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

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Abstract

Background—Neurocysticercosis (NCC) prevalence among people with epilepsy (PWE) has not been reported in Bhutan. We sought to provide an initial assessment of disease burden and evaluate the yield of diagnostic tests for *Taenia solium* among PWE in Bhutan.

Methods—We prospectively enrolled PWE at the referral hospital in Thimphu, Bhutan (2014–2015). Serum was tested for anti-*Taenia solium* IgG using enzyme-linked immunoassay (ELISA), enzyme-linked immunoelectrotransfer blot (EITB), and circulating parasite antigen. Results were compared to brain magnetic resonance imaging (MRI). Participants were categorized as definite (MRI and EITB positive), probable (MRI or EITB positive), or not having NCC.

Results—12/205 [6%, 95%CI: 2%, 9%] and 40/205 [20%, 95%CI 14%, 25%] participants met diagnostic criteria for definite and probable NCC. 25/205 [12%] with positive EITB did not have NCC on MRI, and 15/205 [7%] participants with positive MRI had negative EITB. Participants with NCC-suggestive lesions on MRI had an average of 1.2 cysts, predominantly parenchymal [26/27, 96%] and in the nodular/calcified stage [21/27, 78%]. People with NCC were more likely to be older (mean 23.8 years vs. 28.9 years, $p=0.01$), but no other statistically significant associations were identified.

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Conclusions—NCC is associated with 6% to 25% of epilepsy in a cohort from Bhutan. Diagnostic strategies combining EITB and MRI could aid diagnosis of NCC among PWE since neither test could identify all NCC cases.

Keywords

epilepsy; seizure; neurocysticercosis; magnetic resonance imaging; Bhutan; laboratory; immunoblot; enzyme-linked immunosorbent assay

Background

Neurocysticercosis (NCC) 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 and humans acting as intermediate hosts. Once it enters the central nervous system, the parasite, initially as a vesicular cyst, and upon degeneration, as a calcified cyst, can cause seizures, a condition persisting for years after the initial infection¹. Diagnosis of NCC can be challenging, and typically relies on clinical findings and neuroimaging among people living in endemic areas. Strong suspicion of NCC allows for the treatment of the parasite with anti-parasitic agents, which reduces seizure frequency in infected patients with viable cysts². Population-based efforts to prevent the initial infection can curtail *T. solium* infection and its neurologic sequelae³.

Awareness of epilepsy is growing in the Kingdom of Bhutan (population 720,679), with more than 1,215 epilepsy cases reported in 2014⁴. The prevalence of NCC and its etiologic relationship to seizures have not been previously reported in Bhutan. Geographically close areas, including Nepal and parts of northern India, have a high prevalence of NCC. In Nepal, studies of people with epilepsy (PWE) using neuroimaging have reported findings consistent with NCC in 16-32%^{5,6} of adults and 9-12% of children^{7,8}. In northern India, studies in pig farming communities report 15% of individuals had asymptomatic neuroimaging findings consistent with NCC, and 48% of PWE fulfilled criteria for definite or probable NCC^{9,10}. Given the high prevalence of NCC in surrounding regions, we postulated that infection with *T. solium* would be high among PWE in Bhutan and that a high proportion would have radiological and laboratory findings indicative of current or prior infection. To date, data related to NCC in Bhutan 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 as high as 22.8% but a >5 fold difference in prevalence depending on the location of origin (odds ratio (OR) 5.4, 1.2, 23.9)¹¹. As a preventable and treatable cause of seizures among PWE in Bhutan, an understanding of the contribution of NCC to the epilepsy burden is critical to guide future targeted preventative and therapeutic strategies.

Given the challenges inherent to making the diagnosis of NCC in any location, we also aimed to evaluate the yield of several diagnostic strategies in Bhutan to help inform future efforts to recognize this condition. Brain imaging is the standard to diagnose NCC¹². Enzyme-linked immunoelectrotransfer blot (EITB) is highly specific for cysticercosis and

seropositivity is associated with evidence of viable NCC on brain imaging in large cross-sectional studies^{13,14}. Serum enzyme-linked immunoassay (ELISA) is another available, inexpensive test, though prior studies have reported a high rate of false positive results in both endemic and non-endemic populations¹⁵. Antigen assays have also been used, and may be a means to monitor response to anti-parasitic treatment, particularly in patients with subarachnoid NCC¹⁶.

Methods

Ethics Approvals

The study was reviewed and approved by the Research Ethics Board, convened by the 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.

