

RISK FACTORS FOR EPILEPSY IN RURAL LAO PDR: A CASE-CONTROL STUDY

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Abstract. The objective of this study was to assess the major etiologic categories of epilepsy in a rural district of the Lao PDR. Thirty-one newly identified patients with confirmed active epilepsy were compared with 124 controls, matched for gender, age and village residence. Risk factors for epilepsy were investigated with particular focus on cysticercosis serology. A history of head trauma (OR=4.7, p=0.05), family history of epilepsy (OR=12.8, p=0.03), and the use of human feces to fertilize domestic vegetable gardens (OR=4.9, p=0.04) were significantly associated with epilepsy. The study did not confirm any direct relation between epilepsy and cysticercosis serology. The cysticercosis seroprevalence was nil in the epilepsy group. This is the first study in the Lao PDR on epilepsy risk factors representing important data for the sub-region. Parasitic, environmental, and behavioral factors of this traditional population deserve further studies to explain the missing link between epilepsy and cysticercosis.

INTRODUCTION

Epilepsy is a worldwide public health problem with an estimated global prevalence around 8 per thousand (WHO, 2005). In tropical countries this prevalence is much higher, between 10.0 and 15.0 per thousand according to the International League Against Epilepsy, reaching up to 50.0 per thousand in particular areas (Commission on Tropical Diseases of ILAE, 1994; de Bittencourt *et al*, 1996; Preux and Druet-Cabanac, 2005).

A majority of epilepsy cases are considered idiopathic. Secondary (or symptomatic) epilepsy results from various conditions: perinatal distress, head trauma, cerebrovascular disease, brain tumors, and central nervous

system (CNS) infections (Arruda, 1991; Hui and Kwan, 2004; Miskov *et al*, 2005). The prevalence of these conditions is determined in each area by factors including environment, cultural practices, quality of prevention and care, and socio-economic development. According to the ILAE (Commission on Tropical Diseases of ILAE, 1994) CNS infections are the leading cause of epilepsy in tropical developing countries, and include tuberculosis, schistosomiasis, AIDS, and cysticercosis; the latter ranking number one in many areas.

Epilepsy prevalence data are scarce in Southeast Asia, but have shown rates around 8.0 per thousand or below, which are unexpectedly low for areas where taeniasis due to *Taenia solium* has been documented (Wandra *et al*, 2000, 2003; Erhart *et al*, 2002; Rajshekhar *et al*, 2003; Willingham *et al*, 2003; Chen *et al*, 2004; Dorny *et al*, 2004; Joshi *et al*, 2004; Ito *et al*, 2004, 2005; Ikejima *et al*,

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2005; Tran *et al*, 2006; Somers *et al*, 2006).

We performed a case-control study in rural Lao PDR to assess the main determinants of epilepsy, with a special focus on cysticercosis serology.

MATERIALS AND METHODS

Study area

The study was carried out in eight villages of the Hinheub District, Vientiane Province, Lao PDR. The district is located 120 km north of Vientiane capital town. Rice farming, gardening, fishing and breeding, notably pig rearing, are the main subsistence activities of these communities.

Definition and identification of cases and controls

A two-step community screening was used to identify and ascertain the frequency of epilepsy cases (Preux *et al*, 2000; Tran *et al*, 2006); terms and classification according to recommendation of the Commission on Epidemiology and Prognosis of the International League Against Epilepsy (ILAE, 1993). A total of 33 new patients with active epilepsy (last seizure within 5 years) were confirmed among 4,310 interviewed villagers, of whom 31 consented to participate in the study. For each case, four controls were matched for gender, age (\pm 5 years) and village residence.

Procedures in cases and controls

Cases and controls were interviewed regarding risk factors for epilepsy. The questionnaire addressed family history of epilepsy, perinatal events (from the caretaker of the children), history of head trauma, and risk factors for cysticercosis and taeniasis, including the presence of pigs in the household, the use of latrines, the consumption of pork meat, and the use of human feces as fertilizer.

Each subject underwent a general and neurological clinical examination. Brain CT scans and other imaging procedures were not

available for cases and controls. Each subject provided a stool sample, which was preserved in sodium-acetyl-formalin solution and examined using a standard concentration technique (Yang and Scholten, 1997). Venous blood (5 ml) was drawn from all subjects, and the serum was stored at 4°C for 6 hours, then frozen at -20°C. Frozen sera were flown in dry ice to Asahikawa Medical School, Japan.

