Accuracy of serological testing for the diagnosis of prevalent neurocysticercosis in outpatients with epilepsy, Eastern Cape Province, South Africa

Foyaca-Sibat, Humberto; Cowan, Linda D.; Carabin, Hélène; Targonska, Irene; Anwary, Mushtaq A.; Serrano-Ocaña, Gilberto; Krecek, Rosina C.; Willingham Iii, Arve Lee

Published in:
PLoS Neglected Tropical Diseases

DOI:
10.1371/journal.pntd.0000562

Publication date:
2009

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Foyaca-Sibat, H., Cowan, L. D., Carabin, H., Targonska, I., Anwary, M. A., Serrano-Ocaña, G., ... Willingham Iii, A. L. (2009). Accuracy of serological testing for the diagnosis of prevalent neurocysticercosis in outpatients with epilepsy, Eastern Cape Province, South Africa. PLoS Neglected Tropical Diseases, 3(12). https://doi.org/10.1371/journal.pntd.0000562
Accuracy of Serological Testing for the Diagnosis of Prevalent Neurocysticercosis in Outpatients with Epilepsy, Eastern Cape Province, South Africa

Humberto Foyaca-Sibat\(^1\), Linda D. Cowan\(^2\), Hélène Carabin\(^2\)*, Irene Targonska\(^1\), Mushtaq A. Anwary\(^1\), Gilberto Serrano-Ocaña\(^3\), Rosina C. Krecek\(^4\), A. Lee Willingham, III\(^6\)

\(^1\) Walter Sisulu University, Mthatha, South Africa, \(^2\) Department of Biostatistics and Epidemiology, College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, United States, \(^3\) Dora Nginza Hospital, Port Elizabeth, South Africa, \(^4\) Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis, \(^5\) Department of Zoology, University of Johannesburg, Auckland Park Campus, Auckland Park, South Africa, \(^6\) WHO/FAO Collaborating Center for Parasitic Zoonoses, Danish Center for Experimental Parasitology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark

Abstract

**Background:** Few studies have estimated prevalence of neurocysticercosis (NCC) among persons with epilepsy in sub-Saharan Africa. While the limitations of serological testing in identification of NCC are well known, the characteristics of persons who are misdiagnosed based on serology have not been explored. The first objective of this pilot study was to estimate the prevalence of NCC in epilepsy outpatients from an area of South Africa endemic for cysticercosis. The second objective was to estimate the accuracy of serological testing in detecting NCC in these outpatients and characterize sources of disagreement between serology and neuroimaging.

**Methodology/Principal Findings:** All out-patients aged 5 or older attending the epilepsy clinic of St. Elizabeth’s Hospital in Lusikisiki, Eastern Cape Province, between July 2004 and April 2005 were invited to participate. Epidemiological data were collected by local study staff using a standardized questionnaire. Blood samples were tested by ELISA for antibody and antigen for **Taenia solium**. Four randomly chosen, consenting participants were transported each week to Mthatha for brain CT scan. The proportion of persons with epilepsy attending St. Elizabeth clinic with CT-confirmed NCC was 37\% (95\% CI: 27.4\%–48\%). Using CT as the gold standard, the sensitivity and specificity of antibody testing for identifying NCC were 54.5\% (36.4\%–71.9\%) and 69.2\% (52.4\%–83.0\%), respectively. Sensitivity improved to 78.6\% (49.2\%–95.3\%) for those with active lesions. Sensitivity and specificity of antigen testing were considerably poorer. Compared to false negatives, true positives more often had active lesions. False positives were more likely to keep pigs and to have seizure onset within the past year than were true negatives.

**Conclusions/Significance:** The prevalence of NCC in South African outpatients with epilepsy is similar to that observed in other countries where cysticercosis is prevalent. Errors in classification of NCC using serology alone may reflect the natural history of NCC.

Citation: Foyaca-Sibat H, Cowan LD, Carabin H, Targonska I, Anwary MA, et al. (2009) Accuracy of Serological Testing for the Diagnosis of Prevalent Neurocysticercosis in Outpatients with Epilepsy, Eastern Cape Province, South Africa. PLoS Negl Trop Dis 3(12): e562. doi:10.1371/journal.pntd.0000562

Editor: Kirsten E. Lyke, University of Maryland School of Medicine, United States of America

Received March 23, 2009; Accepted October 30, 2009; Published December 8, 2009

Copyright: © 2009 Foyaca-Sibat et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The authors thank the Izumi Foundation for financial assistance; USAID in South Africa for funding; Medical Research Council. These agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: helene-carabin@ouhsc.edu

Introduction

Neurocysticercosis (NCC) results when the central nervous system (CNS) is invaded by the larval stage of **Taenia solium**, a zoonotic cestode transmitted between humans and pigs. Humans infected with the adult tapeworm, a disease called taeniasis, contaminate the environment with the parasites’ eggs contained in the proglottids that are shed in their feces. Pigs become infected by consuming these eggs in contaminated feed or water. Infected pigs may shed thousands of eggs per day, a single carrier can be responsible for the infection of multiple individuals and pigs in a community [1,2].

