NEUROCYSTICERCOSIS AND EPILEPSY IN CAMEROON

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Abstract:

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 ELISA both for circulating antigen (Ag-Elisa) and antibody detection (Ab-Elisa). *T. solium* antigens were detected in the sera of 1.2% of the epileptics whereas specific antibodies against the parasite were present in 44.6% of patients. Significantly more seropositives in Ab-Elisa were recorded in Batibo than in Bandjoun and Batibo whereas a borderline significant difference was recorded with increasing age. Furthermore, 50% of patients with late-onset epilepsy showed antibodies against cysticercosis. *T. solium* cysticercosis appears to be an important cause of epilepsy in Cameroon.

Key-words: *Taenia solium*, cysticercosis, epilepsy, Cameroon, ELISA, circulating antigen, antibody

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 (Senanayake & Roman, 1993). Among parasitic infections, neurocysticercosis (NCC) which is 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 et 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 (International League Against Epilepsy, 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 which 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 thousand and may be as high as 40 per thousand in some regions (Preux et al., 2000).

Although epilepsy is known as a frequent condition in Cameroon, data on its prevalence and on the etiologic 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 of the region using two serological tests (Elisa for antigen and for antibody 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 as a serious health problem. Epileptic patients attending the health centres in the region were sensitised about the study by the medical authorities. After this information campaign, the study was carried out in the first half of January 2002 in the health centres of these localities.

During consultation, the patients were clinically examined. A history of epilepsy was obtained from each patient or from the person who accompanied him (in case of young or mentally handicapped patients) and a form was completed (Name, sex, age, village, year of first seizure, relative frequency of seizures). A patient was considered as epileptic when he fulfilled the epilepsy case definition of the International League against Epilepsy (1993), i.e. two 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 for further analysis.

Antibody detection Elisa (Ab-Elisa)

An antibody detection ELISA using a recombinant antigen was carried out according to Sako et al. (2000). Recombinant *T. solium* antigens (1.0 µg/ml) were loaded onto 96 well microplates (Maxisorp, Nunc, Copenhagen). Peroxidase labeled goat anti-human IgG (H+L) (10967133, Zymed Laboratories, Inc. California, USA) was used as the secondary antibody in a dilution of 1:5000. ABTS (2,2'-azino-di(3-ethyl-benzthiazoline-6-sulfonate)) (KPL, USA) was used as peroxidase substrate (Sako et al., 2000). Negative control sera from uninfected humans were obtained from 40 healthy people from a non-endemic area. Serum samples used as positive controls were from confirmed cysticercosis cases that were positive by immunoblot (Ito et al., 1999). The cut off point was established as the mean + 4 SD of the values from the 40 individuals. This corresponded to about three times the optical density of the pooled negative control sera.

Antigen detection Elisa (Ag-Elisa)

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 one reference positive serum from the region of Dschang in Cameroon (Nguekam et al., 2003b). The cut-off value was determined by comparing the optical density (OD) of each sample with the mean of a series of 8 negative reference samples using a modified Student test (Sokal & Rohlf, 1981) at a probability level of P=0.001.

Statistical analysis

Random Effect Logistic Regression with as random effect the locality was used to determine the significance of localities, sex and age. In this way possible clustering effects of the results within locality were allowed for. Analyses were conducted in Stata using the binary Ab-Elisa results as a response (Statacorp, 2001).

Results

Based on the clinical history of each patient, 504 epileptics 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 only for 460 patients, of which only 4.3% had late-onset epilepsy (i.e. epilepsy after the age of 30 years as defined by Palacio et al., 1998). Burns and injuries due to epileptic crises were recorded in 11.3% of patients. Reliable information was not available in order to allow a classification into generalised or partial seizure types.

