Trans R Soc Trop Med Hyg 2016; **110**: 517–526
doi:10.1093/trstmh/trw066

Neurocysticercosis in Bhutan: a cross-sectional study in people with epilepsy

Kate Brizzi[a], Sonam Pelden[b], Tshokey Tshokey[b], Damber K. Nirola[c], Megan B. Diamond[a], Joshua P. Klein[d,e], Lhab Tshering[c], Sonam Deki[c], Dechen Nidup[f], Veronica Bruno[a], Pierre Dorny[g], Hector Hugo Garcia[h] and Farrah J. Mateen[a,e,*], for the Bhutan Epilepsy Project

[a]Massachusetts General Hospital, Boston, MA, USA; [b]Department of Laboratory Medicine, Jigme Dorji Wangchuck National Referral Hospital, Bhutan; [c]Department of Psychiatry, Jigme Dorji Wangchuck National Referral Hospital, Bhutan; [d]Brigham and Women's Hospital, Boston, MA, USA; [e]Harvard Medical School, Boston, MA, USA; [f]Department of Radiology, Jigme Dorji Wangchuck National Referral Hospital, Bhutan; [g]Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; [h]Cysticercosis Unit, Instituto de Ciencias Neurologicas, Department of Microbiology, Universidad Peruana Cayetan Heredia, Lima, Peru

*Corresponding author: Present address: 165 Cambridge Street, #627, Neurology Clinical Research Institute, Massachusetts General Hospital, Boston, MA 02114 USA; Tel: +1 617 724 8653; E-mail: fmateen@partners.org

Received 5 August 2016; revised 11 September 2016; accepted 7 October 2016

Background: We sought to provide an assessment of the burden of neurocysticercosis among people with epilepsy (PWE) in Bhutan and evaluate the yield of various tests for *Taenia solium*.

Methods: PWE were enrolled at the National Referral Hospital in Thimphu (2014–2015). Serum was tested for anti-*Taenia solium* IgG using ELISA (Ab-ELISA), enzyme-linked immunoelectrotransfer blot (EITB), and parasite antigen. Results were compared to brain MRI. Participants were categorized as definite neurocysticercosis (MRI and EITB positive), probable neurocysticercosis (MRI or EITB positive), or without neurocysticercosis. Logistic regression models were constructed to explore clinicodemographic associations.

Results: There were 12/205 (6%, 95% CI 2%, 9%) definite and 40/205 (20%, 95% CI 14%, 25%) probable neurocysticercosis cases. 25/205 (12%) with positive EITB did not have neurocysticercosis on MRI, and 15/205 (7%) participants with positive MRI had negative EITB. Participants with neurocysticercosis-suggestive lesions on MRI had an average of 1.2 cysts (parenchymal 26/27; nodular/calcified stage 21/27). In a multivariable analysis, present age (OR 1.05, 95% CI 1.01,1.09, p=0.025) was positively associated with (combined probable or definite) neurocysticercosis while mesial temporal sclerosis on MRI (OR 0.294, 95% CI 0.144, 0.598, p=0.001) was negatively associated.

Conclusions: Neurocysticercosis was associated with 6–25% of epilepsy in a Bhutanese cohort. Combining EITB and MRI would aid the diagnosis of neurocysticercosis among PWE since no test identified all cases.

Keywords: Bhutan, Enzyme-linked immunoelectrotransfer blot, Epilepsy, Magnetic resonance imaging, Neurocysticercosis, Seizure

Introduction

Neurocysticercosis is a leading cause of epilepsy worldwide, particularly in endemic areas of Asia, Africa and Latin America. Caused by the tapeworm *Taenia solium*, the disease is spread through fecal-oral transmission from tapeworm carriers, with pigs acting as intermediate hosts and humans acting as aberrant intermediate hosts. Once the parasite enters the central nervous system, it resides as a vesicular cyst which can be a cause of seizures. In some patients, vesicular cysts degenerate into calcified cysts, leading to seizures that may occur for years after the initial infection.[1]

Diagnosis of neurocysticercosis can be challenging and typically relies on clinical and neuroimaging findings among people living in endemic areas. Strong suspicion for neurocysticercosis allows for the treatment of the parasite with anti-parasitic agents, which reduces seizure frequency in infected patients with viable cysts.[2] Population-based efforts to prevent the initial infection can curtail *T. solium* infection and its neurologic sequelae.[3]

Awareness of epilepsy is growing in the Kingdom of Bhutan (population 720 679), with more than 1215 epilepsy cases reported in 2012.[4] The prevalence of neurocysticercosis and its etiologic relationship to seizures have not been previously reported in Bhutan. To date, reports are limited to the seroprevalence of antibodies to *T. solium* among Bhutanese refugees resettled in the United States of America (2007–2008), showing an antibody prevalence among refugees to be as high as 22.8% but a >5 fold difference in prevalence depending on the location of origin (OR 5.4, 95% CI 1.2, 23.9).[5] Geographically close areas, including Nepal and parts of northern India, have a high prevalence of neurocysticercosis. In Nepal, studies of people with epilepsy (PWE) using neuroimaging have reported findings consistent with neurocysticercosis in 16–32%[6,7] of adults and 9–12% of children.[8,9] In northern India, studies in pig farming communities found 15% of individuals had asymptomatic neuroimaging findings consistent with neurocysticercosis, and 48% of PWE fulfilled criteria for definite or probable neurocysticercosis.[10,11] Given the high prevalence of neurocysticercosis in surrounding regions, we postulated that infection with *T. solium* would also be high among PWE in Bhutan and that a high proportion of PWE would have radiological and laboratory findings indicative of current or prior infection. As a preventable and treatable cause of seizures, an understanding of the contribution of neurocysticercosis to the epilepsy burden is critical to guide targeted preventative and therapeutic strategies.