The manifestations of NCC are protean, but approximately 80\% to 90\% of persons with symptomatic NCC experience seizures at some time in the course of their illness [3,4], making this manifestation a reasonable starting point for identifying NCC cases in populations at risk. Acute symptomatic seizures (seizures occurring only in the presence of an acute central nervous system (CNS) process) in NCC are thought to result from inflammatory responses in the brain in response to a degenerating cyst [5]. Calcified intraparenchymal cysts may serve as a focus for remote...
symptomatic seizures as late sequelae of NCC [5], but there is not always concordance between the location of calcified cysts and a seizure focus identified by electroencephalogram [6].

The proportion of persons with acute symptomatic seizures associated with NCC who go on to develop epilepsy (recurrent, unprovoked seizures) is unknown, and determining the proportion of seizures attributable to NCC is problematic in the absence of repeated serological testing and concurrent diagnostic neuroimaging. In spite of these difficulties, NCC is considered to be the leading preventable cause of epilepsy in developing counties [7–9]. Some of the studies used to support this contention have not discriminated between acute symptomatic seizures and epilepsy or have counted as NCC cases persons with seizures and positive serology for *T. solium* (for example, Garcia et al., 1993 [10]; Nsengiyumva et al. 2003 [11]). In the absence of neuroimaging evidence of brain lesions compatible with NCC, it is not possible to determine whether infection with *T. solium* may be the cause of the epilepsy. This is especially of concern in areas endemic for cysticercosis where many people may be exposed to the eggs of the parasite, including exposure after the onset of epilepsy. The relation between number, location and stage of NCC lesions and parasite, including exposure after the onset of epilepsy. This is especially of concern in areas endemic for cysticercosis where many people may be exposed to the eggs of the parasite, including exposure after the onset of epilepsy. The relation between number, location and stage of NCC lesions and the presence and type of clinical manifestations further complicates the discrimination between acute symptomatic seizures and epilepsy in the field [10,12–15].

Alternatives to serology for identifying lesions of NCC in the brain are computerized tomography (CT) or magnetic resonance imaging (MRI) of the brain. The results of the imaging combined with epidemiological and serological results have been suggested by an international panel of experts for the diagnosis of NCC [16]. Reliance on neuroimaging studies for the diagnosis of NCC is not generally feasible, however, especially in developing countries where the disease is likely to be most prevalent and CT and MRI are often not available. In addition, it is not a reasonable method for case identification in community-based field studies of the epidemiology of NCC. Development of alternative, valid strategies for identifying persons with NCC would greatly facilitate such studies.

The first objective of this pilot study was to estimate the proportion with NCC in persons attending an outpatient clinic for epilepsy in an area of South Africa endemic for cysticercosis. A second objective was to estimate the accuracy of antibody and antigen serological testing in detecting cases of NCC in patients with epilepsy.
of eight negative controls. The optical density (OD) of each serum sample is compared with the mean OD of a series of eight samples from non-infected individuals at a probability level of $P<0.001$ to determine the result in the test [22]. The calculation is based on Student’s t-test.

In some analyses, the results of the antigen-ELISA and antibody-ELISA were considered together to define a positive or negative result. If both measures were positive, the combined measure was considered positive; if both were negative, the combined measure was negative. A positive result to either test was considered positive, even if the other result was missing. Similarly, a negative result to either test was considered negative, even if the other result was missing. These assumptions were considered reasonable since among patients with both antigen and antibody results available, 95% (38/40) of those with a negative antibody result also had a negative antigen result. Only one person had data missing for both antigen and antibodies.

Selection of Participants for the CT-Scan Examination

Each week, four randomly chosen patients with clinically diagnosed seizure disorder and who gave written consent were transported for CT scan of the brain (Toshiba Astion TSX-021A) to the Nelson Mandela Academic Hospital (NMAH), a teaching hospital affiliated with the Walter Sisulu University for Technology and Science in Mthatha. The transportation, per diem and cost of the CT were covered by the research project and did not incur any costs to the patients. When estimating the proportion of cases associated with NCC, it is essential that seizure patients be sampled randomly for CT in order to avoid persons suspected of having NCC being preferentially referred. The random sampling was done by first randomly selecting a day of the week (Monday through Friday). The first four eligible patients at St. Elizabeth’s seen on the randomly chosen day were referred to the NMAH. If there were not four eligible patients on that day, the remaining number required to reach a total of four were chosen from the first eligible patients seen the following day. If the random day was Friday and there were not four eligible patients on that day, then the remainder were selected on the following Monday. CT scans were done with and without radiographic contrast material for all referred patients unless contrast was contraindicated (e.g., allergy to contrast media, pregnancy). Scans were read by radiologists (IT, MAA) at the NMAH as part of usual clinical care. Readings were done without knowledge of serological results. For this pilot study, results of the CT scans were used as the “gold standard” for classification of NCC. We recognized that imaging is not a true gold standard for the diagnosis of NCC but there were no other imaging facility available to the patients in Mthatha. It is reasonable, however, to assume that the CT-scan will be a much better test for identifying lesions in the brain and a serological test. Using the CT report results, cases were classified as “NCC” (either evidence of visible cysticerci, colloidal cysts or presence of cerebral calcifications with a specific CT diagnosis of NCC) or “not NCC” (no diagnosis of NCC and no evidence of cerebral calcifications of any kind). Serology results were not used in making these designations. Persons with CT scans interpreted as “cerebral calcifications” but without mention of NCC and who had no evidence of active cysticerci were included in one set of estimates of the proportion of epilepsy associated with NCC but were excluded from analyses of NCC in order to reduce the possibility of misclassification.

