Evaluating the Recombinant T24H Enzyme-Linked Immunoelctrotransfer Blot Assay for the Diagnosis of Neurocysticercosis in a Panel of Samples from a Large Community-Based Randomized Control Trial in 60 Villages in Burkina Faso

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Abstract. Current guidelines for the diagnosis of neurocysticercosis (NCC) recommend the use of the lentil lectin-bound glycoprotein enzyme-linked immunoelctrotransfer blot assay (LLGP-EITB) as the reference standard for serological testing. In response to the drawbacks involved with the use of the LLGP-EITB, a recombinant T24H antigen (rT24H) EITB assay was developed, with promising results. However, the test has yet to be evaluated among individuals from sub-Saharan Africa (SSA). The aim of the present study was to investigate the performance of the rT24H EITB assay for the detection of NCC cases in a panel of serum samples ($N = 366$, of which 173 patients presented with epileptic seizures and/or severe chronic headaches, and 193 matched manifestation-free participants) collected as part of a large community-based trial in Burkina Faso. A perfect agreement between the rT24H EITB and the native gp24 (and its homodimer, gp42) LLGP-EITB was found (kappa value of 1.0). Furthermore, among patients with the neurological manifestations of interest who underwent a computed tomography scan, the rT24H EITB and native antigen LLGP-EITB had a comparable ability to correctly identify NCC cases with multiple viable (rT24H: sensitivity: 80.0%), single viable (66.7%), and calcified/degenerating cysts only (25.0%), albeit for multiple viable and calcified cysts, the rT24H estimated sensitivity seemed lower, but more uncertain, than previously reported. The rT24H EITB specificity was high (98.2%) and in line with previous studies. This study confirms the value of the recombinant rT24H EITB as an alternative to the native antigen LLGP-EITB for the diagnosis of NCC in a SSA community setting.

INTRODUCTION

Invasion of the brain with the larval stage of the tapeworm *Taenia solium* causes neurocysticercosis (NCC), a neglected zoonotic disease targeted for control by the World Health Organization.¹ In endemic areas, the disease is a major cause of epilepsy, with an estimated 29% of people with epilepsy being affected.² Other signs and symptoms of NCC include progressively worsening severe chronic headache (SCH) and cognitive decline.³

Currently, experts advise using a combination of neuroimaging results, the presence of specific clinical/neurological presentations, results from immunodiagnostic tests in serum or cerebrospinal fluid, and/or epidemiological factors, among others, to diagnose NCC.^{4,5} The lentil lectin-bound glycoprotein enzyme-linked immunoelctrotransfer blot assay (LLGP-EITB)⁶ is considered the most accurate immunodiagnostic test for NCC diagnosis, with a positive test result being considered a major diagnostic criterion.⁴ The LLGP-EITB detects antibodies against glycoproteins found in the soluble fraction of an extract of *T. solium* cysticerci⁷ and has an excellent test performance with a reported sensitivity of 94%

and specificity of 100% for multiple enhancing intracranial lesions.⁸ The LLGP-EITB assay is, however, expensive and impractical, especially for application in low resource and community-based settings because of its need for collection of cysts from naturally infected pigs and extensive expertise with the assay technique.⁹

To overcome these drawbacks, the Centers of Disease Control and Prevention of the United States (CDC) developed and evaluated an EITB assay based on the representative recombinant or synthetic forms of antigens (Ag) from each of the three distinct antigenic protein families of the LLGP-EITB assay: gp50, gp24, and 8-kDa.⁷ In an evaluation study mainly analyzing serum panels of NCC patients from Peru, the EITB assay based solely on the recombinant T24H (rT24H) Ag, belonging to the gp24 antigenic protein family, was 99% sensitive and 100% specific in detecting NCC cases with more than one viable cyst.⁷ The agreement between the rT24H EITB and the full native antigen EITB assay (LLGP-EITB) was almost perfect (kappa value = 0.89).⁷ Based on this agreement, and excellent performance of the rT24H in the same and earlier studies using the rT24H in a multiple assay format,^{10–12} the authors concluded that the recombinant T24H in a single Ag assay could be a valuable alternative for the native combination Ag LLGP-EITB.⁷