Given the challenges inherent to making the diagnosis of neurocysticercosis in any location, we also aimed to evaluate the yield of several diagnostic strategies to help inform future efforts to recognize this condition. Brain imaging is the standard to diagnose neurocysticercosis.[12] Enzyme-linked immunoelectrotransfer blot (EITB) is highly specific for cysticercosis and seropositivity is associated with evidence of viable neurocysticercosis on brain imaging in large cross-sectional studies.[13,14] Serum ELISA (Ab-ELISA) is another available, inexpensive test, though prior studies have reported a high rate of false positive results in both endemic and non-endemic populations.[15] Antigen assays (Ag-ELISA) have also been used, and may be a means to monitor response to anti-parasitic treatment, particularly in patients with subarachnoid neurocysticercosis.[16]

Methods

Setting and participants

Participants were recruited at the Jigme Dorji Wangchuck National Referral Hospital (JDWNRH) in the capital city of Thimphu, Bhutan. The JDWNRH is the country's tertiary referral center for all 20 districts, providing care to approximately 13 000 total patients per year.[3] Participants reporting ≥1 seizure were prospectively recruited during their clinical visits to the JDWNRH and from an existing hospital-based registry of adults (>18 years old) and children (<1 to 18 years) with clinically diagnosed epilepsy. Epilepsy in this registry is defined as any patient with a witnessed or self-reported history of one or more seizures with no evidence of a competing diagnosis such as cardiac dysrhythmia or exclusively febrile seizures. All participants were recruited to the present study between July 2014 and August 2015 and resided in districts across Bhutan.

Data collection

A schematic diagram of testing is shown in Figure 1. Serum from each participant was collected and processed at the laboratory at JDWNRH by trained laboratory personnel (SP, TT). Serum was stored in a −80°C freezer for later processing. Samples were tested by on-site laboratory personnel for *T. solium* IgG using an ELISA (Ab-ELISA, DRG International Inc., Springfield, NJ, USA). All positive results at 1:64 dilution were re-tested for confirmation. Initially positive results that were negative on confirmatory testing were interpreted as negative. Positive tests after confirmatory testing were interpreted as positive. All positive results were tested for *Echinococcus* IgG on re-testing (NovaLisa Echinococcus IgG, NovaTec Inc, Dietzenbach, Germany), as recommended by the manufacturer, to identify possible cross reactivity with other parasites.

Serum was also sent to the laboratory of the Cysticercosis Working group in Peru where EITB using lentil-lectin purified glycoprotein parasite antigens[14] and antigen detection using a monoclonal antibody (B158/B60) based ELISA assay[17] were performed. Samples were considered positive on EITB if one or more antibody bands were present, and positive for circulating Ag-ELISA if the optic density of the sample (run in duplicate) was above a cut-off calculated using known negative sera. Testing for Ag-ELISA was performed on samples positive on EITB. The rationale for this was that EITB may be an indicator for exposure to infection while Ag-ELISA may be indicative of active infection.

Participants prospectively underwent brain MRI (Siemens 1.5 Tesla) at the JDWNRH if they had not had brain imaging performed prior to 2013. MRI, for study purposes, was not performed on children under 5 years of age. Brain MRI included at least T1, T2, and T2-fluid-attenuated inversion recovery (FLAIR) sequences. The images were read by a neuroradiologist (JPK) at Brigham and Women's Hospital in Boston, USA who was unaware of the results of serum testing and confirmed with reports from a radiologist in Bhutan (DN) when available. MRI

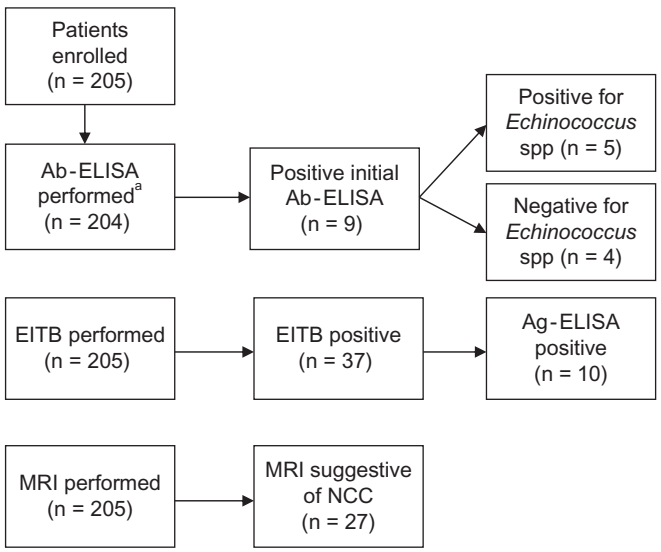

Figure 1. Schematic of testing. Ab-ELISA: ELISA; Ag-ELISA: Antigen ELISA; EITB: enzyme-linked immunoelectrotransfer blot; NCC: neurocycticercosis.