Data Management and Analysis

All interview data were independently entered in an Access® database by two individuals. Questions for which there was a disagreement of entries of more than 5% were reviewed and corrected. Questions with disagreements of less than 5% were considered acceptable. Sixty percent of all entries had a mismatch of 2% or less. Prevalence proportion ratios, sensitivity, specificity and predictive values were estimated. The 95 percent confidence intervals (95% CI) of the field-based diagnosis of NCC were calculated assuming a binomial distribution and using the exact Clopper-Pearson method [23]. Data were examined for confounding and interaction for all covariates, but since neither was present, univariate results are reported. All analyses were conducted using SAS version 9.1 (SAS Institute, Inc., Cary, North Carolina).

Consent Procedures

Two signed consent forms were used in this study. The first consent form asked participants for their agreement to be interviewed and have a blood sample drawn for the serological analysis. A second consent was used for those patients selected at random to have a CT-scan in Mthatha. This project was approved by the Institutional Review Boards of the University of Oklahoma Health Sciences Center, the Walter Sisulu University, and St. Elizabeth Hospital.

Results

A total of 296 individuals with suspected epilepsy were seen at the outpatient clinic in St. Elizabeth of whom 281 were diagnosed with at least one seizure according to the clinical report. Sociodemographic and clinical characteristics as well as serological results from these patients are shown in Table 1. Ages ranged from 5 to 76 years with 31 (11.0%) cases being children (i.e., <16 years of age). The seroprevalence of antibodies and antigens to the larval

| Characteristic | Category | N   | %    | 95% CI |
|----------------|----------|-----|------|--------|
| Number of participants | 281 |     |      |        |
| Age range | 5–76 yrs. |     |      |        |
| Age group | Children (<16 yrs) | 31 | 11.0 | 7.6–15.3 |
| Gender | Female | 163 | 58.0 | 52.0–63.8 |
| Self-reported frequency of seizures | Single seizure | 17 | 6.1 | 3.6–9.5 |
| | More than one seizure | 244 | 86.8 | 82.3–90.6 |
| | No answer | 20 | 7.1 | 4.4–10.8 |
| Primary seizure type | Partial seizures | 131 | 46.6 | 40.7–52.6 |
| Reported duration of seizures+ | Generalized seizures | 150 | 53.4 | 47.4–59.3 |
| | ≤1 year (incident) | 63 | 28.9 | 23.0–35.4 |
| | 2–4 years | 50 | 22.9 | 17.5–29.1 |
| | 5–9 years | 37 | 17.0 | 12.2–22.6 |
| | ≥10 years | 68 | 31.2 | 25.1–37.8 |
| T. solium antibody* | Positive | 89 | 32.6 | 27.1–38.5 |
| T. solium antigen* | Positive | 15 | 7.9 | 4.5–12.8 |
| Brain CT | Yes | 111 | 39.5 |     |

*95% confidence interval.

1 of those tested (antibody testing, n = 273; antigen testing, n = 188).

2 of those who answered duration question and had available seizure frequency data (n = 218 of 281).

doi:10.1371/journal.pntd.0000562.t001
stage of *T. solium* were 32.6% (95% CI: 27.1–38.5%) and 7.9% (95% CI: 4.5–12.0%), respectively. Generalized seizures were reported slightly more often than partial seizures. Of the generalized seizures, tonic-clonic were most often reported, and the most commonly observed partial seizures were either complex partial seizures with motor manifestations (n = 79, 20.1%) or partial seizures secondarily generalized (n = 40, 11.3%). A total of 244 patients (86.8%, 95% CI: 82.3–90.6%) met our definition of epilepsy. Among those with epilepsy, the onset of seizures was within the past year in 29.9% (95% CI: 23.0–35.4%) of cases, while another 31.2% (95% CI: 25.1–37.8%) had been having seizures for 10 years or longer at the time of this study.