In Japan, ELISA and Immunoblot were independently carried out by a similar procedure, except that the antigens used were isoelectrically purified glycoproteins (GPs) (Rotofor, BioRad, USA) extracted from *T. solium* glycoproteins (Ito *et al*, 1998).

Statistical analysis

Data was entered in Epidata freeware, version 3.02 (www.epidata.dk, Odense Denmark) and analyzed with STATA, version 8.2 (Stata Cooperation, College Station, TX, USA). Proportions, means and their standard deviations (SD) were calculated to compare cases and controls. Odd ratios and the 95% confidence intervals were calculated for the risk factors using the matched analysis procedure. The significance threshold was set at 0.05.

Ethical clearance was obtained from the Ministry of Health, Lao PDR. Informed consent was obtained from each of the subjects prior to enrollment in the study.

RESULTS

Study population

A total of 31 newly diagnosed cases of active epilepsy, and 124 controls were enrolled. There were no significant differences between cases and controls in gender ($p=1.00$) or age ($p=0.82$). The male/female ratio was 1.2/1 for both groups. The mean ages of cases and controls were 22.2 years (SD=12.5 years) and 22.7 years (SD=11.8 years), respectively. All controls had their residency in the village of the corresponding matched case.

Clinical description

Generalized seizures (19 cases, 61.3%) were commoner than partial seizures (9 cases, 29.0%); 3 cases (9.7%) were not-classifiable; 38.7% of patients had more than one seizure type, and the first seizure appeared before the age of 20 in 28 patients (90.3%). Nine patients (29.0%) had idiopathic epilepsy, 8 (25.8%) had symptomatic epilepsy, and 14 (45.2%) had cryptogenic epilepsy.

Risk factors for epilepsy

Table 1 summarizes the main risk factors. A family history of epilepsy (OR=12.8, $p=0.03$), use of human-feces for fertilizer (OR=4.9, $p=0.04$), and a history of head trauma (OR=4.7, $p=0.05$) were significantly associated with epilepsy. Pork meat consumption was significantly less frequently reported in the epilepsy group than in controls (OR=0.1, $p<0.01$).

Stool examination and serology

The stool examination showed a total absence of *T. solium* in both cases and controls. However, it disclosed many other parasites, as 76% of stool samples were positive for at least one intestinal parasite, with no significant differences between cases and con-

trols (OR=0.8, $p=0.61$). The cysticercosis serologic tests found 4 ELISA positive and 8 ELISA intermediate results. Of these 12, 6 were confirmed by blot, all belonging to the control group (4.8%).

DISCUSSION

Lao PDR is a developing country in terms of sanitation, prevention, and access to care. The epilepsy prevalence rate of 7.7 per thousand found in a recent community study (Tran *et al*, 2006) is only slightly higher than the rates reported for more developed Asian countries and in developed western countries.

As shown in previous studies a family history of epilepsy or of head trauma were major associated factors. We found both these factors in 12.9% of cases, more than 5 fold higher than in the control group.

The proportion of those with head injury was similar to that found in a population-based survey of epilepsy in Taiwanese adult patients (Chen *et al*, 2006). Head injury is a known etiology of epilepsy in adolescents and adults (Annerggers and Coan, 2000; Chadwick, 2000). It may be caused by a variety of mechanisms, but the probability of epilepsy development,

Table 1
Potential risk factors for epilepsy in Lao PDR, 2004.

Risk factors	Cases (n=31) (%)	Controls (n=124) (%)	OR	95% CI	p
Familial history of epilepsy	4 (12.9)	3 (2.4)	12.8	1.4-119.5	0.03
History of head trauma	4 (12.9)	4 (3.2)	4.7	1.0-21.4	0.05
Peripheral blood eosinophils ($\geq 500/\text{mm}^3$)	19 (61.3)	85 (68.6)	0.6	0.2-1.7	0.35
Cysticerci serology test positive (Asian, South American mixed antigens)	0 (0.0)	6 (4.8)	-	-	-
Intestinal taeniasis	0 (0.0)	0 (0.0)	-	-	-
Pork consumption	27 (87.1)	123 (99.2)	0.1	0.0-0.6	0.01
Undercooked pork consumption	12 (38.7)	50 (40.3)	0.7	0.3-1.7	0.49
Use of human-feces fertilizers	29 (93.6)	94 (75.8)	4.9	1.1-22.1	0.04
Latrine availability	25 (80.6)	111 (89.5)	0.4	0.1-1.4	0.13
Outdoor defecation	30 (96.8)	112 (90.3)	3.9	0.4-34.7	0.22
Domestic outdoor pig raising	24 (77.4)	92 (74.2)	1.2	0.4-3.5	0.68

from 1.5 to 17.0 fold, is relating to the gravity of trauma (Annegers *et al*, 1998). Patients in our study were young adults; most of them developed the disease during childhood. Rural, mountainous environment and houses on piles explain the high risk for trauma in these children.

