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Epilepsy is not caused by cysticercosis in The Gambia

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Summary

OBJECTIVE To determine whether epilepsy is caused by *Taenia solium* cysticercosis in The Gambia.
METHODS Case–control study testing samples collected from 210 people with epilepsy and 420 matched controls by sex and age ±5 years from 69 different places around the country during the period October 2008–March 2009. All serum samples were subjected to an antigen detection ELISA (Ag-ELISA) and electro-immunotransfer blot (EITB), and the seropositives were further CT-scanned to determine the presence of cysticerci in the brain.
RESULTS Although not significantly different ($P = 0.668$), circulating *Taenia* antigen was found by Ag-ELISA in 1.4% (95% CI: 0.3–4.1) of people with epilepsy and in 1.9% (95% CI: 0.8–3.7) of the controls. A non-significant ($P = 0.4718$) odds ratio of association 0.75 (95% CI: 0.13–3.15) between epilepsy and the presence of *Taenia* antigens was found. All 630 serum samples turned out seronegative by the EITB test. There were no intracranial cysts or cyst-like structures detected among the nine CT-scanned Ag-ELISA seropositives.
CONCLUSION Epilepsy appears not to be caused by cysticercosis in The Gambia.

keywords The Gambia, neurocysticercosis, antigen-ELISA, electro-immunotransfer blot, computed tomography, epilepsy, case–control study

Introduction

Human cysticercosis is caused by the metacestode (cysticercus) of *Taenia solium*. Several years after infection, at the time when death of the larvae causes inflammatory reactions, epileptic seizures become the commonest sign of neurocysticercosis infection. Extraneural cysticercosis (subcutaneous, muscular, cardiac cysticercosis) usually causes no major symptoms (Garcia *et al.* 2003). Several studies have shown that neurocysticercosis is one of the major causes of epilepsy in developing countries (Preux & Druet-Cabanac 2005). Carabin *et al.* (2006) did report 34 662 neurocysticercosis-associated cases of epilepsy and an overall monetary burden of US $18.6–34.2 million in East Coast Province of South Africa in 2004. Nsengiyumva *et al.* (2003) found a link between cysticercosis infection and epilepsy with an odds ratio of 3.8 in Kiremba area in Burundi. Within the same area in Burundi, Prado-Jean *et al.* (2007) found that both antigen and antibody ELISA detected a significantly higher number of seropositives for cysticercosis among people with epilepsy than among controls.

Although no reports on human cysticercosis in The Gambia have been published so far, a cross-sectional survey, from October 2007 to February 2008 using Ag-ELISA, showed a cysticercosis seroprevalence rate of 6.2% in farm pigs within Western region and Kanifing Municipality of The Gambia (Secka *et al.*, unpublished results). These data suggest that cysticercosis in humans might also occur in The Gambia. We conducted a case–control study to examine which proportion of people with epilepsy and matched controls in The Gambia test positive for cysticercosis and to look for any association between exposure to cysticercosis and epilepsy.

Methods

Sample collection and storage

The case–control study was carried out between October 2008 and February 2009. A total of 210 epileptic volunteers and 420 matched controls by sex, age ±5 years and same area of residency were sampled. The epileptic volunteers were identified by two of the authors (O.H and O.N.). An individual was considered epileptic when he or she fulfilled the definition of epilepsy of the International League against Epilepsy (ILAE 1989), 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. Cases were randomly selected among patients coming for treatment to the Royal Victoria Teaching Hospital (RTVH) in Banjul or other outpatient clinics and health centres in The Gambia. Diagnosis of epilepsy was based on a history of seizures. The seizures were not characterised. Because of the lack of equipment in many rural clinics, electroencephalography was not performed. The study volunteers came from 69 communities, villages and towns around the country. No census map was available to allow randomised selection among eligible controls. Upon signing a consent form, a blood sample was obtained and the serum transferred into labelled cryo-vial tubes and kept at −20 °C until tested. A questionnaire was administered to every sampled volunteer.

Diagnostic tests

Two cysticercosis diagnostic tests were applied during this study. The first one was a monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (Ag-ELISA) to detect circulating antigens of *Taenia solium*. The Ag-ELISA developed by Brandt *et al.* (1992) was performed as described by Rodriguez *et al.* (2009). The sera were tested at a final dilution of 1:4. The cut-off was calculated using a modified Student *t*-test (Sokal & Rohlf 1981) by comparing the optical density of each serum sample with a series of eight local negative serum samples at a probability level of $P < 0.001$. A serum sample was considered positive when the ratio (OD of test sample/OD cut-off) was ≥1.2.