Since the referral of patients for CT was done randomly, the characteristics of those with and without CT are expected to be similar. There were more patients sent for CT for whom seizure frequency was unknown (13.5% vs. 2.9%) and more children aged less than 16 (16.3% vs 7.2%), but no other statistically significant differences were observed in demographic, seizure type or duration, or serological results between those who were or were not referred for CT. The distributions of demographic and clinical characteristics of those referred as compared to those not referred for a CT scan are given in Table S1.

A total of 92 patients who met the study’s definition of epilepsy received a CT scan. Of those, 34 (37.0%, 95% CI: 27.1–47.7%) had a CT diagnosis of NCC; 20 (21.7%; 95% CI: 13.8–31.6%) of these showed calcification with a diagnosis of NCC and 14 (15.2%; 95% CI: 8.6–24.2%) had either visible cysticerci or colloidial lesions (subsequently referred to as ‘‘active’’ NCC). In these two groups, the proportions with new-onset seizures were 31.6% (95% CI: 12.6–56.6%) and 46.2% (95% CI: 19.2–74.9%), respectively. If persons with epilepsy who had CT evidence of cerebral calcifications not diagnosed as NCC (n = 19) were included as possible cases of NCC, the proportion of epilepsy associated with NCC would be 43/92 or nearly 47%. These 19 uncertain cases were excluded from further analysis of NCC as these lesions may be due to other cerebral infections such as tuberculosis. Some cases of NCC may have been missed by this approach, which would under-estimate the proportion of seizure cases associated with NCC. Excluding them has the benefit, however, of reducing the chances of misclassification within the NCC group, which increases the validity of the comparisons between NCC and non-NCC epilepsy cases.

Characteristics of those with calcified NCC lesions, ‘‘active’’ NCC, and those with no CT abnormalities are summarized in Table 2. Those with ‘‘active’’ lesions were less often female and more often seropositive than those with calcified lesions or those with no CT abnormalities.

Prevalence proportion ratios for selected characteristics were calculated comparing those with: 1) any definite NCC diagnosis to those with no CT abnormality, and 2) those with ‘‘active’’ NCC to those with no abnormality (Table 3). The small number of cases in each group is reflected in the wide 95% CI. When compared to those with epilepsy who had no CT abnormalities, the proportion of incident cases of epilepsy tended to be higher in those with NCC (PPR = 1.97, 95% CI: 0.87–4.42) and even higher in those with ‘‘active’’ NCC (PPR = 2.39, 95% CI: 0.97–5.89). The prevalence of a positive antibody test was higher in those with NCC (PPR = 1.77, 95% CI: 1.01–3.12), especially in those with ‘‘active’’ NCC (PPR = 2.55, 95% CI: 1.48–4.40) as compared to those without NCC. Compared to those with calcified NCC lesions, the prevalence of seropositivity for antibody to *T. solium* was 2.55 times higher (95% CI: 1.48–4.40) in those with ‘‘active’’ NCC.

The validity of serological testing for antibodies or antigens as diagnostic tools for NCC in persons with epilepsy was estimated separately (Table 4). The predictive value for NCC of a positive antibody test was 60.0% (95% CI: 40.6–77.3%). Hence, 40% (95% CI: 22.7–59.4%) of those testing positive to the antibody ELISA but without NCC would be falsely attributed to NCC (false positives) based on serology alone. When this analysis was confined to those who had ‘‘active’’ NCC, the sensitivity of epilepsy in patients with epilepsy was higher (78.6%, 95% CI: 49.2–95.3%). However, since the prevalence of ‘‘active’’ NCC is lower than that of all NCC, the predictive value of a positive antibody test in these patients was reduced to only 47.8% (95% CI: 26.8–69.4%), therefore leading to 52.2% false positives.

The predictive value of a positive antigen test was similar to that for antibody at 66.7% (95% CI: 22.2–95.7%) but the sensitivity was very poor at 17.4% (95% CI: 5.0–38.8%). When the validity of antigen testing was examined only among those with ‘‘active’’ NCC lesions, the predictive value for a positive antigen test was 50.0% (95% CI: 6.8–93.2%).

### Table 2. Comparison of characteristics in epilepsy patients in those with a CT diagnosis of NCC calcifications, those with ‘‘active’’ NCC and those with no CT abnormalities.

| Characteristics | Category | NCC Dx** (n = 20) | ‘‘Active’’ NCC (n = 14) | No abnormality (n = 39) |
|-----------------|----------|-------------------|------------------------|------------------------|
| Age group       | ≥16 years | 15 (75%)          | 11 (78.6%)             | 32 (82.1%)             |
| Gender          | Female   | 12 (60%)          | 6 (42.9%)              | 24 (61.5%)             |
| Seizure type    | Partial  | 6 (30%)           | 7 (50%)                | 18 (46.2%)             |
|                 | Generalized | 14 (70%)        | 7 (50%)                | 21 (53.8%)             |
| Reported duration of seizures | ≤1 year (incident) | 6 (31.6%) | 6 (46.2%) | 7 (19.4%) |
|                 | 2–4 years | 3 (15.8%)         | 2 (15.4%)              | 10 (27.8%)             |
|                 | 5–9 years | 5 (26.3%)         | 2 (15.4%)              | 6 (16.7%)              |
|                 | ≥10 years | 5 (26.3%)         | 3 (23.1%)              | 13 (36.1%)             |
| T. solium antibody | Positive | 7/19 (36.8%)     | 11/14 (78.6%)          | 12/39 (30.8%)          |
| T. solium antigen | Positive | 2/13 (15.4%)    | 2/10 (20%)             | 2/22 (9.1%)            |

See text for complete definition of the result groupings. **Dx = diagnosis.