Box 1. Relevant diagnostic criteria for neurocysticercosis (from Del Brutto et al.[20])

Degrees of diagnostic certainty

Definitive criteria:
(1) Lesions consistent with neurocysticercosis on neuroimaging studies
(2) Positive serum immunoblot
(3) Presence of clinical manifestations of neurocysticercosis (seizures)
(4) Endemic region

Probable criteria:
(1) Lesions consistent with neurocysticercosis on neuroimaging OR Positive serum immunoblot
(2) Presence of clinical manifestations of neurocysticercosis (seizures)
(3) Endemic region

images, determined to have cysts consistent with neurocysticercosis, were further classified by a US-based neuroradiologist (JPK) by cyst stage (viable, degenerating, or nodular/calcified),[18] intracranial location of the cysts (parenchymal, intraventricular, or subarachnoid), number of cysts and the presence or absence of associated cystic edema. Mesial temporal sclerosis was also determined subjectively and was considered as present if found unilaterally or bilaterally.

Concurrently, participants completed questionnaires about their seizure history and perceptions of epilepsy: http://www.massgeneral.org/research/researchlab.aspx?id=1663&display=educational-resources (Supplementary File 1).[19] Participants were specifically asked about their understanding of the contribution of infections to seizures and their personal history of a brain infection, including awareness of a diagnosis of neurocysticercosis. Each participant was reimbursed 500 Bhutanese Ngultrum (approximately US$10) for travel expenses.

Categorization

Degree of certainty in the diagnosis of neurocysticercosis was categorized by at least one neurologist (KTB) on previously published diagnostic criteria by Del Brutto et al[20] (Box 1). In this study, participants with imaging highly suggestive of neurocysticercosis, positive serum antibodies on EITB and a history of seizures met criteria for definite neurocysticercosis based on two major criteria and one minor criterion. Participants with either an MRI suggestive of neurocysticercosis or positive EITB, all of whom had a history of seizures, met criteria for probable neurocysticercosis. Probable and definite neurocysticercosis were considered mutually exclusive categories.

Predictors of interest were predetermined based on associations reported in prior neurocysticercosis studies, the usefulness of the measured variable in this lower literacy population, and the presence of variability in the measure. Age was defined continuously as the age at study enrollment (date of enrollment minus date of birth), while age at first seizure was reported retrospectively by the participant in years with corroboration in the medical record whenever possible. Urban residence was defined as living in the capital city of Thimphu which is the only city in Bhutan where >100 000 people reside (population 111 306, National Statistics Bureau, Bhutan, 2013). Educational attainment level was reported as no school, primary school, secondary school, high school and college or above. Children in schools for people with disability were grouped with primary school. Occupational farming was based on self-report and could include any type of farming, with or without pig farming. The number of antiepileptic drugs was based on the number of drugs taken concurrently at the time of study enrollment based on a list of known antiepileptic drugs (Supplementary File 1). Number of antiepileptic drugs was meant to provide a very broad estimate of epilepsy severity in this context and was categorized as 2 vs 1 vs 0.

Statistical analysis

All data were entered into Excel XP worksheets (Microsoft Corp., Redmond, WA, USA) and analyzed using SAS version 9.3 (Cary, NC, USA) and Stata version 11 (StataCorp LP, College Station, TX, USA) software.

Clinical and demographic variables of interest were reported descriptively by their mean, median, and ranges as appropriate. Comparison between participant subgroups was made using a two-sample t-test, a Fisher's exact test, or a test of two proportions as appropriate. Missing data were considered to be missing completely at random. No imputation procedures occurred.

Logistic regression models were constructed using a priori assumptions of the relevance of gathered clinical and demographic variables on the possible association of having neurocysticercosis and their clinical impact for future patient populations in lower income settings. The outcome was dichotomized as either having diagnosed neurocysticercosis (probable or definite) or not having neurocysticercosis. Models were constructed using exploratory analyses with each possibly associated variable reported alone, then age-adjusted, then in a model of basic demographic features, and finally in a model with combined demographic and medical care variables. Thus, the final logistic regression models included demographic features (participant's present age, sex, age at first seizure, residence in the urbanized capital city of Thimphu (vs not), and the stated occupation as a farmer (vs not). The additional medical care variables of exploratory interest included the number of presently taken antiepileptic drugs and presence of mesial temporal sclerosis.

The 95% CIs and their associated point estimates are given for the ORs. A p-value of 0.05 or less was considered statistically significant and two-tailed probabilities were used. Goodness of fit of the logistic regression models was tested using the method of Hosmer-Lemeshow[21] and considered acceptable if the χ^2 value was >0.05.

Clinical follow up

Participants with definite neurocysticercosis (based on the criteria described above) and a positive Ag-ELISA test suggestive

of active infection were contacted and notified of their results. They were then evaluated by a neurologist or psychiatrist (VB, FJM, DKN) who acquired additional clinical history and determined if anti-parasitic treatment was indicated. Participants with *Echinococcus* spp. were also contacted by the research team and advised to seek evaluation. The recommendation to visit the local allopathic health provider or return to the JDWNR Hospital to meet with the study's physicians were both provided. The ultimate treatment decision was deferred to the clinician performing the assessment. If indicated, treatment with albendazole, the only available antiparasitic medication in Bhutan, was provided free of charge through the Royal Government.

Ethics approval

The study was reviewed and approved by the Research Ethics Board, convened by the Royal Bhutanese Ministry of Health, as well as the Partners Healthcare and University of Ottawa Institutional Review Boards. Informed written consent was obtained from all participants or their next of kin proxies.

Results

Demographic characteristics

The final analysis included 205 participants, excluding 63 who did not undergo either brain MRI or EITB, almost always for logistical reasons, such as lack of transportation, scheduling issues, or difficulty reaching participants by phone. A comparison of participants who completed both the brain MRI and EITB with those who were excluded due to inability to complete both tests is provided in Supplementary File 2. The two groups were comparable with the exceptions of more women and more patients with awareness of brain infection as cause of seizures did not complete both tests. Participants resided in all of the 20 geopolitical districts of Bhutan.

