticercal antigens in serum and urine was performed as described by Dorny et al.<sup>6</sup> and Castillo et al.,<sup>14</sup> respectively. Briefly, some modifications in the serum protocol were carried out for analysis of urine samples. ELISA plates were coated with the capturing mAb B158C11A10 in bicarbonate buffer at  $5 \mu\text{g/ml}$ , washed and blocked with PBS to which Tween 20 and 1% newborn calf serum were added. Unlike serum samples, urine samples were not pre-treated using trichloroacetic acid (TCA), however they were diluted 1:2 in blocking buffer and were pre-incubated with the biotinylated mAb B60H8A4 for 1 h. Next, pre-treated urine samples were added to the wells and were incubated at  $37^{\circ}\text{C}$  on a shaker for 15 min. The plates were emptied, dried without washing, and another  $100 \mu\text{l}$  of the urine-mAb mixture was added. The plates were then incubated overnight at  $4^{\circ}\text{C}$  without shaking. Afterwards, the same procedure as for serum samples was followed, that is washing, incubation with streptavidin-horseradish peroxidase diluted at 1:10 000 in blocking buffer, washing and incubation with ortho-phenylenediamine (in distilled water with hydrogen peroxide). Finally, to stop the reaction,  $50 \mu\text{l}$  of 4 N  $\text{H}_2\text{SO}_4$  was added to each well. Eight negative and two positive control serum samples were run on each plate. The plates were read using an automated spectrophotometer at 490 nm with a reference of 655 nm. The optical density of each serum/urine sample was compared with a sample of negative serum/urine samples ( $N=8$ ) at a probability level of  $P=0.001$  to determine the result in the test.<sup>18</sup>

### 2.4. Data analyses

Agreement between the serum and urine Ag-ELISA results was determined by calculating the positive and negative agreement indices (AI).<sup>19,20</sup> Credibility interval estimates (95%) were calculated using the Bayesian method proposed by Graham and Bull.<sup>21</sup>

Moreover, a multinomial Bayesian model adapted from Berkvens et al.<sup>22</sup> was used (Supplementary Appendix 1) to estimate the characteristics (sensitivity and specificity) of the serum and urine Ag-ELISAs to detect infected individuals. A Bayesian latent class analysis was selected as the method of choice because none of the tests described in this study is a gold standard. The Bayesian approach allows prior information on test sensitivity and specificity to be combined with the diagnostic test results at hand. Assuming good test result reproducibility, various prior information scenarios can be evaluated. Prior information on the test characteristics was obtained from the available literature (Castillo et al.<sup>14</sup> for the urine Ag-ELISA and Praet et al.<sup>7</sup> for the serum Ag-ELISA) and was adapted by experts at the Institute of Tropical Medicine (Antwerp, Belgium) to be expressed as conditional probabilities (Table 1). The model allows estimating the credibility intervals for differences between the estimated characteristics of the same test between countries and between tests. 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.<sup>7,23</sup>

**Table 1**Prior information on the detection of infected individuals in Ecuador and Zambia (uniform distributions)<sup>a</sup>

Sensitivity of the Ag-ELISA for detection of infected individuals (th1[2] and th2[2] in the model; see Supplementary Appendix 1)	[0.8–1]
Specificity of the Ag-ELISA for detection of infected individuals (th1[3] and th2[3] in the model; see Supplementary Appendix 1)	[0.97–1]
Probability of having a positive result for the urine Ag-ELISA if the individual is infected and positive for the serum Ag-ELISA (th1[4] and th2[4] in the model; see Supplementary Appendix 1)	[0.82–1]
Probability of having a negative result for the urine Ag-ELISA if the individual is not infected and negative for the serum Ag-ELISA (th1[6] and th2[6] in the model; see Supplementary Appendix 1)	[0.99–1]

<sup>a</sup> Other probabilities are not constrained and were left as uniform distributions [0–1] (see Supplementary Appendix 1).**Table 2**

Serum and urine Ag-ELISA results for samples from Ecuador

Urine	Serum		Total
	+ve	–ve	
+ve	8	82	90
–ve	13	645	658
Total	21	727	748

**Table 3**

Serum and urine Ag-ELISA results for samples from Zambia

Urine	Serum		Total
	+ve	–ve	
+ve	27	141	168
–ve	14	508	522
Total	41	649	690

The analysis was conducted in WinBUGS and R.<sup>24,25</sup> 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)].<sup>22</sup>

### 3. Results

#### 3.1. Antigen detection in serum and urine

Of the 748 samples collected in Ecuador, 21 (2.8%) were positive on serum Ag-ELISA and 90 (12.0%) were positive on urine Ag-ELISA. Eight samples (1.1%) were positive on both serum and urine Ag-ELISA (Table 2).