### Table 3. Prevalence proportion ratios (and 95% confidence intervals) comparing characteristics of those with any NCC (n = 34) to those with no CT abnormality (n = 39) and of those with ‘‘active’’ NCC (n = 14) to those with no CT abnormality.

| Characteristic | All NCC vs no CT abnormality | ‘‘Active’’ NCC vs no CT abnormality |
|---------------|-------------------------------|--------------------------------------|
| Age <16 yrs   | 1.31 (0.74–1.18)             | 1.19 (0.36–3.99)                     |
| Male Gender   | 1.22 (0.72–2.09)             | 1.49 (0.81–2.71)                     |
| Partial Seizures | 0.83 (0.48–1.43)          | 1.08 (0.58–2.02)                     |
| Incident epilepsy* | 1.97 (0.87–4.42)        | 2.39 (0.97–5.89)                     |
| Ab positive   | 1.77 (1.01–3.12)**          | 2.55 (1.48–4.40)                     |
| Ag positive   | 1.91 (0.39–9.41)³            | 2.20 (0.36–13.47)²                   |

*Seizure onset within past year (n = 68).
³Based on 72 observations.
²Based on 53 observations.
¹Based on 39 observations.
⁷Based on 32 observations.

See text for complete definition of the result groupings. **Dx = diagnosis.
Table 4. Estimated validity of field-based criteria (seropositive to antibodies and antigens of cysticercosis separately) for the diagnosis of all NCC and “active” NCC in persons with epilepsy.

| FIELD-BASED RESULT | CT SCAN RESULT | All NCC | “Active” NCC | NO CT Abnormality |
|--------------------|----------------|---------|--------------|------------------|
| **Antibody**       |                |         |              |                  |
| Antibody positive  | 18             | 11      | 12           |                  |
| Antibody negative  | 15             | 3       | 27           |                  |
| Field diagnosis validity (%) |  | | | |
| Sensitivity: 54.5 (36.4–71.9) | 78.6 (49.2–95.3) | 69.2 (52.4–83.0) |
| Positive predictive value (%) | 60.0 (40.6–77.3) | 47.8 (26.8–69.4) |
| Negative predictive value (%) | 64.3 (48.0–78.4) | 90.0 (73.5–97.9) |
| **Antigen**        |                |         |              |                  |
| Antigen positive   | 4              | 2       | 2            |                  |
| Antigen negative   | 19             | 8       | 20           |                  |
| Field diagnosis validity (%) |  |  |  | |
| Sensitivity: 17.4 (5.0–38.8) | 20.0 (2.5–55.6) | 90.9 (70.8–98.9) |
| Positive predictive value (%) | 66.7 (22.2–95.7) | 50.0 (6.8–93.2) |
| Negative predictive value (%) | 51.2 (34.8–67.6) | 71.4 (51.3–86.8) |

doi:10.1371/journal.pntd.0000562.t004

Serological studies might be more useful in identifying cases of NCC if only those with new onset (incident) seizures were assessed. Unfortunately, there were only 14 patients with new onset seizures who had a CT-scan and either antigen or antibody serology performed. Nonetheless, analysis in this small group suggested that being seropositive for *T. solium* may be a good field diagnostic tool for NCC among incident seizure cases (predictive value of positive serology = 80%, 95%CI: 44%–98%), although 40% (95%CI: 5%–85%) of those whose seizures are not related to NCC will be falsely categorized by serology as NCC.

A series of comparisons was made between those who were correctly and incorrectly classified as NCC by serology (antibody ELISA or antigen ELISA) in order to see whether there were characteristics that distinguished them. Serological true positives (CT positive for NCC and seropositive) and false negatives (CT positive for NCC but seronegative) as well as serologically true negatives (CT negative for NCC and seronegative) and false positive (CT negative for NCC and seropositive) are compared in Table 5. The small numbers in each group are reflected in the wide 95% CI. More true positives had ‘active’ lesions (PPR = 3.06, 95%CI: 1.04–8.97) than false negatives. Persons with epilepsy who are falsely identified as cases of NCC based on serology were more often present or past owners of pigs (PPR = 1.35, 95%CI: 1.08–1.69) and had a higher prevalence of incident seizures (PPR = 1.23, 95%CI: 1.02–1.47) compared to those correctly identified by serology as epilepsy not due to NCC.