A positive family history of epilepsy is another classical risk factor for epilepsy. It increased the risk of developing epilepsy by 3 fold in a case-control study of childhood epilepsy in Iran (Asadi-Pooya, 2005). Consanguinity, socially accepted by the Lao tradition beyond the second degree of kinship, may also play a role in epilepsy, as previously shown in sub-Saharan Africa (Preux and Druet-Cabanac, 2005). A history of peri-natal distress could not be traced accurately in our series because only children over 5 years were included in the study. However, this factor may not be excluded since 80% to 90% of deliveries in rural Lao PDR take place at home in unsafe conditions, resulting in high infant and mother mortality rates (70/1,000 and 405/100,000, respectively) (National Statistic Center Lao PDR, 2005).

In several tropical developing countries, neurocysticercosis is the leading cause of epilepsy (ILAE, 1994). Cysticercosis and human taeniasis have been previously reported in Lao PDR as well as in neighboring Vietnam and Cambodia (Dorny *et al*, 2004), but their relationship to epilepsy have never been assessed.

Neuroimaging, the gold standard for the diagnosis of cerebral cysticercosis was not available in our setting. The study could not definitively assess the rate of cerebral cysticercosis in our series of patients, however, we attempted to assess the relationship between epilepsy and cysticercosis using seroprevalence rates as an indirect marker. In this context, the study demonstrated a negative result.

In Africa, South America, India and Indonesia, much higher cysticercosis seropre-

valence rates have been found, at around 30% for people with epilepsy and 5% in the general population (Preux *et al*, 1996; Wandra *et al*, 2000,2003; Rajshekhar *et al*, 2003; Garcia *et al*, 2003; Ito *et al*, 2005; Krecek *et al*, 2004; Ngowi *et al*, 2004; Del Brutto *et al*, 2005). A similar result was also found in the general population from a mountainous region in Vietnam (Dorny *et al*, 2004). Our sample size was based on these prevalences. In reality we found a result of 4.8% positive in the controls.

Unexpectedly, no positive results were found in the group of epileptic cases. The conclusion is that the seroprevalence of cysticercosis in the population with epilepsy in Lao PDR may not be as high as that found in those African or South American studies. It was not sufficient to state the importance of cysticercosis as the etiology of epilepsy in Lao PDR due to the sample size.

The risk factors for cysticercosis infection include exposure to human feces with *Taenia* eggs, either directly or via food products (Willingham *et al*, 2003). In the present study, a significant correlation was found between epilepsy and using human feces as fertilizer in the vegetable garden. This practice is a potential risk factor since most people in this region favor raw vegetables.

In Lao PDR, a recent questionnaire investigation found that pork meat, especially pork fat, was traditionally believed to be related to seizures, and therefore largely avoided once the disease commenced, not only by the patients but also by the family members, in some cases (unpublished personal data). This fact may explain why our patients with epilepsy had significantly lower pork consumption than the controls (OR=0.1, $p < 0.01$). This dietary change, not found in other countries, may interrupt the parasite life cycle and cause a negative serological result after several years. Other studies of patients with new onset epilepsy may reveal this paradox.

All the above data are consistent with a low or negligible *T. solium* transmission rate in Lao PDR. Similar findings have been documented in the neighboring Vietnam where human taeniasis and cysticercosis have decreased remarkably within the last decade (Willingham *et al*, 2003), with very low persistent rates in coastal and mountain areas, and a quasi complete elimination in urban areas (Somers *et al*, 2006).

In conclusion, in a series of patients with active epilepsy from a rural, deprived area of Lao PDR, the prevalence of taeniasis and the seroprevalence of cysticercosis were found surprisingly low. Traditional practices, such as avoiding pork consumption, may have protected people with epilepsy from intestinal taeniasis. On the other hand, roaming pigs and the use of human feces as fertilizer may have favored the acquisition of cysticercosis. The latter, however, appeared to play little etiologic role, if any. Finally, these low prevalences of epilepsy, taeniasis, and cysticercosis, appear as a paradox in a setting where poverty, poor hygiene, human-animal promiscuity, and an overall high burden of parasitic diseases would all be expected to act as risk factors. To explain this paradox, further studies are needed to address other potential determinants, such as environmental, parasitic, or genetic factors.

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