Detection of antibodies against *Taenia solium* in collected samples was carried out by the enzyme-linked-immunoelectrotransfer blot assay (EITB) using lectin-lentil purified glycoprotein antigens of *Taenia solium* metacestodes (Tsang *et al.* 1989). A serum sample (dilution 1:50) was considered positive if it showed at least one reactive band of antibody to the glycoprotein antigens. To obtain a definitive diagnosis of neurocysticercosis, individuals who tested positive in Ag-ELISA or EITB underwent a normal computed tomography (CT-scan without contrast) of the brain at the RVTH in Banjul.

Data collection and analysis

A direct face-to-face questionnaire was filled for every person with epilepsy and matched control. This questionnaire gathered information about the person's identity, epilepsy history, epilepsy predisposing factors and cysticercosis predisposing factors. Case control odds ratio was used to examine the association between exposure to cysticercosis and epilepsy case. Logistic regression was used in Stata 10 to compare the proportion of cysticercosis seroprevalence in people with epilepsy and controls and to calculate confidence intervals. The exact method was used to calculate confidence intervals when all the samples were negative.

Ethical clearance

Ethical clearance for conducting this study (reference number R08003v2) was obtained from The Gambia Government/Medical Research Council joint Ethics Committee on 10 June 2008.

Results

Questionnaire results

The characteristics of the 210 people with epilepsy are shown in Table 1. All but six were on carbamaze-pine and/or phenobarbital prescription. Malaria infection that could also cause epilepsy was reported to have affected 119 (57%) of the study cases during the past 5 years.

On sanitary issues, all people with epilepsy but one used pit latrines or flush toilets. One volunteer used the bush for defecation. One hundred and forty persons reported to wash their hands with soap and water after toilet, and 100 also did so before eating but most cases eat with spoons. The sources of drinking water were wells and taps, and none used surface water for drinking. Only six cases were

Table 1 Characteristics of people with epilepsy and controls

Characteristics	Epileptic cases	Controls
Sampling sites	69	69
Sample size	210	420
Sex		
Female	96 (45.7%)	185 (44%)
Male	114 (54.3%)	235 (56%)
Religion		
Christians	12 (5.7%)	16 (4.5%)
Muslims	198 (94.3%)	401 (95.5%)
Age category (years)		
≤20	69 (33%)	157 (37.4%)
21–40	114 (54%)	206 (49%)
41–60	24 (11%)	52 (12.4%)
>60	3 (1%)	5 (1.2%)
Top four Ethnic groups		
Mandinkas	65 (31%)	175 (41.7%)
Wolofs	41 (19.5%)	83 (19.8%)
Jolas	39 (19.1%)	64 (16.2%)
Fullas	29 (13.8%)	52 (12.4%)
Total sampled Ethnic groups	17	13
Number of positives		
Ag-ELISA	1.4 (0.3–4.1)*	1.9 (0.8–3.7)*
Electro-immunotransfer blot	0 (0–1.4)*	0 (0–0.7)*
CT-scan	0 (n = 3)	0 (n = 6)

*95% confidence interval.

rearing pigs in their households, and only 13 consumed pork in cooked or grilled form.

The characteristics of the 420 matched non-epileptic controls are summarised in Table 1. Similarly to the people with epilepsy, all controls used pit latrines or flush toilets except for three individuals that used the bush for defecation. Three hundred and thirty nine control volunteers reported to wash their hands with soap and water after defecation, and 40 also did so before eating. However, spoons are used by most controls when eating. Both the people with epilepsy and controls used the same source of drinking water. Only four persons reported to rear pigs, and only 10 consumed pork in cooked or grilled form.

Cysticercosis and epilepsy

The results of the serological tests and brain CT-scans are shown in Table 1. There is no significant difference in the results of the serological tests and the CT-scan between people with epilepsy and controls. The association of exposure to cysticercosis and epilepsy found in this study has an odds ratio of 0.75 (95% CI: 0.13–3.15). All samples were negative at EITB, and no *Taenia solium* cysticerci were detected in CT-scans of the brain of 9 out of 11 Ag-ELISA seropositives. The two others refused to undergo a CT-scan.