Discussion

The source population for this study included persons seeking medical care for symptomatic seizures (acute seizures) or recurrent asymptomatic seizures (epilepsy) at an outpatient clinic of St. Elizabeth Hospital, a rural hospital in Lusikisiki, Eastern Cape Province, South Africa. While the study includes consecutive, unselected epilepsy patients, this group does not represent a community-based sample of persons with epilepsy since only those seeking care are represented. The characteristics of these patients with respect to age distribution and seizure types were similar, however, to those observed in community-based studies of epilepsy conducted in sub-Saharan Africa [9,11,24].

Nearly one-third of epilepsy patients were seropositive to *T. solium* antibodies. This proportion is within the range of that reported from endemic countries of Sub-Saharan Africa using the antibody-ELISA test [25,26].

When CT results are used to identify NCC, the proportion of epilepsy patients with evidence of NCC was 37%, which is higher than what would have been identified based on either ELISA test. This proportion is also comparable to many reports based on neuroimaging findings from other endemic regions [25,27]. Only 15% of those with seizures had lesions with either a demonstrated scolex or that were described as colloidal or cystic; seizures in these patients may be more correctly classified as acute symptomatic seizures.

One of the aims of this pilot study was to estimate the validity of serological testing for *T. solium*, as a readily available classification tool in the field, in identifying persons with NCC among those with epilepsy or recurrent seizures. When using CT-scan results as the “gold standard”, only 60% of those seropositive to the antibody to *T. solium* truly had NCC and nearly one-third of those truly without NCC would be wrongly identified as NCC based on serology alone. Also, more than one-third of those seronegative to the antibodies of *T. solium* had NCC, and would thus not have been diagnosed using this field-based criterion. Some of the relatively poor sensitivity and specificity of serology in correctly identifying persons with NCC may be a result of the antibody ELISA test used in this study, which is based on purified antigens. However, in a study conducted using 67 sera and 53 CSF samples from confirmed cases of NCC in Chile, the sensitivity and specificity of the ELISA using a purified antigen was 97% and 98.3% for the serum samples, respectively [28]. We have used a commercially available kit which may have slightly different accuracy values. Other antibody ELISA tests using crude extract of the metacestodes have shown some limitations. In a double blind, head-to-head comparison in the same population in which only 6% of NCC cases had calcified lesions, EITB was more sensitive than antibody-ELISA based on crude extract of the metacestode in detecting antibody to *T. solium* (86% vs. 41% respectively) but approximately equal in specificity (93% v. 96%, respectively) [13]. In populations in which the proportion of NCC with calcified or single lesions is high, both the ELISA test using crude extract of the metacestode [13] or the EITB [29,30] have significantly reduced sensitivity.

We found that antigen testing was very poor for identifying persons with NCC, even among those with active disease. This is likely due to the natural history of NCC in which the incubation period for CNS manifestations is not clearly understood but appears to be extremely variable; symptoms can occur many years after the primary infection [31]. While the breakdown of cysts in the brain and subsequent release of parasite antigen is thought to trigger an inflammatory response which results in acute CNS manifestations in NCC [5], antigens in the CNS may not be present at detectable levels in the serum. This would be contrary, however, to what has been demonstrated for antibodies detectable by EITB, in which samples from sera were more often positive than samples from cerebral spinal fluid [13]. Unfortunately, this pilot study was too small to examine the
Table 5. Distribution and associated prevalence proportion ratios (PPR) of demographic and clinical characteristics of 33 epilepsy patients with CT-diagnosed NCC lesions and 39 patients without CT-scan lesions of NCC according to serological field-based diagnosis (antigen or antibody ELISA positive).

| Characteristic | Field-based classification | True positive (n = 18) | False negative (n = 15) | PPR* (95% CI) | True negative (n = 27) | False positive (n = 12) | PPR** (95% CI) |
|---------------|---------------------------|------------------------|------------------------|---------------|------------------------|------------------------|---------------|
| Age<16 yrs    |                           | 5 (27.8%)              | 2 (13.3%)              | 2.08 (0.47–9.24) | 4 (14.8%)              | 3 (25.0%)              | 1.69 (0.44–6.40) |
| Male gender   |                           | 9 (50.0%)              | 6 (40.0%)              | 1.25 (0.58–2.71) | 7 (25.9%)              | 5 (41.7%)              | 1.61 (0.67–4.26) |
| Generalized Seizure |                     | 11 (61.1%)            | 10 (66.7%)            | 0.92 (0.35–1.53) | 11 (40.7%)            | 7 (58.3%)              | 1.43 (0.74–2.77) |
| Active NCC by CT |                         | 11 (61.1%)            | 6 (40.0%)             | 3.06 (1.04–8.97)   | NA                     | NA                     | NA             |
| Ever kept pigs |                           | 14 (82.4%)            | 11 (78.6%)            | 1.05 (0.74–1.49) | 20 (74.1%)            | 12 (100%)              | 1.35 (1.08–1.69)  |
| Reported duration of seizures<1 year |                   | 8 (47.1%)             | 4 (28.6%)             | 1.65 (0.62–4.34)  | 22 (81.5%)            | 12 (100%)              | 1.23 (1.02–1.47)  |

*PPR: prevalence proportion ratio calculated as % true positive with factor × % false positive with factor.
**PPR: prevalence proportion ratio calculated as % false positive with factor ÷ % true negative with factor.

