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Seroprevalence and risk factors of human cysticercosis and taeniasis prevalence in a highly endemic area of epilepsy in Bangoua, west Cameroon

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ABSTRACT

Cysticercosis caused by the larvae of *Taenia solium* is a serious and emerging threat to public health in the endemic areas as well as in the non-endemic areas. Neurocysticercosis, an affection of the central nervous system is a leading cause of epilepsy in endemic areas. This study was carried out to investigate human cysticercosis, taeniasis and risk factors, and also their association with epilepsy in Bangoua, west Cameroon where epilepsy is highly prevalent. Out of 384 people investigated, 12 (3.1%) exhibited antibody response against low molecular weight antigens of *T. solium* by ELISA. Immunoblot revealed that six persons (1.6%) were seropositive with the same antigens. Among 61 epileptic patients, only one was seropositive by immunoblot and the study did not find any statistically significant difference ($P > 0.05$) in seropositivity to *T. solium* between epileptic persons (1/61, 1.6%) and non-epileptic group (5/323, 1.5%). In addition, cysticercosis was associated with households eating pork meat from pigs slaughtered at home, but not with other factors. The risk factors including pig farming, the consumption of pork meat, vegetables, and non-drinkable water were attenuated by the relatively good hygiene and pig husbandry practices of the population. No egg of *Taenia* was found in stool by microscopic examination. All data obtained in this study suggested that cysticercosis might not be the principal causative agent of epilepsy in this area.

1. Introduction

Cysticercosis caused by the larvae of *Taenia solium* is the food-borne parasite with the greatest global impact (Robertson et al., 2013; WHO, 2010), since it is reemerging in a number of non-endemic countries due to increased travel to disease endemic areas and immigration of tapeworms carriers (Del Brutto, 2012; Schantz et al., 1992; Sorvillo et al., 2011; Yanagida et al., 2012). The poor living conditions and the management of pig husbandry in rural communities in developing countries greatly contribute to maintain the life cycle of the parasite between human and pig (Bruno et al., 2013,5; Ito et al., 2014,2005; Winkler, 2012; Zoli et al., 2003a). On the other hand, at least 80% of people with epilepsy in the world live in resource-poor countries where most of them are affected by neurocysticercosis (NCC), a cysticercosis of the central nervous system, with the incidence varying from 5 to 50% in epileptic patients (Bruno et al., 2013; Garcia et al., 2004; Moyano et al., 2014; Rottbeck et al., 2013; Winkler et al., 2009; Zoli et al., 2003b).

Both human and porcine cysticercosis have been reported in endemic areas in Cameroon (Assana et al., 2010; Nguekam et al., 2003; Zoli et al., 2003a) and previous findings demonstrated that NCC is responsible for a considerable proportion of epilepsy in endemic communities (Praet et al., 2009; Winkler, 2012; Zoli et al., 2003b). Although the recognition of the status of *T. solium* infections as a serious and emerging threat to public health is increasing in the endemic areas (WHO, 2010), the data on incidence in humans are still very scarce in most endemic areas in Cameroon. Therefore, the identification of endemic communities through epidemiological studies would help to implement adequate surveillance, monitor-

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ing and reporting systems in order to reduce sufferings due to this disease.

Bangoua is a rural area in west Cameroon where the association of living conditions and life style factors represent a risk for acquiring parasitic infections. In this area, people rear pigs and eat pork meat, and epilepsy occurs frequently. However, no information is available concerning the occurrence of cysticercosis and the cause of epilepsy in this area. This study was conducted in order to investigate the impact of *T. solium* cysticercosis and its association with epilepsy, to detect taeniasis carriers, and to evaluate risk factors for human cysticercosis based on questionnaires in the population living in Bangoua. Immunodiagnostic methods have been very useful to identify the spots of cysticercosis in the absence of neuroimaging that is computerized tomography (CT) and magnetic resonance imaging (MRI), which are inaccessible in many poor endemic countries (Garcia et al., 2012; Ito et al., 2006). Thus, in this study, immunological techniques with specific low molecular weight antigens (LMWAgs) of *T. solium* (Sako et al., 2013) were used to examine people with or without epilepsy in this area.

2. Materials and methods

2.1. Description of the study area

The study was conducted in Bangoua, west Cameroon (5° 12′ 0″ North, 10° 29′ 0″ East). It is located at about 265 Km west of Yaounde, the capital city of Cameroon. Bangoua is 74 Km^2 with a total population of approximately 17000 and a density of 230 inhabitants per Km^2. The annual rainfall of the study area is 1324 mm and the average temperature is 15–25 °C, with an altitude of about 1383 meters above sea level. The inhabitants of Bangoua use agriculture and trade to lead their livelihood. Agriculture and trade play an important role in daily sustenance, where the farming system is characterized by small-scale production of mixed crops and livestock.

