Short Report

Taenia solium cysticercosis in a village in northern Viet Nam: seroprevalence study using an ELISA for detecting circulating antigen

Annette Erhart[1], Pierre Dorny[1]*, Nguyen Van De[2], Ha Viet Vien[2], Dang Cam Thach[2], Nguyen Duy Toan[2], Le Dinh Cong[2], Stanny Geerts[1], Niko Speybroeck[1], Dirk Berkvens[1] and Jef Brandt[1] *[1]Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium; [2]National Institute of Malariology, Parasitology and Entomology (NIMPE), BC 10 200 Tu Liem, Hanoi, Viet Nam*

Abstract

An enzyme-linked immunosorbent assay (ELISA) for detecting circulating *Taenia solium* antigen was evaluated in Viet Nam; 12 of 210 people gave a positive result, including 5 persons with epilepsy. Cysticercosis was confirmed in 9 persons. Agreement between the ELISA, computerized tomography scanning and biopsy examination was high.

Keywords: cysticercosis, *Taenia solium*, diagnosis, enzyme-linked immunosorbent assay, Viet Nam

Introduction

Foci of human cysticercosis are suspected to occur in rural areas around Hanoi in northern Viet Nam. Occasionally, diagnoses of cysticercosis due to *Taenia solium* in patients with late onset epilepsy or subcutaneous nodules are confirmed in Hanoi by imaging techniques or biopsy. Because computerized tomography (CT) scans are expensive and available only in Hanoi, the prevalence of *T. solium* in these rural areas remains largely unknown.

Methods

A limited cross-sectional survey was organized in a small village (Ty Dien village, Phu Hoa commune, Luong Tai district in Bac Ninh province) 50 km northeast of Hanoi. A census was held on 15 December 1999 and 2096 people, belonging to 434 households, were enumerated. From these, 100 households were randomly selected using a computer-generated sample list (Epi-Info 6; CDC, Atlanta, Georgia, USA). Participation was requested of family members above 12 years of age including permission to take blood and stool samples, clinical inspection for palpable nodules, and collection of medical histories in relation to epilepsy and the presence of adult tapeworms. Of the 273 eligible persons, 254 were present for the household survey and were given an individual code number. Only 230 were present on the next day for individual clinical examination and sampling. Blood and stool samples could be collected from 210 persons (76·9% of the population above 12 years of age in the 100 families). Samples were taken after the individual had given informed consent. Stool samples were examined for the presence of taeniid eggs and other parasitic infections by a formalin–ether concentration method. The prevalence of cysticercosis was assessed by an enzyme-linked immunosorbent assay (ELISA) for the detection of circulating *T. solium* antigen in serum samples that had been subjected to trichloroacetic acid precipitation (DE JONGE *et al.*, 1987). Originally, this assay was developed for the detection of bovine cysticercosis; it is based on a 'sandwich' of 2 monoclonal antibodies raised against excretory/secretory products of *T. saginata* metacestodes (BRANDT *et al.*, 1992; DORNY *et al.*, 2000). Preliminary studies have indicated the usefulness of this assay for the detection of *T. solium* metacestodes in humans and pigs (unpublished results). The cut-off level was calculated by comparing the optical density of each sample with the mean of a series of 8 negative human serum samples at a probability level of $P = 0{\cdot}001$ (SOKAL & ROHLF, 1981). For the present study, negative serum samples were taken from healthy Vietnamese individuals.

Results

Eggs of *Taenia* spp. were not found in any stool sample. The prevalences of *Ascaris lumbricoides* and *Trichuris trichiura* were 57·0% and 45·6%, respectively. Other helminth infections, including hookworm and trematodes, and protozoal infections had a low prevalence. Twelve people (5·7%) gave a positive ELISA result; 5 of them had a history of late onset epilepsy (Table 1). Subcutaneous nodules were found in 2 of these 5 epileptic patients and in 3 other seropositive persons without nervous signs. Subcutaneous nodules were not found in any of the seronegative people. The age of the seropositive subjects ranged between 13 and 76 years (median 40 years). Two of the seropositive persons (029/01 and 029/04) belonged to the same family.

All seropositive persons, and 8 seronegative persons without a medical history suggestive of cysticercosis who were randomly selected using a computer-generated sample list (Epi-Info 6), were offered a neuro-imaging CT scan in a hospital in Hanoi. Nine of the 12 seropositive persons and all the seronegative persons agreed to undergo CT scanning. The 9 seropositive subjects included the 5 epileptic patients. The radiologist had no prior knowledge of the serological results. Living cysticerci were demonstrated in the brains of 4 of the 5 epileptic patients; in the fifth, only one calcified cyst was seen. CT scans of 2 seropositive persons without nervous symptoms or palpable nodules revealed the presence of living cysticerci in the brain which, according to SANCHEZ *et al.* (1999), is not an unusual observation. Biopsy of a subcutaneous nodule in 2 patients without neurological symptoms demonstrated a living cysticercus. In total, a diagnosis of cysticercosis was confirmed in 9 of the 12 seropositive patients by biopsy and/or CT scan. The 3 remaining persons refrained from further co-operation. No evidence of neurocysticercosis was found in any of the 8 seronegative persons (data not shown).