Discussion

Serology and imaging techniques

This study is the first report on human cysticercosis in The Gambia. Based on the Ag-ELISA that is 94.4% sensitive and 97.6% specific (Erhart *et al.* 2002; Prado-Jean *et al.* 2007), the occurrence of cysticercosis was 1.75% (95% CI: 0.87–3.10) (11 out of 630); EITB and CT-scan did not detect any cases. However, these seropositives were weak positives, with a positivity ratio between 1.2 and 1.6. The fact that no *Taenia solium* cysticerci were detected by cerebral CT-scan of nine Ag-ELISA seropositives does not necessarily mean that these persons did not harbour any cysts. Cysticerci might have been present in their muscles.

In this study, seropositives were only found by the Ag-ELISA. Assuming that these were true positives and given the excellent correlation between the presence of living cysticerci and positive Ag-ELISA results (Garcia *et al.* 2000; Erhart *et al.* 2002; Nguekam *et al.* 2003), this would indicate that only living cysts were present in these individuals. It is known that living cysticerci in the brain are less frequently causing epileptic seizures than dead or degenerating cysts (Carpio & Hauser 2002). Although no cysts were found by CT-scan in the Ag-ELISA positive individuals, one has to keep in mind its limitations in detecting brain cysticerci (Nash *et al.* 2005).

Concerning the EITB, Tsang *et al.* (1989) reported a sensitivity of 98% and a specificity of 100% for detecting antibodies to cysticercosis. However, other authors reported much lower sensitivities (26%) in people with light infections (Wilson *et al.* 1991). If the infections diagnosed through Ag-ELISA are genuine, they must be light given the low positivity ratios. Therefore, it is not surprising that they were not detected by EITB.

The small number of cases of cysticercosis found in this study confirms findings in three rural communities in the West Province of Cameroon (0.4%, 1.0% and 3.0% using Ag-ELISA; Nguekam *et al.* 2003) and is similar to the 1.3% overall cysticercosis seroprevalence found in Benin (Houinato *et al.* 1998). This low prevalence is not very surprising, because the majority of the study population is Muslim and only few people are involved in pig rearing or admit to eating pork. On the other hand, in the Western region of The Gambia, where the majority of the samples were taken, the seroprevalence of porcine cysticercosis using Ag-ELISA was 6.2% (Secka *et al.*, unpublished results), which indicates that the environment is contaminated by *T. solium* eggs. Unfortunately, we were not able to collect and examine faecal samples of the study population during this survey. Given the fact that the prevalence of *T. solium* carriers is often very low in regions with a similar seroprevalence of human cysticercosis (0.13% *T. solium* carriers among more than 3000 people examined in West-Cameroon; Vondou *et al.* 2002), the chances to detect *T. solium* eggs in the 630 people of this study would have been very low.

Cysticercosis is not frequently found in Jews and Muslims because there is generally no close contact between man and pigs and because pork is not eaten, which lowers the chances to become infected with an adult tapeworm and eventually contaminates the environment with parasite eggs. Only two cases of neurocysticercosis have been reported in Malaysia, a Muslim-dominated country (Norlinah *et al.* 2003).

Association between cysticercosis and epilepsy

Although cysticercosis is one of the important causes of epilepsy in many developing countries, this appears not to be the case in The Gambia. The Ag-ELISA detected even more seropositives in the controls than in people with epilepsy, eight and three, respectively, but this was not significantly different ($P = 0.668$). A low statistically insignificant odds ratio of 0.75 ($P = 0.4718$) was found for the association between cysticercosis and epilepsy contrary to many other African countries where high OR were found (Preux & Druet-Cabanac 2005). In Burundi, for instance, an odds ratio of 3.8 showed a strong link between cysticercosis

infection and the occurrence of epilepsy (Nsengiyumva *et al.* 2003). Using the data of the case–control study of the latter authors, Prado-Jean *et al.* (2007) calculated the matched OR between epileptic cases and controls for an Ab-ELISA and an Ag-ELISA for cysticercosis and obtained figures of 3.6 and 2.9, respectively. We can conclude from the results of this case–control study that epilepsy is not caused by *T. solium* cysticercosis in The Gambia.

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