A total of 205 participants had both EITB and brain MRI performed (Table 1). The average age of participants was 25.1 years and 118/205 (57.6%) were female. Most participants described themselves as 'unemployed' 57/205 (27.8%), and 22/205 (10.7%) described their employment as farming. There were 20/205 (9.8%) participants from the urban region of Thimphu. The other 185/205 (90.2%) of participants were from areas throughout Bhutan that are less urban than Thimphu. All participants reported seizures and 188/205 (91.7%) were presently taking at least one antiepileptic drug. Forty participants (40/205, 19.5%) reported a history of neurologic infection, but only 6/205 (2.9%) reported an infection caused by neurocysticercosis or a parasite. Just half of self-reported neurocysticercosis participants (3/6, 50.0%) had either MRI or EITB consistent with neurocysticercosis at the time of study participation. These three participants with positive diagnostic studies for neurocysticercosis and self-reported neurocysticercosis were a participant with a single parenchymal, viable cyst and negative EITB; a participant with two parenchymal, calcified cysts and positive EITB; and a participant with at least seven parenchymal cysts in calcified, degenerating, and viable stages and positive EITB.

Prevalence of neurocysticercosis

There were 12 participants (n=1 <18 years old) with definite neurocysticercosis. An additional 40 participants (n=5 <18 years old) had probable neurocysticercosis. Definite and probable neurocysticercosis comprised 5.9% (95% CI 2, 9%) and 19.5% (95% CI 14, 25%) of the cohort of PWE, respectively. In combination, the proportion of PWE with definite and probable neurocysticercosis was 25% (95% CI, 19%, 31%). The tested associations of clinical and demographic factors with the diagnosis of combined probable and definite neurocysticercosis are given in Table 2.

Serum Ab-ELISA

A summary of the results of diagnostic tests is provided in Table 3. A total of 204 participants had the Ab-ELISA completed. There were 9/204 (4.4%) of PWE serologically positive for *T. solium* IgG on initial testing, with four of these nine positive cases positive for *Echinococcus* spp IgG on confirmatory testing. None of the participants with positive Ab-ELISA had findings on MRI suggestive of neurocysticercosis.

EITB

One or more bands was present on nitrocellulose paper in 37/205 (18.1%) of participants, with 33/37 (89%) samples showing more than one band. There was little overlap in the results of the EITB and Ab-ELISA testing, with only two participants demonstrating both a positive EITB and positive Ab-ELISA.

Antigen test

Serum Ag-ELISA was performed in the participants with positive EITB, and 10/37 (27%) had a positive Ag-ELISA, suggestive of infection with viable cysts (Table 4). Six participants with positive Ag-ELISA test returned for follow up: 1. a participant with one parenchymal, calcified cyst who was previously treated; 2. a participant with >10 cysts in viable and degenerating stages who had previously been treated with albendazole and steroids twice; 3. a participant who had positive EITB and antigen but no cysts on MRI; 4. a second participant who had positive EITB and antigen test but no cysts on MRI; 5. a participant who was previously treated with a congenital cyst on MRI but no evidence of neurocysticercosis; 6. and a participant with two parenchymal, calcified cysts who was previously treated with albendazole and steroids. Participants 3 and 4 were offered treatment with anti-parasitic medications after a review of their clinical history.

Neuroimaging

There were 27/205 (13.2%) participants with findings on MRI consistent with neurocysticercosis (Table 5). The majority of the participants had parenchymal lesions, although three participants had intraventricular lesions and one had a sulcal convexity subarachnoid cyst. There were no cases with basal subarachnoid involvement. Twenty-one participants had cyst-associated edema, with 18 showing minimal edema and three with severe edema. Other findings on brain MRI, as interpreted

Table 1. Characteristics of participants

	NCC negative n=153	NCC probable n=40	NCC definite n=12	Total n=205
Demographic				
Mean age, yrs (range)	23.8 (0.5–72.0)	27.8 (2.0–62.0)	32.6 (20.0–58.0)	25.1 (0.5–72.0)
Female (%)	88 (57.5)	22 (55.0)	8 (66.7)	118 (57.6)
Urban population (%)	13 (8.5)	5 (12.5)	2 (16.7)	20 (9.8)
Occupation (%)				
Business/Technology	18 (11.8)	5 (12.5)	4 (33.3)	27 (13.2)
Student	27 (17.7)	6 (15.0)	0 (0.0)	33 (16.1)
Farmer	13 (8.5)	6 (15.0)	3 (25.0)	22 (10.7)
Monk/Nun	6 (3.9)	2 (5.0)	1 (8.3)	9 (4.4)
Civil servant	8 (5.2)	5 (12.5)	0 (0)	13 (6.3)
Teacher	4 (2.6)	0 (0)	0 (0)	4 (2.0)
Healthcare	2 (1.3)	0 (0)	0 (0)	2 (1.0)
Unemployed	40 (26.1)	13 (32.5)	4 (33.3)	57 (27.8)
Missing	35 (22.9)	3 (7.5)	0 (0.0)	38 (18.5)
Clinical				
Mean age at first seizure, yrs (range)	14.4 (0.0–69.0)	16.7 (1.5–58.0)	18.2 (8.0–38.0)	15.1 (0–69.0)
Self-reported seizure frequency (no. seizures/month, %)				
0 seizures in past month	62 (40.5)	19 (47.5)	8 (66.7)	89 (43.4)
1–4 seizures/month	39 (25.5)	9 (22.5)	1 (8.3)	49 (23.9)
5–9 seizures/month	7 (4.6)	4 (10.0)	2 (16.7)	13 (6.3)
≥10 seizures/month	12 (7.8)	3 (7.5)	0 (0)	15 (7.3)
Missing	33 (21.6)	5 (12.5)	1 (8.3)	39 (19.0)
Timing of last seizure (%)				
Within the last week	42 (27.5)	9 (22.5)	1 (8.3)	52 (25.4)
Within the last month	44 (28.8)	14 (35.0)	2 (16.7)	60 (29.3)
Within the last year	25 (16.3)	5 (12.5)	3 (25.0)	33 (16.1)
>1 year ago	41 (26.8)	12 (30.0)	6 (50.0)	59 (28.8)
Missing	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.5)
On AEDs (%)	138 (90.2)	38 (95.0)	12 (100.0)	188 (91.7)
On more than one AED (%)	54 (35.3)	13 (32.5)	3 (25.0)	70 (34.2)
Mean number of AEDs (range)	1.4 (0.0–5.0)	1.4 (0.0–3.0)	1.3 (1.0–2.0)	1.4 (0.0–5.0)
Infection history				
Self-reported history of brain infection (%)	25 (16.3)	9 (22.5)	6 (50.0)	40 (19.5)
History of neurocysticercosis (%)	3 (2.0)	1 (2.5)	2 (16.7)	6 (2.9)
Awareness that infections can cause seizures (%)	71 (46.4)	21 (52.5)	5 (41.7)	97 (47.3)