Discussion

A reference standard for the detection of *T. solium* cysticercosis is not available. Therefore, our results were assessed by calculating confidence intervals for positive and negative agreement indices (GRAHAM & BULL, 1998). Considering the outcome of 2 tests in general (Table 2), the values a, b, c and d denote the observed frequencies for each possible combination of ratings by tests 1 and 2. The proportion of specific agreement for the positive ratings (p_s^+) (the positive agreement index or positive A.I.), and for the negative ratings (p_s^-) (the negative A.I.) were calculated as follows (using the notation shown in Table 2).

$$p_s^+ = \frac{2a}{2a+b+c} \text{ and } p_s^- = \frac{2d}{2d+b+c}$$

These proportions are interpretable as estimated conditional probabilities and do not have the limit-

*Author for correspondence; phone +32 3 2476394, fax +32 3 2476268, e-mail pdorny@itg.be

Table 1. Results of clinical examination of 12 persons giving positive results in an ELISA to detect circulating *Taenia solium* antigen

Patients[a]						
				Subcutaneous nodules		
Code	Sex	Age (years)	Cysts (CT scan)[b]	No. present	Biopsy[c]	Late onset epilepsy
051/02	f	37	1 live	2	Live	No
029/01	m	46	2 live, 1 calcified	1	Live	Yes
023/01	m	51	1 calcified	2	Dead	Yes
029/04	m	76	1 live, 1 calcified	0	–	Yes
078/03	f	19	1 live	0	–	No
007/01	m	29	2 live	0	–	Yes
100/02	f	29	4 live	0	–	Yes
077/01	m	47	1 live	0	–	No
011/01	m	40	None	5	Live	No
082/01	m	57	nd	2	nd	No
043/01	m	40	nd	0	–	No
085/05	f	13	nd	0	–	No

[a]The first 3 digits of the code indicate the household; f = female, m = male.
[b]No. of cysts revealed by computerized tomography scan; nd = not done.
[c]nd = not done.

Table 2. Summary of binary ratings in two tests[a]

	Test 1		
Test 2	+	–	Total
+	a	b	$a+b$
–	c	d	$c+d$
Total	$a+c$	$b+d$	N

[a]Test 1 = antigen ELISA; test 2 = biopsy and/or computerized tomography scan; see text for further explanation.

ations of the kappa statistic (κ). Confidence interval estimates (95%) were calculated using the Bayesian method proposed by GRAHAM & BULL (1998), which result in acceptable coverage properties for the aforementioned positive and negative A.Is. In this analysis a non-informative prior distribution was used because no specific prior knowledge about the long-run cell probabilities was available. The antigen ELISA results and a combination of CT scan results and the presence or absence of subcutaneous nodules produced a positive A.I. of 100% (87%–100%) and a negative A.I. of 100% (85%–100%).

Exact logistic regression showed that there was no difference in infection rates of males and females ($P = 0{\cdot}38$) and no influence of age on infection ($P = 0{\cdot}74$). There was a borderline association ($P = 0{\cdot}054$) between epilepsy and infection.

Conclusion

The antigen ELISA seems to be a valuable tool in epidemiological studies of human cysticercosis. It may also be valuable as a confirmatory or initial test for individual diagnosis. Studies on bovine cysticercosis indicate that this test demonstrates infection with viable cysts only; this may also apply to human cysticercosis. The test may also be of use in the follow-up of neurocysticercosis patients. In Latin America, GARCIA *et al.* (1998, 2000) and CORREA *et al.* (1999) also demonstrated the usefulness of serum antigen detection in cerebrospinal fluid or serum for clinical diagnosis and monitoring of neurocysticercosis. Validation of the present monoclonal antibody-based antigen ELISA is in progress; preliminary results showed that serum samples from 34 of 36 cases of cysticercosis (94·4%), who had been confirmed by CT scan, biopsy of subcutaneous nodules, or both, gave positive results. No cross-reaction was observed with sera from patients with parasitologically and/or serologically confirmed infections with *Schistosoma* ($n = 3$), hydatid cysts ($n = 10$), *Ascaris* ($n = 38$), *Trichuris* ($n = 38$), filaria ($n = 3$), *Entamoeba* ($n = 3$), *Plasmodium* ($n = 7$) and *Trypanosoma* ($n = 8$).

This preliminary survey confirmed the suspicion of an important focus (prevalences of 4·3% and 5·7% by conventional techniques or serology, respectively) of human cysticercosis in a rural area of northern Viet Nam. Further epidemiological studies, i.e. cross-sectional surveys in different villages of this province and other provinces of northern Viet Nam, are needed to get a better estimate of the real prevalence, distribution and transmission patterns of this highly debilitating parasitic disease. For this purpose, the antigen ELISA would be an invaluable diagnostic tool for mass screening for cysticercosis.