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 year³. Participants reporting 1 seizure were prospectively recruited using an existing registry of adults (>18 years old) and children (<1 to 18 years) with epilepsy at the JDWNRH. All participants were recruited between July 2014 and August 2015 and resided in districts across Bhutan.

Data Collection

A schematic 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 degree Celsius freezer for later processing. Samples were tested by on-site laboratory personnel for *T. solium* IgG using an Enzyme Linked Immunoassay (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, 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 antigens¹⁴ and antigen detection using a monoclonal antibody (B158/B60) based ELISA assay¹⁷ were performed. Samples were considered positive on EITB if one or more antibody bands were present, and positive for circulating antigen if the optic density of the sample (run in duplicate) was above a cut-off calculated using known negative sera.

Participants underwent brain magnetic resonance imaging (MRI; Siemens 1.5 Tesla) prospectively at the JDWNRH if they had not had brain imaging performed prior to January 1, 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. Brain MRI images were read by a neuroradiologist (JPK) at Brigham and Women's Hospital in Boston, U.S.A. who was unaware of the results of serum testing and confirmed with reports from a radiologist in Bhutan (DN) when available. MRI images determined to have cysts consistent with NCC were further classified by cyst stage (viable, degenerating, nodular/calcified)¹⁸, intracranial location of the cysts (parenchymal, intraventricular, subarachnoid), number of cysts, and the presence or absence of associated cystic edema.

Concurrently, participants completed questionnaires about their seizure history and perceptions of epilepsy, refined by the study investigators to be relevant to the local context¹⁹. Participants were asked about their understanding of the contribution of infections to seizures and their personal history of brain infections, including awareness of a diagnosis of NCC. Each participant was reimbursed 500 Bhutanese Ngultrum (~10 USD) for travel.

Data Analysis

Degree of certainty in the diagnosis of NCC was categorized by at least one neurologist (KTB) on previously published diagnostic criteria by Del Brutto *et al*²⁰ (Box 1). In our study, participants with imaging highly suggestive of NCC, positive serum antibodies on EITB and a history of seizures met criteria for *definite* NCC based on two major criteria and one minor criterion. Participants with either an MRI suggestive of NCC or positive EITB, all with a history of seizures, met criteria for *probable* NCC. Probable and definite NCC were considered mutually exclusive categories. The final analysis included 205 participants, excluding 63 who did not undergo either brain MRI or EITB, almost always for logistical purposes. There were no significant differences in clinic-demographic characteristics between those that completed the MRI and EITB and those that were excluded due to inability to complete testing. All data were entered into Excel XP worksheets (Microsoft) and analyzed using SAS 9.3 software (Cary, North Carolina, USA). Logistic regression models were constructed to assess the possible clinical and demographic associations that may exist with positive diagnostic test results. A *p*-value of 0.05 or less was considered statistically significant and two-tailed probabilities were used.

Clinical follow up

Participants with definite NCC (based on the criteria described above) and a positive antigen 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.

Results

Demographic Characteristics

205 participants had both and EITB and brain MRI performed (Table 1). The average age of participants was 25.1 years and 118/205 [58%] were female. Most participants described themselves as “unemployed” 57/205 [28%] and 22/205 [11%] described their employment

as farming. There were 20/205 [10%] participants from the urban region of Thimphu, population 111,306 [National Statistics Bureau, Bhutan, 2013]. All participants reported seizures and 188/205 [92%] were presently taking at least one antiepileptic drug. Forty participants [40/205, 20%] reported a history of neurologic infection, but only 6/205 [3%] reported an infection caused by NCC or a parasite. Just half of self-reported NCC-infected participants (3/6, 50%) had either MRI or EITB consistent with NCC at the time of study participation. These three participants with positive diagnostic studies for NCC and self-reported NCC were 1) a participant with a single parenchymal, viable cyst and negative EITB, 2) a participant with two parenchymal, calcified cysts and positive EITB, 3) a participant with at least 7 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 NCC. An additional 40 participants ($n=5$ <18 years old) had probable NCC. Definite and probable NCC 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 people with epilepsy with definite and probable NCC was 25% [95% CI, 19%, 31%]. NCC was significantly associated with older age [$p=0.01$], but not statistically significantly associated with sex, occupation, or location of residence in the capital city (Table 1).

Serum ELISA

There were 9/205 [4%] of PWE serologically positive for *T. solium* IgG on initial testing, with 4/9 of these positive cases positive for *Echinococcus* spp IgG on confirmatory testing. None of the participants with positive ELISA had findings on MRI suggestive of NCC.