AED: antiepileptic drug; NCC: neurocysticercosis

by a neuroradiologist, included congenital cysts or cortical dysplasia in 23 participants (11.2%) and evidence of prior stroke in 9 participants (4.4%). Nodular/calcified cysts were the most frequent cyst stage seen. All participants with actively degenerating cysts on neuroimaging had positive EITB serology (Table 4).

Most participants with MRI evidence of neurocysticercosis had few cysts, with an average of 1.2 cysts (SD 0.48, median 1.0). All participants with ≥1 cysts on brain MRI (9/9 participants) had positive EITB serology. A majority of participants with multiple cysts (7/9) had positive Ag-ELISA. Positive Ag-ELISA was seen in participants who collectively had cysts in all stages; however, proportionally more participants with cysts in viable (2/7, 29%) or degenerating stages (3/4, 75%) compared to nodular/calcified stage (5/21, 24%) had positive Ag-ELISA, but this result did not reach statistical significance (p=0.251).

The logistic regression models are shown in Table 2 and included all participants. In the full multivariable analysis, only present age (OR 1.05, 95% CI 1.01,1.09, p=0.025) was positively associated with (combined probable or definite) neurocysticercosis while the presence of mesial temporal sclerosis on MRI (OR 0.294, 95% CI 0.144, 0.598, p=0.001) was negatively associated. No statistically significant association with occupational farming, age at first seizure, sex, number of antiepileptic medications, educational attainment level, or urban residence was found.

Table 2. Association of clinical and demographic variables with having neurocysticercosis versus not[a] in people with epilepsy in Bhutan

	Univariate	p-value	Age-adjusted	p-value	Model 1	p-value	Model 2	p-value	Model 3	p-value
	OR		OR		OR (95% CI)		OR (95% CI)		OR (95% CI)	
Present age	1.03	0.007	NA	NA	1.02 (0.997, 1.07)	NS	1.05 (1.01, 1.09)	0.025	1.04 (0.999, 1.08)	NS
Sex	0.995	NS	1.00	NS	1.02 (0.562, 1.87)	NS	1.29 (0.645, 2.57)	NS	NA	NA
Age at first seizure (continuous)	1.02	0.045	0.993	NS	0.989 (0.950, 1.03)	NS	0.965 (0.921, 1.01)	NS	0.968 (0.925, 1.01)	NS
Resides in Thimphu, yes vs no	0.719	NS	0.836	NS	0.963 (0.358, 2.59)	NS	0.833 (0.230, 2.48)	NS	NA	NA
Educational level[b]	1.16	NS	1.15	NS	1.22 (0.961, 1.55)	NS	1.19 (0.906, 1.58)	NS	1.23 (0.931, 1.63)	NS
Farmer, yes vs no	2.28	NS	1.77	NS	2.06 (0.813, 5.20)	NS	1.52 (0.535, 4.30)	NS	1.63 (0.565, 4.69)	NS
No. of AEDs (2 vs 1 vs 0)	1.21	NS	1.21	NS	NA	NA	1.58 (0.936, 2.65)	NS	NA	NA
Mesial temporal sclerosis on MRI brain, present vs not	0.296	<0.001	0.308	0.001	NA	NA	0.294 (0.144,0.598)	0.001	0.260 (0.124, 0.542)	<0.001
Self-reported history of brain infection, present vs not	1.55	NS	1.57	NS	NA	NA	NA	NA	NA	NA
NCC by history, present vs not	1.55	0.029	1.46	NS	NA	NA	NA	NA	2.01 (1.24, 3.24)	0.004

AED: antiepileptic drugs; NA: not applicable; NCC: neurocysticercosis; NS: not significant.
Model 1: Age, age at first seizure, sex, urban residence in Thimphu, educational level, farmer. (goodness of fit, χ^2, p=0.412).
Model 2: Also including mesial temporal sclerosis, no. of AEDs (goodness of fit, χ^2, p=0.351).
Model 3: Statistically defined model that includes only variables that had a p-value of <0.20 in univariate OR association testing.
[a] Neurocysticercosis was defined as probable or definite versus not (goodness of fit, χ^2, p=0.265).
[b] None (31% of participants), primary school (31%) or school for disability (1%), secondary school (13%), high school (15%), or college (9%).

