

Neurocysticercosis and epilepsy in Cameroon

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Abstract

During January 2002 the frequency of *Taenia solium* cysticercosis was studied in a series of 504 epileptic patients from 3 rural localities in the West and North-West provinces of Cameroon using an enzyme-linked immunosorbent assay for both circulating antigen (Ag-ELISA) and antibody (Ab-ELISA) detection. *Taenia solium* antigens were detected in the sera of 1.2% of the patients whereas specific antibodies against the parasite were present in 44.6% of the patients. Significantly less seropositive results in Ab-ELISA were recorded in Batibo than in Bandjoun and Bamendjou, whereas a borderline significant difference was recorded with increasing age. Furthermore, 56.2% of patients with late-onset epilepsy showed antibodies against cysticercosis. *Taenia solium* cysticercosis appears to be an important cause of epilepsy in Cameroon.

Keywords: cysticercosis, *Taenia solium*, epilepsy, enzyme-linked immunosorbent assay, Cameroon

Introduction

Epilepsy is a major problem in tropical developing countries (de Bittencourt *et al.*, 1996). The incidence and prevalence of the disorder in these countries are high because of poor standards of neonatal care and high rates of infectious and parasitic diseases (Senayake & Roman, 1993). Among parasitic infections, neurocysticercosis, an infection of the central nervous system by *Taenia solium* larvae, has been reported as a major cause of epilepsy in many Latin American and African countries (Dumas *et al.*, 1990; Van As & Joubert, 1991; Del Brutto *et al.*, 1992; Garcia *et al.*, 1993; Garcia-Noval *et al.*, 2001).

The prevalence of active epilepsy in tropical countries as a whole is between 10 and 15 per 1000 inhabitants (ILAE, 1994). According to the African Declaration on Epilepsy adopted during the Dakar conference (5 and 6 May 2000), epilepsy is the most common serious chronic brain disorder, estimated to affect at least 50 million people in the world of whom 10 million live in Africa alone (WHO, 2000). Studies in Africa have shown that the prevalence of epilepsy on the continent ranges between 15 and 25 per 1000 and may be as high as 40 per 1000 in some regions (Preux *et al.*, 2000).

Although epilepsy is known as a frequent condition in Cameroon, data on its prevalence and aetiological factors are unavailable. Given the fact that *T. solium* is endemic in the western highland region of the country (Nguekam *et al.*, 2003b), we studied the frequency of cysticercosis in epileptic patients attending health centres in the region using 2 serological tests (enzyme-linked immunosorbent assay for antigen [Ag-ELISA] and for antibody [Ab-ELISA] detection).

Materials and Methods

Study area and patients

In the rural localities of Batibo (North-West Province), Bamendjou and Bandjoun (West Province), epilepsy is known to be a serious health problem. Epileptic patients attending health centres in the 3 localities were informed about the study by the medical authorities and, after this information campaign, the study was carried out in the first half of January 2002.

During consultation, the patients were clinically examined. A history of epilepsy was obtained from each patient or from the person who accompanied them (in

the case of young patients or those with mental illness) and a form was completed (name, gender, age, village, year of first seizure, and relative frequency of seizures). A patient was considered as epileptic when they fulfilled the epilepsy case definition of the International League against Epilepsy (ILAE, 1993), i.e. 2 or more epileptic seizures occurring more than 24 h apart and not post-partum or caused by fever, cranial trauma, or metabolic disorder. Burns and injuries related to seizures were also inclusion criteria. After having obtained informed consent, blood samples were collected and the serum was frozen at -20°C for further analysis.

Enzyme-linked immunosorbent assay for antibody detection

An Ab-ELISA using a recombinant antigen was carried out according to Sako *et al.* (2000). Recombinant *T. solium* antigens (1.0 $\mu\text{g}/\text{mL}$) were loaded on to 96-well microplates (Maxisorp, Nunc, Copenhagen, Denmark). Peroxidase-labelled goat anti-human immunoglobulin G (IgG) (H + L) (10967133, Zymed Laboratories, Inc., California, USA) was used as the secondary antibody in a dilution of 1:5000 and ABTS (2,2'-azino-di(3-ethylbenzothiazoline-6-sulphonate)) (KPL, Gaithersburg, MD, USA) was used as a peroxidase substrate (Sako *et al.*, 2000). Negative control sera were obtained from 8 uninfected people from the region of Dschang, Cameroon (Nguekam *et al.*, 2003b). Serum samples used as positive controls were from confirmed cysticercosis cases positive by immunoblot (Ito *et al.*, 1998). The cut-off point was established as the mean + 4 SD of the optical density (OD) values from the 8 uninfected controls. This corresponded to about 3 times the OD value of the pooled negative control sera.