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Short Report

Envenoming due to snake bite during pregnancy

S. L. Seneviratne[1], C. E. de Silva[3], M. M. D. Fonseka[1], A. Pathmeswaran[2], S. B. Gunatilake[1] and H. J. de Silva[1] *Departments of [1]Medicine and [2]Community and Family Medicine, Faculty of Medicine, University of Kelaniya, P. O. Box 6, Ragama, Sri Lanka; [3]Base Hospital, Polonnaruwa, Sri Lanka*

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Snake bite during pregnancy appears to be uncommon (DUNNIHOO *et al.*, 1992). In a large series of hospital admissions due to snake bite reported from South Africa, pregnant women accounted for 0·4% of cases (MCNALLY & REITZ, 1987), while in a similar study from India they accounted for 1% of cases (BHAT, 1974). As a result of its being uncommon, the effects and outcome of snake bite envenoming in human pregnancy are not well known. From the documented cases it appears to be associated with high fetal wastage, mainly due to abortion, and significant maternal morbidity (MALZ, 1967; DUNNIHOO *et al.*, 1992). Few maternal deaths have been reported in the literature (SUTHERLAND *et al.*, 1982; DUNNIHOO *et al.*, 1992). There are several possible mechanisms for abortion following snake bite during pregnancy: they include direct effects of the venom on the fetus, fetal hypoxia due to maternal shock, placental bleeding due to coagulopathy, venom-induced uterine contractions, and pyrexia and cytokine release which occur following tissue damage. Case reports also suggest that adverse effects on the fetus seem more severe early in pregnancy (MALZ, 1967; DUNNIHOO *et al.*, 1992). It is possible that some constituents of snake venom may cross the placenta, and there are case reports which suggest that venom may affect the fetus in the absence of, or before, manifestation of serious maternal envenoming (JAMES, 1985). In addition to the well-known local and systemic effects of snake bite envenoming, there are reports of other maternal complications peculiar to the pregnant state: supine hypotension syndrome leading to maternal death following a bite by the Australian common or eastern brown snake *Pseudonaja textilis* (see SUTHERLAND *et al.*, 1982); abruptio placentae with evidence of fibrin deposition and microthrombus formation in the spongy layer which led to placental cleavage and separation following a *Bothrops* bite (ZUGAIB *et al.*, 1985).

Antivenom serum (AVS) is the only specific treatment for snake bite envenoming (WARRELL, 1996). The use of AVS in pregnancy should balance its risks and benefits. AVS therapy may be life-saving for the mother. However, both anaphylaxis, which is a well known complication of AVS therapy, as well as its treatment with adrenaline, may compromise placental circulation (ENTMAN & MOISE, 1984; DUNNIHOO *et al.*, 1992).

Snake bite envenoming is a major health problem in Sri Lanka, which has one of the highest snake bite fatality rates in the world (SRI LANKA, 1999). The aim of this paper is to present the outcome in 39 patients with bites due to venomous snakes during pregnancy. To our knowledge, this is the largest single series of this uncommon clinical problem.

We performed a prospective study of all pregnant women admitted with envenoming following a snake bite to the University Medical Unit, Colombo North Teaching Hospital, Ragama, Sri Lanka from September 1996 to April 2001, and to the Base Hospital, Polonnaruwa from July 1997 to December 1999. The principles of AVS therapy for snake bite envenoming were as for non-pregnant women; AVS was administered for systemic envenoming (spontaneous bleeding and/or bedside whole blood clotting time prolonged more than 20 min; neurotoxicity; shock; impaired consciousness; myotoxicity; renal impairment) and/or significant local envenoming (swelling of more than half the bitten limb). Management also included regular obstetric observation (including ultrasound scanning), and the patients were kept in hospital for at least 10 d following the snake bite. They were then followed up until the end of their pregnancy.

During the study period, 39 pregnant women were admitted with envenoming (local and/or systemic) due to snake bite. They formed 1·8% of the 2178 snake bite admissions (male:female ratio = 7·1:2·9) to the 2 units during the study period. The snake was positively identified in 24 cases (61·5%) (dead snake brought to hospital; immunodiagnosis was not available). Eleven patients were bitten by highly venomous snakes (*Bungarus ceylonicus* or *Daboia russelii russelii*) (Table), 14 by a hump-nosed viper (*Hypnale hypnale*), which is considered a moderately venomous snake, and a further 14 by an unidentified snake. Details of clinical presentation, AVS therapy and outcome are given in the Table. Eighteen patients were given Haffkine polyspecific AVS (Haffkine Laboratories, Mumbai, India) which is raised against the venoms of cobra (*Naja naja*), Russells's viper (*Daboia russelii*), saw-scaled viper (*Echis carinatus*) and common krait (*Bungarus caeruleus*). The

Address for correspondence: Prof. H. J. de Silva, Department of Medicine, Faculty of Medicine, P.O. Box 6, Thalagolla Road, Ragama, Sri Lanka; fax +94 1 958337, e-mail hjdes@sri.lanka.net