*Reported duration of seizures based on 31 observations for the true positive, false negative comparison.

The data available for the present study made it difficult to separate symptomatic seizures from epilepsy. We did include as epilepsy only those who self-reported having had more than one seizure episode and clearly, the duration of seizures was very long for many individuals, but the presence of some acute symptomatic seizures cannot be ruled out. It is interesting to note that the predictive value of positive serology for antibody to T. solium was highest in those with either visible cystic or colloidal lesions on CT, suggesting that antibody testing might be useful for identifying acute symptomatic seizures, but not epilepsy.

The use of serological testing as a method for identifying cases of NCC in the field will continue to be inadequate because of the natural history of NCC. Although not fully understood because of the absence of cohort data, available cross-sectional and case-control information has helped to define some aspects of the disease which impact on the validity of any attempt to measure it in population settings. These characteristics include the evolving nature of the CNS lesions and how the location, stage and number of lesions may affect the clinical manifestations of NCC [5,10,12,14,15]; the unknown nature of the balance between intensity of infection and measurable antibody levels vs. CNS invasion; the duration of measurable antibody and antigen levels in relation to the presence of observable CNS involvement [10,32]. Given these limitations, it is unlikely that even improved serology will alone be sufficient for the valid identification of persons with NCC.

Supporting Information

Table S1  Comparison of those referred or not referred for CT scan.
Found at: doi:10.1371/journal.pntd.0000562.s001 (0.03 MB DOC)

Checklist S1  STROBE checklist.
Found at: doi:10.1371/journal.pntd.0000562.s002 (0.08 MB DOC)

Acknowledgments

We thank Ted Nash for his thoughtful comments on an earlier version of this manuscript.
Author Contributions
Conceived and designed the experiments: LDC HC RCK ALWI. Performed the experiments: HFS IT MAA RCK. Analyzed the data: LDC HC. Contributed reagents/materials/analysis tools: LDC HC GSO RCK. Wrote the paper: HFS LDC HC GSO RCK ALWI. Assisted in obtaining local IRB approval: HFS. Provided clinical data on patients and access to patient population: GSO.