2.2. Sample collection

2.2.1. Ethics

The study was approved by the Cameroon National Ethics Committee and the ethical approval was sought and obtained from the ethic committee.

2.2.2. Study design

A household survey conducted from February to March 2014 was carried out for the assessment of the prevalence of taeniasis and cysticercosis and their association with epilepsy in this area, and to evaluate risk factors for human cysticercosis based on questionnaires. People from all age (1–90 year-old in this study) without sex distinction, living in the area from at least 6 months and willing to participate to the study were enrolled. All people who refused to participate were excluded. One to eleven people were sampled per household, with an average of 2.61 people per household. Each person from the households was approached individually to obtain his or her informed consent before questionnaire administration. Informed consent was sought from parents or guards of children. The household based sampling was done through questionnaire seeking information about age, sex, family size, source of water and its handling, presence and utilization of latrines, education level, personal hygiene, level of awareness to parasitic infections, deworming history, expulsion of 'noodle-like worms' (tapeworms), prevailing husbandry systems, pork consumption, history of epilepsy and other factors associated with *Taenia* infections. The clinical status of people without epilepsy was reviewed by the physician and epileptic patients were examined by a neurologist in the field to confirm the seizure.

2.2.3. Sample size determination and sampling procedure

The sample size was determined with a confidence level of 95% and a margin of error of 5%. Blood and fecal samples from respondents in households including people with and without epilepsy were collected for further analysis. Fecal samples were investigated by microscopic examination for the search of parasitic ova and the serum samples obtained from blood were analyzed by serology using *T. solium* LMWAgs (Sako et al., 2013). Although some of the epileptic patients were recorded in the biggest hospital of the study area and were currently receiving treatment from this hospital, they were offered free antiepileptic drugs during this study. In addition, participants with stool test positive with any parasitic egg received free treatment with antiparasitic drugs. Albendazole was administered as appropriate by medical staff. Serum samples from healthy donors in Japan were used to determine the cut-off value.

2.3. Stool examination

Stool samples were analyzed for the presence of parasites ova by using the formalin-ether concentration technique. The processed stool samples were examined microscopically for the presence of parasitic organisms, especially *Taenia* ova and any other intestinal parasites.

2.4. Serological analyses

2.4.1. ELISA

ELISA was performed as previously reported (Sako et al., 2013). Briefly, 96-well microplates were coated with 100 μl of 1 μg/ml of *T. solium* LMWAgs in PBS and incubated overnight at 4 °C. The antigens were removed from the plates and the wells were blocked with 300 μl of blocking solution (20 mM Tris–HCl, pH 7.6, 150 mM NaCl, 1.0% casein, 0.1% Tween 20) at 37 °C for 1–2 h. After rinse the wells twice with PBS containing 0.1% Tween 20 (PBST), 100 μl of serum samples diluted 1:100 in blocking solution were added to the wells and incubated at 37 °C for 1 h. The wells were incubated with 100 μl of recombinant protein G conjugated with peroxidase (Invitrogen, USA) diluted 1:2000 with blocking buffer at 37 °C for 1 h. For color development, the plates were incubated with 100 μl of substrate (0.4 μM 2,2′-azino-di-[3-ethyl-benzthiazoline sulfonate] in 0.2 M citric acid buffer, pH 4.7) for 30 min at room temperature. The optical density (OD) was determined at 405 nm of each well using microplate reader (Immuno Mini NJ-2300, Biotec, Japan). The cut-off value was determined as the mean of OD plus 4 standard deviations of sera from 37 healthy donors.

2.4.2. Immunoblot

The LMWAgs (60 μg/mini gel) were treated with a SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 2.0% SDS, 50 mM dithiothreitol and 10.0% glycerol) at 100 °C for 5 min and separated in a 15.0% polyacrylamide gel. The separated proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane sheet (Millipore) and then blocked with blocking solution (20 mM Tris–HCl, pH 7.6, 150 mM NaCl, 1.0% casein, 0.1% Tween 20). Each sheet was probed with each ELISA positive serum samples and serum samples with OD value close to the cut-off value diluted 1:20 in blocking buffer at room temperature for 1 h. The sheets were incubated with the peroxidase-conjugated recombinant protein G (Invitrogen, USA) diluted 1:2000 in blocking buffer. The substrate (4-Chloro-1-Naphtol/Phosphate) was used for color development