We are not aware of any other study having compared the performance of the rT24H in a single assay format and the

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native Ag EITB assay for NCC diagnosis. Furthermore, no published study has evaluated the performance of rT24H EITB assay as a serological test for NCC diagnosis in individuals from sub-Saharan Africa (SSA). Therefore, the aims of this study were to 1) investigate the agreement between the rT24H EITB assay versus the CDC LLGP-EITB and a commercialized EITB and 2) evaluate the performance of the rT24H EITB for the detection of NCC in people with single or recurrent seizures and SCH, in a panel of serum samples collected in a large scale community-based randomized controlled trial in Burkina Faso.

MATERIALS AND METHODS

Study design and participants. The sera originated from a subgroup of participants in the baseline cross-sectional component of a large community-based randomized controlled trial (Évaluation du Fardeau Économique de la Cysticercose au Burkina [EFECAB]), conducted in 60 villages in Burkina Faso.¹³ Each consenting participant was asked to answer a screening questionnaire including questions about the presence of epileptic seizures and SCH (see Supplemental material).

An epileptic seizure was defined as an unprovoked seizure without an apparent cause. Participants with either recurring or single epileptic seizures were included. SCHs were defined as symptoms arising more than weekly for more than 2 weeks with each episode lasting at least 3 hours, and progressively worsening in severity with time. Headaches had to be severe enough to require painkillers or to prohibit working, playing, attending school, or partaking in daily activities.¹⁴

Sera of all participants who were, based on the individual screening questionnaire and a medical examination by a physician of those screened positive, classified as having

single or recurrent epileptic seizures, or SCH, referred to as “patients with neurological manifestations,” were included in the test panel. In addition, sera from randomly selected individuals who declared no symptoms on the questionnaire and were not examined by a physician, frequency matched by age groups, gender, and village to the cases, were included, referred to as “manifestation-free participants.”

Samples and analytical procedures. A total of 199 and 199 serum samples of participants initially classified by the study physician as patients with neurological manifestations of interest or manifestation-free participants, respectively, were selected (Figure 1). Of these, enough sera remained to analyze 182 and 184 samples of participants with and without neurological manifestations, respectively. Following further examination of patient files by the neurologist, nine individuals initially being classified as patients with neurological manifestations were reclassified as being manifestation-free participants, leaving sera of 173 patients with neurological manifestations and 193 manifestation-free participants available for the analysis ($N = 366$). This panel of sera was subjected to analysis with the LLGP-EITB run at the CDC as well as the rT24H and rGP50 EITB assays, using procedures described earlier.^{6,7} For the LLGP-EITB, results for reactions to any of the antigenic protein families, gp24 (including reactions to its homodimer gp42), gp 50, and 8-kDa, were presented. The samples were also subjected to a commercial LLGP-EITB assay, the Qualicode™ Cysticercosis EITB Kit, following manufacturer’s instructions (Immunetics Inc, Boston, MA), yet because of a prolonged interruption in diagnostic kit delivery by the distributor, only 223 samples (103 patients with neurological manifestations, 120 manifestation-free participants) could eventually be analyzed.

Neuroimaging. Brain imaging results obtained by enhanced computed tomography (CT) scans both with and

FIGURE 1. Sampling scheme. The participants’ responses to the screening questionnaire were reviewed by the study physician and reexamined by the neurologist thereafter. Participants were classified as being “patients with neurological manifestations” or “manifestation-free participants.” The commercial enzyme-linked immunoelectrotransfer blot (EITB) test panel was smaller than the other serum test panels because of the shortage of diagnostic kits from the distributor. See also MATERIALS AND METHODS.

without contrast were available for 151 of 173 patients with neurological manifestations; the remaining 22 individuals refused to undergo a CT scan. The presence of NCC lesions on CT scans and their classification was evaluated by three radiologists; scans for which there was a disagreement were additionally evaluated by a neurologist. CNS lesions were classified as “uncertain” in situations where no agreement could be reached among the radiologists and the neurologist, “calcified/degenerating cysts” when only calcified or degenerating cysts were identified, and “single viable cysts” or “multiple viable cysts” when one or more live cysts with or without scolex were identified. Patients with single or multiple viable cysts could also harbor degenerating or calcified cysts, yet for these patients, only the presence of the viable cysts was described, and sera were categorized accordingly.