Enzyme-linked immunosorbent assay for antigen detection

Serum samples were examined in duplicate using a monoclonal antibody-based ELISA for the detection of circulating *T. solium* antigen (Brandt *et al.*, 1992), slightly modified according to Nguekam *et al.* (2003b). Each ELISA run included 8 negative reference sera and 1 positive reference serum from the region of Dschang in Cameroon (Nguekam *et al.*, 2003b). The cut-off value was determined by comparing the OD of each sample with the mean of the series of 8 negative reference samples using a modified Student's *t* test (Sokal & Rohlf, 1981) at a probability level of $P = 0.001$.

Statistical analysis

Random effect logistic regression with the locality as the random effect was used to determine the significance of localities, gender, and age. In this way, possible clustering effects of the results within locality were

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allowed for. Analyses were conducted using Stata Statistical Software 7.0 (StataCorp, College Station, TX, USA) using the binary Ab-ELISA results as a response.

Ethical considerations

Due to the absence of an ethical review committee at the University of Dschang and at the District Hospital of Batibo, Cameroon, formal ethical clearance could not be given. However, the protocol of the trial was discussed in detail before the start of the study with all authors involved. Furthermore, all the epileptic subjects who were positive in the Ag-ELISA and might thus harbour living cysts were offered free treatment with albendazole.

Results

Based on the clinical history of each patient, 504 epileptic subjects from the 3 localities were included in the study. Their age ranged from 3 to 73 years with a mean of 20.0 ± 10.5 years. Data on the initial episode of epilepsy were available for only 460 patients, of whom 15.9% (73/460) had late-onset epilepsy (i.e. epilepsy after the age of 18 years). Burns and injuries due to epileptic crises were recorded in 11.3% of patients. Reliable information was not available to allow a classification into generalized or partial seizure types.

Antigens of *T. solium* metacestodes were detected in only 6 (1.2%) of the epileptic patients whereas antibodies against this parasite were present in 225 (44.6%) patients. Results of both Ag-ELISA and Ab-ELISA detection according to age, locality and gender are presented in the Figure and Tables 1 and 2. The Ab-ELISA results were significantly different between Batibo and the localities of Bamendjou and Bandjoun ($P < 0.001$) (Table 1). No significant differences in the Ab-ELISA were present between gender (Table 2) and with increasing age at onset of epilepsy (Table 3). However, a borderline significant difference ($P = 0.072$) was observed with increasing age (Figure).

Comparison of the results of the Ag-ELISA and Ab-ELISA showed that of 6 positive sera in the Ag-ELISA 3 tested positive and 3 tested negative in the Ab-ELISA.

Discussion

In this study, 504 epileptic patients were examined

Table 1. Frequency of seropositive results for cysticercosis by enzyme-linked immunosorbent assay for antigen (Ag-ELISA) and for antibody (Ab-ELISA) detection in epileptic patients according to locality, Cameroon, January 2002

Locality	No. examined	Ag-ELISA Positive (%)	Ab-ELISA Positive (%)
Batibo	345	4 (1.2)	121 (35.1)
Bamendjou	98	2 (2)	65 (66.3)
Bandjoun	61	0 (0)	39 (63.9)
Total	504	6 (1.2)	225 (44.6)

Table 2. Frequency of seropositive results for cysticercosis by enzyme-linked immunosorbent assay for antigen (Ag-ELISA) and for antibody (Ab-ELISA) detection in epileptic patients according to gender, Cameroon, January 2002

Gender	No. examined	Ag-ELISA Positive (%)	Ab-ELISA Positive (%)
Male	281	4 (1.4)	123 (43.8)
Female	223	2 (0.9)	102 (45.7)

Table 3. Frequency of seropositive results for cysticercosis by enzyme-linked immunosorbent assay for antigen (Ag-ELISA) and for antibody (Ab-ELISA) detection in epileptic patients according to the age at onset of epilepsy, Cameroon, January 2002

Age at onset (years)	No. examined	Ag-ELISA Positive (%)	Ab-ELISA Positive (%)
< 18	387	2 (0.5)	154 (39.8)
≥ 18	73	3 (4.1)	41 (56.2)
Unknown	44	1 (2.3)	30 (68.2)

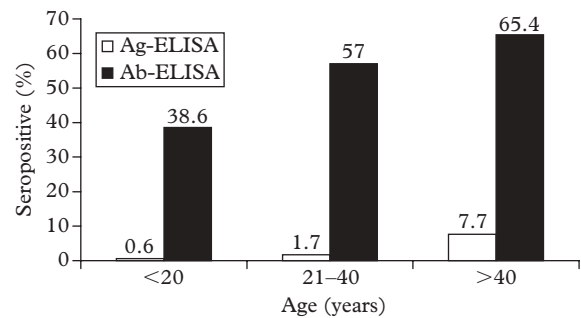


Figure. Frequency of cysticercosis according to enzyme-linked immunosorbent assay for antigen (Ag-ELISA) and for antibody (Ab-ELISA) detection in epileptic patients according to age, Cameroon, January 2002.