Table 1: Frequency of seropositives for cysticercosis (Ag and Ab-ELISA) according to locality

		Ag-ELISA		**Ab-ELISA**	
Localities	**No. examined**	Positives	%	Positives	%
Batibo	345	4	1,2	121	34,8
Banendjou	98	2	2	65	66,3
Bandjoun	61	0	0	39	63,9
Total	504	6	1,2	225	44,6

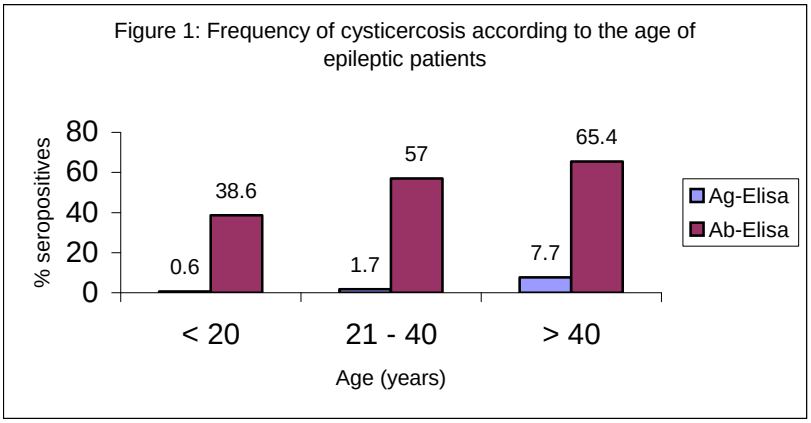
Antigens of *T. solium* metacestodes were detected in only 6 (1.2%) epileptic patients whereas antibodies against this parasite were present in 225 (44.6%) patients. Results of both antigen and antibody detection Elisa according to age, locality and sex are presented in figure 1 and tables 1 and 2. Ab-Elisa results were significantly different between Batibo and localities of Bamendjou and Bandjoun ($P<0.001$) (Table 1). No significant differences in Ab-Elisa were present between sexes (table 2) and with increasing age of epilepsy onset (Table 3). However, a borderline significant difference (P: 0.072) was observed with increasing patient ages (Figure 1)

Table 2: Frequency of seropositives for cysticercosis (Ag and Ab-Elisa) according to the sex of the patients

		Ag-ELISA		**Ab-ELISA**	
Sex	No. examined.	Positives	%	Positives	%
Male	281	4	1,4	123	43,8
Female	223	2	0,9	102	45,7

Table 3: Frequency of seropositives for cysticercosis (Ag and Ab-ELISA) according to the age of onset of epilepsy

		Ag- ELISA		**Ab-ELISA**	
Age of onset	no.examined	positives	%	positives	%
≥ 30	20	2	10	10	50
20 - 29	35	1	2.9	21	60
< 20	405	2	0,50	164	40.5
Unknown	44	1	2,30	30	68,20



Comparison of the results of Ag-ELISA and Ab-ELISA showed that out of 6 positive sera in the Ag-ELISA three tested positive in the Ab-ELISA, whereas the 3 others were negative in the Ab-ELISA.

Discussion

In this study, 504 epileptic patients were examined using an Ag- and an Ab-Elisa for the detection of *T. solium* cysticercosis. The results showed that 1.2% of the epileptic patients harboured circulating antigens, which strongly indicates that viable cysts were 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 as well in cattle (Brandt et al., 1992), in pigs (Nguekam et al., 2003a) as in humans (Erhart et al., 2002). In this group of epileptics, cysticercosis was obviously more linked to the presence of dying or dead cysticerci, since the Ab-ELISA detected 44.6% seropositives among the examined people. This confirms the observations of many different authors that dying and/or degenerated cysticerci are very common in 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 resampled after one to 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 be present 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 CT-scan could be performed to confirm the parasites in the brain, this study confirms previous observations by other authors, that NCC is one of the most important causes of epilepsy in developing countries (de Bittencourt et al., 1996; Carpio et al., 1998). The frequency of epileptics with antibodies against *T. solium* cysticercosis is twofold the 22.3% reported using immunoblot (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 in Colombia (9.82%; Palacio et al., 1998) using EITB .

Only 4.3% of patients involved in the study had late-onset epilepsy. Of them, 10% and 50% were positive in Ag and Ab-Elisa, respectively. Seropositivity increased - although only borderline significantly - with increasing age in both Ag- and Ab-Elisa (Figure 1). 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 sexes in this study. This finding is in contrast with the observation by Cruz et al. (1999) who found a higher proportion of female epileptics with positive Ab-Elisa results and who attributed it to food handling activities and their relationship with infection with cysticerci.

It was surprising that 3 out 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 %) and more probably to the presence of single cysts (Sako et al., 2000). The latter authors reported that serum samples originating from NCC patients harbouring solitary cyst might escape detection. Negative results in ELISA or EITB in cases of single cyst infections have been reported by several authors (Lara-Aguilera et al., 1992; Wilson et al., 1991).

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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