In Zambia, 690 paired samples were collected, of which 41 samples (5.9%) were positive on serum Ag-ELISA and 168 (24.3%) were positive on urine Ag-ELISA (Table 3).

#### 3.2. Agreement between serum and urine Ag-ELISA results

A better agreement was observed in the negative direction (AI of 93.1 and 86.8 for Ecuador and Zambia, respectively) than in the positive direction (AI of 14.4 and 25.8 for Ecuador and Zambia, respectively) (Table 4).

**Table 4**

Positive and negative agreement indices (AI) between serum and urine Ag-ELISA with 95% credibility interval estimates for Ecuador and Zambia

Country	Positive AI (%)	95% credibility interval	Negative AI (%)	95% credibility interval
Ecuador	14.4	7.3–23.3	93.1	92.0–94.0
Zambia	25.8	19.0–33.0	86.8	85.0–89.0

**Table 5**

Sensitivity and specificity and 95% credibility interval estimates of the serum and urine Ag-ELISA for samples from Ecuador and Zambia (expressed as percentages)

	Serum Ag-ELISA	Urine Ag-ELISA
Sensitivity (95% credibility interval)		
Ecuador	90 (80–99)	86 (74–98)
Zambia	90 (80–99)	85 (73–98)
Specificity (95% credibility interval)		
Ecuador	98 (97–100)	88 (86–91)
Zambia	98 (98–99)	78 (75–81)

#### 3.3. Sensitivity and specificity of serum and urine Ag-ELISAs

Prior information used in the model was based on Castillo et al.<sup>14</sup> for the urine Ag-ELISA and Praet et al.<sup>7</sup> for the serum Ag-ELISA. However, when running the model, prior information on the specificity of the urine Ag-ELISA was not in agreement with the Ecuadorian and Zambian test results. Therefore, th1[6] and th2[6] in the model (see Supplementary Appendix 1) were relaxed from [0.99–1] to [0.7–1].

The sensitivity of the serum and urine Ag-ELISAs was not statistically different for both tests in both countries (Table 5). The specificity of the serum Ag-ELISA was not statistically different in both countries and was statistically higher than the specificity of the urine Ag-ELISA in both countries. The specificity of the urine Ag-ELISA was statistically higher in Ecuador than in Zambia (Table 5).

### 4. Discussion

We aimed to explore the usefulness of urine as a sample for the diagnosis of human cysticercosis. Detecting specific Ag in urine is a non-invasive approach in the diagnosis of parasitic infections, and the excretion of cysticercal Ag in urine has already been demonstrated by other researchers.<sup>5,14</sup> Up to now, studies have been conducted in clinical settings with very encouraging results; therefore, the current study aimed to evaluate the use of urine as a specimen in field settings.

As a gold-standard diagnosis was not available in this study, a Bayesian analysis was used to estimate characteristics of both tests. This approach consists of combining data and prior information (knowledge) to estimate posterior parameters (sensitivity and specificity of the diagnostic tests and true prevalence of the disease). Prior information on the parameters can be fixed in a deterministic way or be given a probability distribution. Experts having experience in the diagnosis of the disease can provide this knowledge, or information can be found in publications reporting the results of similar experiments conducted elsewhere.<sup>15</sup> As this model allows estimating the credibility intervals for differences between the estimated characteristics of the same test between countries and between tests, it was considered as a convenient methodology for this study in which different populations are compared.

Results from this study determined similar sensitivities both for the urine and serum Ag-ELISAs in both countries, with an estimated sensitivity of the urine Ag-ELISA of 86%, which is slightly lower than the 91% reported by Castillo et al.<sup>14</sup> However, the estimates for specificity of the urine Ag-ELISA are considerably lower in this study compared with the 100% reported both by Castillo et al.<sup>14</sup> using the same B158/B60 Ag-ELISA and by Parija et al.<sup>5</sup> using the staphylococcal co-agglutination test. However, those two studies were hospital-based with a lower number of samples (one group of NCC patients and one control group), and the methodology used by Parija et al.<sup>5</sup> is different. In addition, the high estimates for test performances recorded were based on a subgroup of individuals with viable NCC, and reduced sensitivities and specificities were noted for the other subgroups. As our research was field-based, a mix of infected and non-infected individuals is expected in the sample. The infected group is likely to comprise individuals with NCC and with extraneural cysticercosis. In addition, cysticerci may be in different stages of development and degeneration. All these factors may impact on the absence/presence and the level of circulating Ag. In a recent paper from Praet et al.,<sup>7</sup> differences in test characteristics have been determined depending on the infection status of the individuals, highlighting the importance of a case definition when determining test sensitivity and specificity.