Data analysis. The agreement between antibody-detecting tests was assessed on the full panel of serum samples obtained from patients with neurological manifestations and matched manifestation-free participants and was expressed by means of the Cohen’s kappa statistic,¹⁵ with a kappa value of 0.01–0.20 indicating “slight” agreement between tests, whereas kappa values of 0.21–0.40, 0.41–0.60, 0.61–0.80, and > 0.81 indicate “fair,” “moderate,” “substantial,” and “almost perfect” agreement, respectively.¹⁶

The performance (sensitivity and specificity) of evaluated tests was assessed in the panel of serum samples of the patients with neurological manifestations who underwent CT imaging with conclusive NCC diagnosis (called “NCC cases”). In the context of this performance assessment, CT imaging was considered as the gold standard. Statistical analyses, including calculation of 95% confidence intervals (95% CI), were conducted using the binom.test, Kappa test, and confusionMatrix commands in R, version 3.3.1.¹⁷

Ethical clearance. Serum samples were collected and imaging was performed in compliance with protocols approved by the University of Oklahoma Health Sciences Center Institutional Review Board (USA) and by the Center MURAZ ethical review panel (Burkina Faso). An informed consent was obtained from adult participants and from parents or legal guardians of minors. All patients received care according to the national guidelines.

RESULTS AND DISCUSSION

The present study aimed to evaluate the performance of the rT24H EITB assay for the detection of NCC cases in a panel of serum samples collected in a large scale community trial in Burkina Faso. As such, it is the first study evaluating the rT24H EITB in samples collected in SSA. Our serum panel included sera of 173 patients with neurological manifestations, identified in a community setting, of which five suffered from single epileptic seizures, 13 from recurrent epileptic seizures and SCH, 75 from recurrent epileptic seizures only, and 80 from SCH only, as well as 193 manifestation-free participants. Of the 366 samples analyzed, 34 were positive to the LLGP-EITB assay run at the CDC (34/366, 9.3%, 95% CI: 6.5–12.7). Of these 34 positive samples, 26 showed reactivity to the native gp24, gp42, and gp50 Ag (26/366, 7.1%, 95% CI: 4.7–10.2), whereas eight only reacted to the native gp50 Ag (8/366, 2.2%, 95% CI: 0.9–4.3). Furthermore, of the 34 positive samples, weak reactions to at least one of the native Ag were observed for nine samples (9/366, 2.5%). Of the 366 samples

analyzed, 26 were found positive on the rT24H EITB assay (26/366, 7.1%, 95% CI: 4.7–10.2), for which weak reactivity occurred in four samples (4/366, 1.1%, 95% CI: 0.3–2.8), all of which had a weak reaction to the native gp24 Ag on the LLGP-EITB. None of the samples showed reactivity to the 8-kDa antigenic family in the LLGP-EITB (0/366, 0.0%, 95% CI: 0.0–1.0).