EITB

One or more bands was present on nitrocellulose paper in 37/205 [18%] of participants, with 33/37 [89%] samples showing more than one band. There was little overlap in the results of the EITB and ELISA testing, with only two participants demonstrating both a positive EITB and positive ELISA.

Antigen test

Serum cysticercosis antigen testing was performed in the participants with positive EITB, and 10/37 [27%] had a positive antigen test, suggestive of infection with viable cyst(s) (Table 3). Six participants with positive antigen test returned for follow up: 1) a participant with 1 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 NCC 6) and a participant with 2 parenchymal, calcified cysts who was previously treated with albendazole and steroids. Participants 3) and 4) were offered treatment with anti-parasitics after review of clinical history.

Neuroimaging

There were 27/205 [13.2%] participants with findings on MRI consistent with NCC infection (Table 2). 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 3 with severe edema. Several patients were found to have congenital cysts upon neuroradiologist review that were not consistent with NCC. Nodular/calcified cysts were the most frequent cyst stage seen. All participants with actively degenerating cysts on neuroimaging had positive EITB serology (Table 3).

Most participants with MRI evidence of NCC had few cysts, with an average of 1.2 cysts [standard deviation 0.48, median 1.0]. Participants with 1 cyst on brain MRI were more likely to have positive EITB serology and more likely to have positive cysticercosis antigen, but these associations also did not reach statistical significance. Positive cysticercosis antigen 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 cysticercosis antigen, but this result did not reach statistical significance. Participants with multiple cysts were more likely to have positive cysticercosis antigen than participants with a single cyst, but this result also did not reach statistical significance.

There was a statistically significantly lower number of participants with mesial temporal sclerosis when comparing PWE with definite or probable NCC versus those without NCC ($p=0.0002$).

Discussion

We provide evidence of a significant burden of NCC among a cohort of PWE in Bhutan. NCC was present in 6% of PWE and possibly up to 25% of this Bhutanese cohort. While we recognize that there are limitations to the generalizability of this cohort to the country's entire population, this study provides the first attempted estimate of the prevalence of NCC among PWE in Bhutan, a region thought to be endemic for *Taenia solium*. By incorporating multiple modalities of diagnostic tests we report the overlapping nature of test results, including potentially false positive tests, cross-reactivity with *Echinococcus* spp., the clinical value of MRI, and confirm the high sensitivity of the EITB. Our participants resided in all 20 geopolitical districts of Bhutan. Although there are limitations to our use of a referral-based cohort, given that the recruitment site at the National Referral Hospital represents the sole referral center in Bhutan, our study provides a reasonable first estimation.

The prevalence of NCC reported here is consistent with a prior report of the prevalence of antibodies to *T. solium* in Bhutanese refugees in the USA¹¹ although the latter did not report whether refugees had a history of seizures or diagnosed epilepsy. It is in some ways unclear if our reported prevalence of NCC in PWE in Bhutan reflects the NCC prevalence reported in other nearby locations. Variations in study population, participant selection, study design, and diagnostic testing limit the ability to directly compare these studies.

There was no association between NCC and any of the seizure or demographic variables collected analyzed, with the exception of older age. The association of NCC with older age is likely related to the increased potential for exposure with time. Possible occupational exposure to pigs (i.e. farmers) among those with epilepsy did not show a statistically significant association with NCC in our study. While this may be due to insufficient sample size, it also reflects the current understanding of NCC transmission, i.e. that NCC is transmitted person-to-person via infected tapeworm carriers rather than via direct exposure to animals carrying *T. solium*^{21,22}.

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 NCC participants had lesions which were parenchymal. Subarachnoid and intraventricular NCC are more likely to present with headache and hydrocephalus as opposed to seizures¹. Expanded inclusion criteria to include people with these symptoms, instead of seizures, would almost certainly reveal additional cases of NCC in Bhutan.

Our study highlights important aspects of the diagnosis of NCC. The serum ELISA used here was not an accurate marker for NCC, 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^{15,23}. 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^{24,25}. EITB testing had a much higher overlap with the MRI results than ELISA, with concurrent findings in 6% of PWE, i.e. the NCC-definite cases. Notably, there remained a significant number of participants with NCC findings on MRI that were not seropositive on EITB. This may be due to the lower sensitivity of EITB in parenchymal NCC with few cysts or calcified cysticerci²⁶. PWE with a higher cyst burden and cysts in actively degenerating stages appeared more likely to have positive EITB serology, although low numbers of these cases 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 NCC, 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 NCC, including superior detection of intraventricular cysts and detection of early edema in the absence of a clearly detectable cyst²⁷. 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, and as a result, this study may underestimate the number of participants with parenchymal NCC or the number of lesions per participant if many brain lesions are calcified. The use of susceptibility-weighted sequences on MRI can improve sensitivity of

the detection of small calcified cysticerci²⁸, but these sequences were not consistently acquired here.