Table 3. Number of neurocysticercosis-positive cases by diagnostic test (n=205)

Diagnostic test used	Number positive for NCC
MRI brain only	27
EITB only	9
Ab-ELISA only	37
MRI+EITB	12
MRI+Ab-ELISA	0
EITB+Ab-ELISA	2

Ab-ELISA: enzyme-linked immunosorbent assay; EITB: enzyme-linked immunoelectrotransfer blot; NCC: neurocysticercosis.

Table 4. Correlation of cyst findings on MRI with positive EITB or Ag-ELISA serology

MRI findings	EITB-positive cases	Antigen positive cases
Cyst stage[a]		
Viable (n=7)	3/7 (43%)	2/7 (29%)
Single cyst present	1/5 (20%)	0/5 (0)
Multiple cysts	2/2 (100%)	2/2 (100%)
Degenerating (n=4)	4/4 (100%)	3/4 (75%)
Single cyst present	None	None
Multiple cysts present	4/4 (100%)	3/4 (75%)
Nodular/calcified (n=21)	10/21 (48%)	5/21 (24%)
Single cyst present	7/18 (39%)	3/18 (17%)
Multiple cysts	3/3 (100%)	2/3 (67%)
Number of cysts		
Single cyst (n=23)	8/23 (35%)	3/23 (13%)
Multiple cysts (n=9)	9/9 (100%)	7/9 (78%)
Location of cysts[b]		
Parenchymal (n=26)	12/26 (46%)	6/26 (23%)
Extraparenchymal[c] (n=3)	2/3 (67%)	2/3 (67%)

Ag-ELISA: antigen ELISA; EITB: enzyme-linked immunoelectrotransfer blot.
[a] Some patients' cysts were classified as being in more than one stage.
[b] Some participants had cysts in more than one location.
[c] Extraparenchymal includes intraventricular and subarachnoid cases.

Discussion

We provide evidence of a significant burden of neurocysticercosis among a cohort of PWE in Bhutan. Neurocysticercosis was present in 6% of PWE and possibly up to 25% of PWE. While this cohort is not generalizable to the country's entire population, our study provides the first estimate of the prevalence of neurocysticercosis among PWE in Bhutan, a region thought to be endemic for *T. solium*. By incorporating multiple modalities of diagnostic tests we report the overlapping nature of the test results, including potentially false positive tests, cross-reactivity with *Echinococcus* spp., and the clinical value of brain MRI. We also confirm the high sensitivity of the EITB. Although there are limitations to the use of a referral-based cohort, given that the recruitment site is at the National Referral Hospital—the sole referral center in Bhutan—and received participants from every geographic district, our study provides a reasonable first estimation of neurocysticercosis in PWE in Bhutan.

The prevalence of neurocysticercosis reported here is consistent with a prior report of the prevalence of antibodies to *T. solium* in Bhutanese refugees in the USA[11] although the latter did not report whether refugees had a history of seizures or diagnosed epilepsy. Comparison of our reported neurocysticercosis prevalence in Bhutan to other studies from nearby locations is difficult. Other reports demonstrate variations in study population, participant selection, study design, and diagnostic testing.

There were two statistically significant associations between neurocysticercosis and the clinical and demographic variables explored on multivariate logistic regression models. The association of neurocysticercosis with older age in our multivariable analysis likely represents the increased potential for exposure to *T. solium* with time. Farming among PWE did not show a statistically significant association with neurocysticercosis in our study. While this may be due to insufficient sample size of pig farmers, it also reflects the current understanding of neurocysticercosis transmission, i.e., that neurocysticercosis is transmitted person-to-person via infected tapeworm carriers rather than via direct exposure to animals carrying *T. solium*.[22,23]

Given that all participants in our cohort had a clinical diagnosis of epilepsy, it is not surprising that nearly all of our definite and probable neurocysticercosis participants had lesions that are parenchymal. Subarachnoid and intraventricular neurocysticercosis are more likely to present with headache and hydrocephalus as opposed to seizures.[1] Expanded inclusion criteria to include people with these symptoms, instead of seizures, would almost certainly reveal additional cases of neurocysticercosis in Bhutan.

Our study highlights important aspects of the diagnosis of neurocysticercosis. The serum Ab-ELISA used here was not an accurate marker for neurocysticercosis, with poor sensitivity and specificity for the presence of cysts on MRI as well as false positive results, suggesting alternative parasitic infections such as *Ecchinococcus spp*. This is consistent with reports from prior studies.[15,24] Ab-ELISA could be improved in sensitivity if performed in the cerebrospinal fluid, although this test is still frequently negative in patients with few cysts or calcified disease.[25,26] EITB testing had a much higher overlap with the MRI results than ELISA, with concurrent findings in 6% of PWE, i.e., the neurocysticercosis-definite cases. Notably, a significant number of participants with neurocysticercosis findings on MRI were not seropositive on EITB. This may be due to the lower sensitivity of EITB in parenchymal neurocysticercosis with few cysts or calcified cysticerci.[27] PWE with a higher cyst burden and with cysts in actively degenerating stages frequently had

Table 5. Neuroimaging features of epilepsy cohort (n=205)