The agreement between the rT24H EITB and the LLGP-EITB test results was almost perfect (kappa value: 0.85; 95% CI, 0.76–0.95), and non-concordance could be attributed entirely to reactions to the native gp50 Ag. Therefore, the recombinant Ag EITB format was confirmed to be a valuable alternative for the LLGP-EITB as suggested in earlier studies.^{7,10–12} The agreement between the combined result of the rT24H and rGP50 versus the LLGP-EITB was again almost perfect (kappa value: 0.90; 95% CI: 0.83–0.98). Non-concordant results for three of six samples were due to the presence of reaction to the rGP50, whereas the other three were due to reactivity to the native gp50 Ag in the LLGP-EITB. It is known that reactions to the gp50 antigenic family alone can be caused by cross-reactions with other parasites, and the presence of a non-specific “bogus band” near or at the location of the true 50-kDa band may render interpretation difficult.¹⁸ Its continued inclusion in the LLGP-EITB is, however, advised because sera from some patients alone exhibit reactivity to this band.⁶ This was also observed in the present study, for two NCC cases with calcified/degenerating cysts only. The agreement between the rT24H EITB and the native Ag gp42 and gp24 LLGP-EITB was perfect (kappa value of 1.0) and higher than the agreement reported earlier.⁷ A commercial EITB was performed in 223 of 366 samples, with a positive test result for 35 samples (35/223, 15.7%, 95% CI: 11.2–21.1). The overall agreement between the commercial EITB and the LLGP-EITB run at the CDC was substantial (kappa value of 0.69, 95% CI: 0.55–0.84), with a discordant result for 16 serum samples, 13 being positive on the commercial EITB and negative on the LLGP-EITB, and vice versa for three other. As far as we are aware, this is the first study comparing the performance of these two tests.

For 28 cases of the 151 patients with neurological manifestations who underwent imaging, NCC was diagnosed upon imaging (28/151, 18.5%, 95% CI: 12.7–25.7), whereas for 10 cases, the NCC diagnosis was uncertain (10/151, 6.6%, 95% CI: 3.2–11.8.2); serum samples of the latter group were excluded from further analysis. Of the NCC cases, 20 had exclusively degenerating or calcified cysts (20/28, 71.4%, 95% CI: 51.3–86.8), whereas three had a single viable and five multiple viable cysts (3/28, 10.7%, 95% CI: 2.3–28.2; 5/28, 17.9%, 95% CI: 6.1–36.9, respectively). Among NCC cases with viable cysts, seven of eight also had multiple degenerating/calcified cysts, as mentioned earlier, sera from these patients were classified based on the presence of viable cysts. For 113 individuals, imaging was negative for NCC (113/151, 74.8%, 95% CI: 67.1–81.5).

The ability to correctly identify NCC cases among patients with neurological manifestations seemed similar for the different antibody-detecting tests (Table 1), although in contrast to earlier studies,^{7,10} we did not observe a superior sensitivity of the rT24H versus the rGP50 EITB assays. Sensitivities for detecting NCC cases with single and multiple viable cysts were similar (66.7–100.0%) for most tests and much higher than for NCC cases with degenerating or calcified cysts only

TABLE 1

Performance of the in-house native LLGP-EITB, recombinant EITB, and commercial EITB test for the detection of neurocysticercosis in serum samples* from patients with neurological manifestations in a large community randomized control trial in 60 villages in Burkina Faso

	Sensitivity		Degenerating/calcified lesions	Specificity
	≥ 2 viable	1 viable		
CDC native Ag LLGP-EITB (N = 141)†				
Any band	4/5 (80.0%; 95% CI, 28.4–99.5)	2/3 (66.7%; 95% CI, 9.42–99.2)	7/20 (35.0%; 95% CI, 15.4–59.2)	111/113 (98.2%; 95% CI, 93.8–99.8)
gp24/gp42	4/5 (80.0%; 95% CI, 28.4–99.5)	2/3 (66.7%; 95% CI, 9.42–99.2)	5/20 (25.0%; 95% CI, 8.66–49.1)	111/113 (98.2%; 95% CI, 93.8–99.8)
gp 50	4/5 (80.0%; 95% CI, 28.4–99.5)	2/3 (66.7%; 95% CI, 9.42–99.2)	7/20 (35.0%; 95% CI, 15.4–59.2)	111/113 (98.2%; 95% CI, 93.8–99.8)
Recombinant Ag EITB (N = 141)†				
rT24H only	4/5 (80.0%; 95% CI, 28.4–99.5)	2/3 (66.7%; 95% CI, 9.42–99.2)	5/20 (25.0%; 95% CI, 8.66–49.1)	111/113 (98.2%; 95% CI, 93.8–99.8)
rGP50	4/5 (80.0%; 95% CI, 28.4–99.5)	3/3 (100.0%; 95% CI, 29.2–100.0)	5/20 (25.0%; 95% CI, 8.66–49.1)	111/113 (98.2%; 95% CI, 93.8–99.8)
rT24H + rGP50	4/5 (80.0%; 95% CI, 28.4–99.5)	3/3 (100.0%; 95% CI, 29.2–100.0)	5/20 (25.0%; 95% CI, 8.66–49.1)	111/111 (98.2%; 95% CI, 93.8–99.8)
Commercial EITB (N = 86)‡				
Commercial EITB	3/3 (100.0%; 95% CI, 29.2–100.0)	3/3 (100.0%; 95% CI, 29.2–100.0)	5/13 (38.5%; 95% CI, 13.9–68.4)	63/67 (94.0%; 95% CI, 85.4–98.3)