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, ELISA, and antigen testing. Given that the JDW National Referral Hospital is the sole referral center for epilepsy in Bhutan, we provide a first estimation of the prevalence of NCC 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. First, participants' responses in the clinical and demographic surveys may have limited reliability. Despite the use of a Dzhongka translator and quality control of surveys, reporting of seizure frequency by participants was not always reliable. Similarly, Bhutanese participants' responses to the question about their location of residence alternated between place of birth and their current area of residence, depending on the participant. Secondly, prior anti-parasitic treatment was not assessed in this study, which may have affected the outcome of serum and imaging results. Third, participants who did not have both an MRI and EITB completed were excluded from analysis. As a result, some patients with one positive test who were missing data were excluded from analysis.

We report NCC 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. Taken together, our results also demonstrate diagnostic approaches utilizing serum ELISA are not helpful in establishing an accurate NCC diagnosis and should be abandoned in favor of a combination diagnostic strategy using neuroimaging and EITB in PWE in endemic regions.

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Box 1**Relevant diagnostic criteria for neurocysticercosis from Del Brutto et al²⁰.****Degrees of diagnostic certainty**

Definitive criteria:

- 1) Lesions consistent with NCC on neuroimaging studies
- 2) Positive serum immunoblot
- 3) Presence of clinical manifestations of NCC (seizures)
- 4) Endemic region

Probable criteria:

- 1) Lesions consistent with NCC on neuroimaging
OR
Positive serum immunoblot
- 2) Presence of clinical manifestations of NCC (seizures)
- 3) Endemic region

Figure 1.
Schematic of testing

Table 1
Characteristics of Participants

	NCC negative n=153	NCC probable n=40	NCC definite n=12	Total n=205
<i>Demographic</i>				
Mean age, yrs (range)	23.8 (71.5)	27.8 (60.0)	32.6 (38.0)	25.1 (71.5)
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]	38 [18.5]
<i>Clinical</i>				
Mean age at first seizure, yrs (range)	14.4 (69.0)	16.7 (56.5)	18.2 (30.0)	15.1 (69.0)
Self-reported seizure frequency [# 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]
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]
Greater than 1 year ago	41 [26.8]	12 [30.0]	6 [50.0]	59 [28.8]
On antiepileptic drugs (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 (5.0)	1.4 (3.0)	1.3 (1.0)	1.4 (5.0)
<i>Infection History</i>				
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]

Table 2
Neuroimaging Features of Epilepsy Cohort (n=205)

	NCC negative n=153	NCC probable n=40	NCC definite n=12
MRI findings of NCC [%]	N/A	15 [37.5]	12 [100.0]
Location of cysts ¹ [%]	N/A		
Parenchymal		15 [37.5]	11 [91.7]
Intraventricular		1 [2.5]	2 [16.7]
Subarachnoid ²		0 [0.0]	1 [8.3]
Stage of cyst ³ [%]	N/A		
Nodular/calcified		11 [27.5]	10 [83.3]
Viable		4 [10.0]	3 [25.0]
Degenerating		0 [0.0]	4 [33.3]
Hippocampal atrophy [%]	96 [62.8]	12 [30.0]	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]

¹ Some participants had cysts in more than one location

² Convexity subarachnoid only; no basal subarachnoid cases seen on imaging

³ Some cysts were transitioning from one stage to the next and are included in more than one designation

Table 3
Distribution of cases by MRI findings and serology

	EITB-positive	Antigen positive
Cyst Stage¹		
<i>Viable</i>	3/7 (43%)	2/7 (29%)
Single cyst present	1/5 (20%)	0/5 (0%)
Multiple cysts	2/2 (100%)	2/2 (100%)
<i>Degenerating</i>	4/4 (100%)	3/4 (75%)
Single cyst present	None	None
Multiple cysts present	4/4 (100%)	3/4 (75%)
<i>Nodular/calcified</i>	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	8/23 (35%)	3/23 (13%)
Multiple Cysts	9/9 (100%)	7/9 (78%)
Location of Cysts²		
Parenchymal	12/26 (46%)	6 (23%)
Extraparenchymal ³	2/3 (67%)	2/3 (67%)

¹ Some cysts were classified as more than one stage

² Some participants had cysts in more than one location

³ Extraparenchymal includes intraventricular and subarachnoid cases