	NCC negative n=153	NCC probable n=40	NCC definite n=12
MRI findings of NCC (%)	NA	15 (37.5)	12 (100)
Location of cysts[a](%)	NA		
Parenchymal		15 (37.5)	11 (91.7)
Intraventricular		1 (2.5)	2 (16.7)
Subarachnoid[b]		0	1 (8.3)
Stage of cyst[c](%)	NA		
Nodular/calcified		11 (27.5)	10 (83.3)
Viable		4 (10.0)	3 (25.0)
Degenerating		0	4 (33.3)
Mesial temporal sclerosis (%)	93 (60.8)	12 (30.0)	4 (33.3)
Other structural abnormalities (%)	42 (27.5)	7 (17.5)	0 (0.0)

NA: not applicable; NCC: neurocysticercosis.
[a] Some participants had cysts in more than one location.
[b] Convexity subarachnoid only. No basal subarachnoid cases seen on imaging.
[c] Some cysts were transitioning from one stage to the next and are included in more than one designation.

positive EITB serology, although our sample size did not allow sufficient statistical power to make this assertion definitively. EITB may also demonstrate discordance with MRI due to involvement of cysticercosis in other areas of the body, prior *T. solium* exposure, or transient asymptomatic infection. Thus, EITB results must be interpreted in the context of a patient's full clinical picture.

Both MRI and CT have been used in the detection of neurocysticercosis, with advantages and disadvantages to both techniques. In our study, MRI was selected as the imaging modality due to the more frequent availability of MRI, minimization of radiation exposure, and ability to detect additional, subtle structural abnormalities of the brain that may be epileptogenic. MRI has several advantages over CT in neurocysticercosis, including superior detection of intraventricular cysts and detection of early edema in the absence of a clearly detectable cyst.[28] Additionally, use of MRI allowed for clearer delineation between congenital and other non-infectious cysts in this epilepsy cohort. MRI may not detect small, calcified cysticerci, potentially underestimating either the number of participants with parenchymal neurocysticercosis or the number of lesions per participant if many brain lesions were calcified. It is expected that mesial temporal sclerosis is not associated with the presence of neurocysticercosis as it is a distinct etiologic cause in PWE, although there is speculation that neurocysticercosis and MTS are interrelated in some patients.

Our study had several strengths. By prospectively utilizing several potentially diagnostic strategies, we provide a graded assessment of diagnostic certainty and compare findings between MRI, EITB, Ab-ELISA, and Ag-ELISA. Given that the JDWNRH is the sole referral center for epilepsy in Bhutan, we provide a first estimation of the prevalence of neurocysticercosis including people from all districts. Interpretation of MRIs by a neuroradiologist allowed for detailed analysis of cyst features which were compared to blood tests. We believe our study is unique in the region for the range of tests incorporated into the estimations provided.

There are also notable limitations. Given that our source population was PWE, participants with neurocysticercosis may have also had other potential causes of epilepsy aside from neurocysticercosis. Findings of neurocysticercosis on neuroimaging and testing do not automatically indicate epileptogenesis. Additionally, participants' responses in the clinical and demographic surveys may have limited reliability, particularly in lower literacy populations. Despite the use of Dzhongka translators (LT, SD), quality control of surveys through dedicated data managers, and follow up calls to participants for clarification of missing or inconsistent responses, the reporting of specific variables such as history of a brain infection by participants led to unclear implications with inability to corroborate data, making them likely unreliable. Similarly, Bhutanese participants' responses to the question about their location of residence alternated between the place of birth and their current area of residence, depending on the participant's interpretation. Participants with transient illness without chronic epilepsy, asymptomatic participants, and participants with hydrocephalus from subarachnoid neurocysticercosis would have been overlooked due to the lack of inclusion in the cohort. Prior anti-parasitic treatment was not assessed in this study, which may have affected the outcome of serum and imaging results. Additionally, serum testing for Ab-ELISA, EITB, and Ag-ELISA, unlike MRI, does not indicate site of infection, and thus may reflect cysts in other locations in the body aside from the brain. Lastly, participants who did not undergo both an MRI and EITB were excluded from analysis. As a result, some participants with one positive test who were missing data were excluded from the analysis.

Conclusions

Neurocysticercosis is an important and likely leading cause of preventable epilepsy in Bhutan. Efforts to curtail *T. solium* transmission at a community level may help to reduce the incidence of epilepsy.[29] Our results also demonstrate diagnostic approaches utilizing serum Ab-ELISA are not helpful in establishing an accurate neurocysticercosis diagnosis and should be abandoned in favor of a combination diagnostic strategy using neuroimaging and EITB in PWE in endemic regions.

Supplementary data

Supplementary data are available at Transactions online (http://trstmh.oxfordjournals.org/).

Authors' contributions: Study conception and design: FJM, DKN, KTB; acquisition of data: SP, TT, LT, SD, DN, VB, DKN; analysis and interpretation of data: MBD, JPK, HHG, KTB, PD, TT, SP, FJM; drafting of manuscript: KTB; critical revision: FJM, MBD, HHG, KTB. All authors read and approved the final manuscript. FJM is guarantor of the paper.

Acknowledgements: We thank the Cysticercosis Working Group for their help in processing and analyzing the patient serum samples, particularly Javier Bustos and Yesenia Castillo for their help with sample management and assay processing. We thank Dr Chencho Dorji, Psychiatrist, at the JDWNRH for his efforts to help establish the Bhutan Epilepsy Project and Sarah J. Clark, BSc for data management.

Funding: This work was supported by Grand Challenges Canada, funded by the Government of Canada (338-04); Partners Center for Expertise in Global Health Travel Grant; Massachusetts General Hospital Center for Global Health Travel Grant.

Competing interests: None declared

Ethical approval: The study was reviewed and approved by the Research Ethics Board, convened by the Royal Bhutanese Ministry of Health, as well as the Partners Healthcare and University of Ottawa Institutional Review Boards.