References
1. Prabhakar S, Singh G (2002) Neurocysticercosis: an overview of clinical presentations. In: Singh G, Prabhakar S, eds. Taenia solium cysticercosis. From basic to clinical science. New York: CAB International. pp 169–176.
2. Pawloski ZS (2002) Taenia solium: Basic biology and transmission. In: Singh G, Prabhakar S, eds (2002) Taenia solium cysticercosis. From basic to clinical science. New York: CAB International. pp 1–13.
3. Bern C, Garcia HH, Evans C, Gonzalez AE, Veraestegui M, et al. (1999) Magnitude of the disease burden from neurocysticercosis in a developing country. Clin Infect Dis 29: 1203–1209.
4. Pal DK, Carpio A, Sander J (2000) Neurocysticercosis and epilepsy in developing countries. J Neurol Neurosurg Psychiatry 68: 137–143.
5. Nash TE, Del Brutto OH, Bunman JA, Corona T, Delgado-Escueta A, et al. (2004) Calcific neurocysticercosis and epileptogenesis. Neurology 62: 1934–1938.
6. Cukiert A, Puglia P, Scapolan HB, Vilela MM, Marino Junior R (1994) Congruence of the topography of intracranial calcifications and epileptic foci. Arq Neuropsiquiat 52: 289–294.
7. Bergen DC (1998) Preventable neurological diseases worldwide. Neuroepidemiology 17: 67–73.
8. Roman G, Sotelo J, Del Brutto O, Eliser A, Dumas M, et al. (2000) A proposal to declare neurocysticercosis an international reportable disease. Bull WHO 78: 399–406.
9. Preux P-M, Druet-Cabanac M (2003) Epidemiology and aetiology of epilepsy in sub-Saharan Africa. Lancet Neurol 4: 21–31.
10. Garcia HH, Gilman R, Martinez M, Tsang VC, Pilcher JB, et al. (1993) Neurocysticercosis and active epilepsy as an index of burden of neurocysticercosis in Vellore district, an old disease. Lancet Neurol 4: 653–661.
11. Nsenyinya G, Druet-Cabanac M, Ramankandrasana B, Bouteille B, Nizibari L, et al. (2003) Cysticercosis as a major risk factor for epilepsy in Burundi, East Africa. Epilepsia 44: 950–955.
12. Garcia HH, Gilman Rb, Catacora M, Verastegui M, Gonzalez AE, et al. (1997) Serial evolution of neurocysticercosis patients after antiparasitic therapy. J Infect Dis 175: 486–489.
13. Prano-Narvaez JF, Meza-Lucass A, Mata-Ruiz O, Garcia-Jeronimo RC, Correa D (2002) Laboratory diagnosis of human neurocysticercosis: Double-blind comparison of enzyme-linked immunosorbent assay and electroimmuno-transfer blot assay. J Clin Microbiol 40: 2115–8.
14. Garcia HH, Del Brutto OH (2005) Neurocysticercosis: updated concepts about an old disease. Lancet Neurol 4: 653–661.
15. Rajeshkhar V, Ragha MV, Prabhakaran V, Oommen A, Mulyi J (2006) Active epilepsy as an index of burden of neurocysticercosis in Vellore district, India. Neurology 67: 2135–2139.
16. Del Brutto OH, Rajeshkhar V, White AC Jr, Tsang VC, Nash TE, et al. (2001) Proposed diagnostic criteria for neurocysticercosis. Neurology 57: 177–183.
17. Statistics South Africa (2004) Census 2001: Primary Tables Eastern Cape 1996 and 2001 Compared. Pretoria: Statistics South Africa. Report Number 05-02-05.
18. Commission on Classification and Terminology. International League Against Epilepsy (1981) Proposal for revised clinical and electroencephalographic classification of epileptic seizures. Epilepsia 22: 409–501.
19. Commission on Classification and Terminology. International League Against Epilepsy (1989) Proposal for classification of epilepsies and epileptic syndromes. Epilepsia 30: 389–399.
20. Dorny P, Brandt J, Zoli A, Geerts S (2003) Immunodiagnostic tools for human and porcine cysticercosis. Acta Trop 87: 79–86.
21. Dorny P, Vercaemen F, Brandt J, Vanstrekeniste W, Berkvens D, et al. (2000) Sero-epidemiological study of Taenia saginata cysticercosis in Belgian cattle. Vet Parasitol 88: 43–49.
22. Sokal RR, Rohlf JF (1981) Biometry: The Principles and Practice of Statistics in Biological Research. New York: WH Freeman Co.
23. Cophop, Pearson S (1934) The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 26: 404–413.
24. Kabore J, Lenganai A, Drabo YJ, Melaku Z, Preux PM, et al. (1995) Clinical aspects of seizure disorders at Ouagadougou Burkina Faso: retrospective study of 532 cases. Afr J Neurol Sci 14: 24–26.
25. Druet-Cabanac M, Ramankandrasana B, Biser S, Dongmo L, Avodé G, et al. (2002) Taenia solium cysticercosis in Africa. In: Singh G, Prabhakar S, eds. Taenia solium cysticercosis. From basic to clinical science. New York: CAB International. pp 129–137.
26. Mafojane NA, Appleton CC, Kreek RC, Michael LM, Willingham AL (2003) The current status of neurocysticercosis in Eastern and Southern Africa. Acta Trop 87: 25–34.
27. Del Brutto OH, Nsizabira L, et al. (2003) Cysticercosis as a major risk factor for epilepsy in Burundi, East Africa. Epilepsia 35: 1934–1938.
28. Delbrutto OH, Noboa CA (1991) Late-onset epilepsy in Ecuador: aetiology and clinical features in 225 patients. J Trop Geograph Neurol 1: 31–34.
29. Salinas P, Sandoval L, Ruijero E, Conteras MC (1996) [Diagnosis of human neurocysticercosis by ELISA-IgG using a purified antigen]. Bol Chil Parasitol 51: 85–90.
30. Wilson M, Bryan RT, Fried JA, Ware DA, Schantz PM, et al. (1991) Clinical evaluation of the cysticercosis enzyme-linked immunoelectrotransfer blot in patients with neurocysticercosis. 164: 1007–9.
31. Singh G, Kaushal V, Rama S, Kaushal RK, Dhamuka AK, et al. (1999) Cysticercus immunoblot assay in patients with single, small enhancing lesions and multilesional neurocysticercosis. J Assoc Physicians India 47: 476–479.
32. Dixon HF, Lipscomb FM (1961) Cysticercosis: an analysis and follow-up of 450 cases. London: Privy Council, Medical Research Council. pp 1–38.
33. Garcia HH, Martinez M, Gilman R, Herrera G, Tsang VCW, et al. (1991) Diagnosis of cysticercosis in endemic regions. Lancet 338: 549–551.
34. Palacios E, Lujamio PS, Jasso RR (1997) Computed tomography and magnetic resonance imaging of neurocysticercosis. Seminars Roentgen 32: 325–334.
35. Garcia HH, Del Brutto OH (2003) Imaging findings in neurocysticercosis. Acta Trop 87: 71–78.