using an Ag-ELISA and an Ab-ELISA for the detection of *T. solium* cysticercosis. The results showed that 6 (1.2%) of the patients harboured circulating antigens, which strongly indicates that viable cysts are present in only a very small number of people. Using the Ag-ELISA a strong correlation has been shown to be present between living cysts and circulating antigen in cattle (Brandt *et al.*, 1992), pigs (Nguekam *et al.*, 2003a), and humans (Erhart *et al.*, 2002). In this group of epileptic subjects, cysticercosis was obviously mainly manifested by the presence of dying or dead cysticerci, since the Ab-ELISA detected 44.6% seropositive subjects among those examined. This confirms the observations of many different authors that dying and/or degenerated cysticerci are very common in epileptic patients with cysticercosis (Sotelo *et al.*, 1985; Garcia-Noval *et al.*, 1996; Nash *et al.*, 2001).

The Ab-ELISA using recombinant antigens, which was used in this study, has been shown to be highly sensitive (89.7%) and 100% specific (Sako *et al.*, 2000). However, the figure of 44.6% should be interpreted with caution, since it has been shown that transient antibodies against *T. solium* occur quite frequently (Garcia *et al.*, 2001). The latter authors did show that about 40% of seropositive people became seronegative when re-sampled after 1–3 years. This phenomenon, which was ascribed to the exposure to eggs of the parasite, which did not develop into a viable infection, might also occur in the study area, which is hyperendemic for *T. solium* (Pouedet *et al.*, 2002; Vondou *et al.*, 2002; Nguekam *et al.*, 2003b).

Taking into account this nuance, it can nevertheless be assumed that cysticercosis is clearly an important cause of epilepsy in this area of Cameroon. Although, unfortunately, no computed tomography (CT) scan could be performed to confirm the presence of parasites in the brain, this study confirms the previous observation by other authors, that neurocysticercosis is one of the most important causes of epilepsy in developing countries (de Bittencourt *et al.*, 1996; Carpio *et al.*, 1998). The frequency of epileptic subjects with

antibodies against *T. solium* cysticercosis is double the 22.3% reported using enzyme-linked immunoelectro-transfer blot (EITB) in Madagascar (Andriantsimahavandy *et al.*, 1997). It also exceeds the figures reported in northern Togo (29.5%) using an ELISA test (Dumas *et al.*, 1990) and those in Peru (12%; Garcia *et al.*, 1993) and Colombia (9.82%; Palacio *et al.*, 1998) using EITB.

About 16% of the patients involved in the study had late-onset epilepsy and 4.1% and 56.2% were positive in the Ag-ELISA and Ab-ELISA, respectively. Seropositivity increased—although with only borderline significance—with increasing age in both Ag-ELISA and Ab-ELISA (Figure). This is in agreement with the observations of Sarti *et al.* (1992, 1994) in community-based studies on taeniasis and cysticercosis in Mexico.

No significant difference was found between the genders in this study. This finding is in contrast with the observation by Cruz *et al.* (1999) who found a higher proportion of epileptic females with positive Ab-ELISA results and attributed it to food handling activities and their relationship with infection with cysticerci.

Three of 6 positive serum samples in Ag-ELISA tested negative in Ab-ELISA. This might be due to the sensitivity of this Ab-ELISA (89.7%) or to the presence of single cysts (Sako *et al.*, 2000). The latter authors reported that serum samples originating from neurocysticercosis patients harbouring a solitary cyst might escape detection. Negative results in ELISA or EITB in cases of single cyst infections have been reported by several authors (Wilson *et al.*, 1991; Lara-Aguilera *et al.*, 1992; Ito *et al.*, 1999; Ohsaki *et al.*, 1999). Another explanation for the discrepancy between the Ag-ELISA and Ab-ELISA results might be sought in the production method of the recombinant antigen. The chimeric antigen was produced in a bacterial system with lack of N-linked glycans. These carbohydrate structures are very immunogenic and contribute strongly to the humoral response of infected persons (Restrepo *et al.*, 2000; Obregon-Henao *et al.*, 2001). Patients whose immune response is mainly directed against the carbohydrates of *T. solium* might therefore escape detection in an Ab-ELISA using recombinant antigen.

On the basis of these results it can be concluded that *T. solium* cysticercosis is an important cause of epilepsy in the localities where the study was conducted. A particularly high frequency of antibodies against the parasite was detected in epileptic patients. Additional studies using both serological and imaging techniques are necessary to investigate the association between this neurological disorder and cysticercosis.

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