Ag = antigens; CI = confidence intervals; LLGP-EITB = lentil lectin-bound glycoprotein enzyme-linked immunoelectrotransfer blot.

*Serum samples of 141 patients with neurological manifestations of interest (i.e., single seizures, epilepsy, or SCH) who underwent contrasted computed tomography imaging resulting in a conclusive diagnosis (i.e., absence/presence NCC).

† All 141 serum samples were analyzed with the in-house native Ag LLGP-EITB and recombinant Ag EITB tests.

‡ A subset of 86 of 141 serum samples were analyzed with the commercial EITB.

(25.0–38.5%) (see Table 1 for 95% CI), the latter being observed in earlier studies as well.^{7,10,12} Earlier studies also reported higher rT24H EITB sensitivities in NCC cases with multiple viable cysts versus those with a single cyst,^{7,10,12} yet, in the present study, sensitivities for detection of these two classes of patients seemed similar. Numerically, the rT24H EITB sensitivities seemed lower than those observed in the earlier work, with reported sensitivities of 94–99% in NCC cases with multiple viable lesions and 45–73% in those with calcified/degenerating cysts.^{7,10,12} By contrast, for cases with single viable cysts the rT24H EITB sensitivity were in line with those reported earlier (44–64%).^{7,10,12} These estimates are, however, highly uncertain as suggested by the wide confidence intervals. The specificity was excellent and comparable for all evaluated antibody detecting tests, except for the commercial EITB test for which it seemed lower (94.0% versus 98.2%). The rT24H EITB specificity estimate was similar to the 98–100% reported in previous studies.^{7,10,12}

Overall, we must emphasize the small number of NCC cases in the present study, inevitably causing great uncertainty surrounding the sensitivity values reported, especially for those cases with viable cysts. Furthermore, our study group is inherently different from the ones in previous studies,^{7,10,12} where serum samples for determination of sensitivity were all obtained at a referral hospital in urban Peru, and thus, in a clinical setting where cases were likely to have more severe symptoms than in this study. Samples for specificity determination in that study were obtained from healthy residents from the United States and Egypt, in whom the presence of NCC is highly unlikely.^{7,10,12} In our study, sampled individuals were all living in rural communities in an endemic area in Burkina Faso. Furthermore, sensitivity and specificity determinations in the present study were based on serum samples from patients with neurological manifestations linked to NCC, all of whom underwent CT imaging. Finally, the use of CT imaging as gold standard for the performance analysis equally has some drawbacks as small calcified cysts can be missed, so could viable cysts.^{19,20}

Our results confirm that the recombinant T24H EITB is a valuable, cheaper, and easier to apply alternative to the native antigen LLGP-EITB, considered the current serological reference test for NCC diagnosis. Furthermore, this is the first study evaluating the performance of the rT24H EITB assay for NCC diagnosis in people with single or recurrent seizures and SCH, in a community-based setting in SSA.

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