References

1 Del Brutto O. Neurocysticercosis. In: Biller J, Ferro JM (eds) Handbook of Clinical Neurology: Vol 121: Neurologic Aspects of Systemic Disease Part III. 2014, p. 1445–59.

2 Garcia HH, Pretell EJ, Gilman RH et al. A trial of antiparasitic treatment to reduce the rate of seizures due to cerebral cysticercosis. N Engl J Med 2004;350:249–58.

3 Carabin H, Traore AA. *Taenia solium* taeniasis and cysticercosis control and elimination through community-based interventions. Curr Trop Med Rep 2014;1:181–93.

4 Bhutan Health Management Information System. Annual Health Bulletin 2013. Thimphu: Ministry of Health; 2013. ISBN: 978-99936-919-0-7

5 O'Neal SE, Townes JM, Wilkins PP et al. Seroprevalence of Antibodies against *Taenia solium* cysticerci among refugees resettled in United States. Emerg Infect Dis 2012;18:431–8.

6 Kafle DR, Oli KK. Clinical profile of patients with recurrent seizure in tertiary care hospital in Nepal. Kathmandu Univ Med J 2014;12:202–6.

7 Ojha R, Shah DB, Shrestha A et al. Neurocysticercosis in Nepal: a retrospective clinical analysis. Neuroimmunol Neuroinflamm 2015;2:167–70.

8 Adhikare S, Sathian B, Koirala DP, Rao KS. Profile of children admitted with seizures in a tertiary care hospital of Western Nepal. BMC Pediatr 2013;13:43.

9 Poudel P, Parakh P, Mehta K. Clinical profile, aetiology and outcome of afebrile seizures in children. J Nepal Med Assoc 2013;52:260–6.

10 Prasad KN, Verma A, Srivastava S et al. An epidemiological study of asymptomatic neurocysticercosis in a pig farming community in northern India. Trans R Soc Trop Med Hyg 2011;105:531–6.

11 Prasad KN, Prasad A, Gupta RK et al. Neurocysticercosis in patients with active epilepsy from the pig farming community of Lucknow district, north India. Trans R Soc Trop Med Hyg 2009;103:144–50.

12 Garcia HH, Rodriguez S, Gilman RH et al. Neurocysticercosis: is serology useful in the absence of brain imaging? Trop Med Int Health 2012;17:1014–8.

13 Moyano LM, Saito M, Montano SM et al. Neurocysticercosis as a cause of epilepsy and seizures in two community-based studies in a cysticercosis-endemic region in Peru. PLoS Negl Trop Dis 2014;8:e2692.

14 Tsang VC, Brand JA, Boyer AE. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). J Infect Dis 1989;159:50–9.

15 Gekeler F, Eichenlaub S, Mendoza EG et al. Sensitivity and specificity of ELISA and immunoblot for diagnosing neurocysticercosis. Eur J Clin Micobiol Infect Dis 2002;21;227–9.

16 Fleury A, Garcia E, Hernandez M et al. Neurocysticercosis: HP10 antigen detection is useful for the follow-up of the severe patients. PLoS Negl Trop Dis 2013;7:e2096.

17 Dorny P, Phiri IK, Vercuysse J et al. A Bayesian approach for estimating values for prevalence and diagnostic test characteristics of procine cysticercosis. Int J Parasitol 2004;34:569–76.

18 Garcia, HH, Del Brutto, OH. Imaging findings in neurocysticercosis. Acta Trop. 2003;87:71–8.

19 Brizzi, K, Deki, S, Tshering, L et al. Knowledge, attitudes and practices regarding epilepsy in the Kingdom of Bhutan. Int Health 2016;8:286–91.

20 Del Brutto OH. Diagnostic criteria for neurocysticercosis, revisited. Pathog Glob Health 2012;106:299–304.

21 Hosmer DW, Hosmer T, Le Cessie S, Lemeshow S. A comparison of goodness-of-fit tests for the logistic regression model. Stat Med 1997;16:965–80.

22 Garcia HH, Nash TE, Del Brutto OH. Clinicial symptoms, diagnosis, and treatment of neurocysticercosis. Lancet Neurol 2014;13:1202–15.

23 Del Brutto OH, Garcia HH. *Taenia solium* cysticercosis—The lessons of history. J Neurol Sci 2015;359:392–5.

24 Diaz JF, Verastegui M, Gilman RH et al. Immunodiagnosis of human cysticercosis (*Taenia solium*): a field comparison of an antibody-enzyme-linked immunosorbent assay (ELISA), an antigen-ELISA, and an enzyme-linked immunoelectrotransfer blot (ETIB) assay in Peru.

The Cysticercosis Working Group in Peru (CWG). Am J Trop Med Hyg 1992;46:610–5.

25 Michelet L, Fleury A, Sciutto E et al. Human neurocysticercosis: comparison of different diagnostic tests using cerebrospinal fluid. J Clin Microbiol 2011;49:195–200.

26 Rodriguez S, Wilkins P, Dorny P. Immunological and molecular diagnosis of cysticercosis. Pathog Glob Health 2012;106:286–98.

27 Singh G, Rajshekhar V, Murthy JM et al. A diagnostic and therapeutic scheme for a solitary cysticercus granuloma. Neurology 2010;75:2236–45.

28 Lerner A, Shiroishi MS, Zee CS et al. Imaging of neurocysticercosis. Neuroimag Clin N Am 2012;22:659–76.

29 Garcia HH, Gonzalez AE, Tsang VC et al. Elimination of *Taenia solium* transmission in Northern Peru. N Engl J Med 2016;374:2335–44.