The lower specificity of the urine test calls for further clarification. In a different study where NCC patients were followed up, it was noticed that after treatment (praziquantel on the first day followed by a 2-week course of albendazole) urine levels remained positive for more than a month after serum levels became negative (P. Dorny, unpublished data). This could be due to the delayed clearance of cysticercal antigens in the urinary system, resulting in negative serum results while urine ELISAs were still positive. This phenomenon needs further research and could explain the lower specificity estimates of the urine Ag-ELISA in this study. Another factor could be the TCA treatment included in the serum protocol and cause of an extra dilution of the sample (TCA treatment and subsequent neutralisation results in a 1:4 dilution). This step is not included in the urine Ag-ELISA and as such the more concentrated urine samples can turn out positive in the urine test while the more diluted serum samples remain under the cut-off. A revision of the cut-off calculation

should perhaps be envisaged. Dehydration occurs fairly often in hot climates (but not under hospital conditions) and causes a concentration of the urine, which could again explain higher levels of Ag if present. The lower specificity estimate for the Zambian samples in comparison with the samples from Ecuador could be indicative of the occurrence of more cross-reactions in Zambia. High prevalences of other parasites such as soil-transmitted helminths and *Schistosoma* spp. were recorded in the Zambian study area (K.E. Mwape et al., unpublished data; K.E. Mwape and G. Zulu, unpublished data). In Ecuador, *Hymenolepis nana*, *Trichuris trichiura*, *Strongyloides stercoralis*, hookworms and *Ascaris lumbricoides* were detected in only four, two, five, two and one individuals, respectively.<sup>15</sup> Although no cross-reactions have been reported for helminths in the serum Ag-ELISA,<sup>19,26</sup> this has not been investigated for the urine Ag-ELISA and as such cannot be excluded. Cross-reactions with *Trypanosoma* spp. were observed before the inclusion of TCA treatment in the serum protocol. As this step is not included in the urine test, this could be a possible interfering factor. However, a recent study indicated the occurrence of only 82 new cases of sleeping sickness in Zambia during the last 10 years.<sup>27</sup>

The current study was a large-scale field study and compared results from two geographically different endemic areas on different continents and therefore gives an improved understanding of the suitability of the urine Ag-ELISA as described by Castillo et al.<sup>14</sup> in cysticercosis diagnosis. Results indicate that in field-based surveys the performance of the urine Ag-ELISA is inferior to the serum Ag-ELISA, particularly regarding its specificity. The urine Ag-ELISA could possibly be an excellent clinical tool in the case of diagnosis/follow-up of individuals with viable NCC,<sup>5,14</sup> however under field conditions the use of serum is preferential at this moment. Alternatively, the urine Ag-ELISA can be used as a first screening tool, followed by confirmatory serum Ag-ELISA. The advantages of urine as a sample specimen, such as the non-invasiveness and the easy acceptability by the community, justify further research to address the poorer specificity of urine Ag detection.

**Authors' contributions:** KEM, NP, IKP, WB-O, PD, RR-H and SG designed the study protocol; KEM, NP, JBM, WB-O, GZ, RR-H, MC-E, PD and SG organised and carried out collection of the samples; KEM, JBM, MC-E, RR-H and GZ carried out the immunodiagnostic tests, and analyses of these data was by the same and NP, SG and PD. All authors contributed to drafting and/or revising of the article and read and approved the final version. SG and NP are guarantors of the paper.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.trstmh.2011.05.010.

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**Conflicts of interest:** None declared.

**Ethical approval:** The study protocol and consent forms were approved by the Institutional Ethics Committee of the University of Zambia (Lusaka, Zambia) and Universidad Central del Ecuador (Quito, Ecuador). In Ecuador, further approval was obtained from the provincial and community authorities and the local health centres. In Zambia, further approval was sought from the Ministry of Health, District Director of Health Office and also from the community leaders before commencement of the study.

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