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Search for antibodies to *Taenia solium* cysticerci in paediatric patients with seizures

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Abstract. Neurocysticercosis is a leading cause of seizures in adults, but in paediatric patients, the diagnosis is controversial. The aim of this study was to search for antibodies to *Taenia solium* cysticerci in paediatric patients with seizures. We retrospectively studied a cohort of 41 serum samples from paediatric patients and 40 serum samples from healthy children. Antibodies were analysed by ELISA (vesicular fluid) and by Western blot (glycoproteins). Clinical, image and socio-demographic data were obtained from the medical records. The frequency of positive by ELISA was of 12% (n=5) in patients with seizures, while no positive samples were found in the healthy group. Results of Western blot were negatives. The analysis of the medical records showed a cyst of unknown origin in 2/5 ELISA positive samples. According to the diagnostic criteria for neurocysticercosis, three minor criteria (positive serology, active seizures and compatible image) were associated to an epidemiological condition (Mexico is endemic for neurocysticercosis); thus, the probable frequency of neurocysticercosis in the studied sample of patients with seizures was 4.9% (2/41 patients). The three remaining positive samples were associated with problems of non-infectious origin. The positivity was associated with the identification of cysts by magnetic resonance imaging (p = 0.047; chi-square), but found no association with the socio-economic characteristics of the patients, family history or to clinical symptoms. In conclusion, scarce frequency of antibodies to *T. solium* cysticerci was determined in paediatric patients with seizures. The low prevalence of antibodies detected in children is an indirect indicator of the interruption of *T. solium* transmission. Further studies are needed to design an algorithm for the conclusive diagnosis of seizures.

INTRODUCTION

Cysticercosis is a zoonotic disease caused by the larva of *Taenia solium*. In Mexico, the official epidemiological data reported by the Ministry of Health show a decrease in the number of notifications reported yearly, from 1,608 in 1995 to 231 in 2009 (Flisser & Correa, 2010). Thus, the search for antibodies to *Taenia solium* cysticerci has become unusual. The immunodiagnosis for neurocysticercosis uncommonly occurs in paediatric patients with seizures due to two main causes; the first one is because, the infection in children is associated to a single cyst; therefore, the clinical manifestations of neurocysticercosis are less complex than in adult or even, have no symptoms. The second reason is associated to the period of incubation; the clinical manifestations of neurocysticercosis appears several years after the infection and, in particular, the seizures appear between the second and the third decade of life, when the cysts are in a

degeneration process derived from the attack of the immune response (García *et al.*, 2006; Sotelo, 2011).

Data regarding the worldwide frequency of neurocysticercosis is variable in the paediatric population and is recorded that seizures are more common in children from developing countries than in children from industrialized countries. In Mexico, two national epidemiological surveys were conducted to search for antibodies to *Taenia solium* cysticerci. The first study was conducted by immunolectroforesis and a prevalence of 1% was found to be associated with overcrowding (Woodhouse *et al.*, 1982). The second was performed by haemagglutination. A prevalence of 0 to 8% was found to be associated in the low socioeconomic communites and those living in rural areas (Larralde *et al.*, 1992). Data about cysticercosis in children are scarce in our national surveys. In an epidemiological study carried out in a rural town in Mexico where 154/155 inhabitants gave consent for a tomography scan of their brain (Fleury *et al.*, 2003). The prevalence in children (0-19 years) was 13.2% (CI 95%, 7.0–21.9), while in adults (20–54 years) it was of 3.2% (CI 95%, 0.4-11.0). In contrast, paediatric autopsy studies revealed 0.5% (18/3424 patients) occurrence of neurocysticercosis in a Paediatric Hospital in México City (Ridaura-Sanz, 1987).

The availability of the ELISA and the Western blot between 1980´s to 1990´s made it possible to carry out a number of serological studies to confirm clinical suspicion. The diagnostic sensitivity of the Western blot, which uses an enriched fraction of glycoproteins rates between 98 and 100% if there are multiple cysts; however, when there is only one or two cysts, sensitivity is around 59 to 65% (Aguilar-Rebolledo *et al.*, 2002; Proaño *et al.*, 2002). Since data regarding antibodies to *Taenia solium* cysticerci in children are not easily available, the aim of this work was to search for antibodies to *Taenia solium* cysticerci in pediatric patients whom present seizures.

MATERIALS AND METHODS

Patients with seizures

We obtained 41 sera from paediatric patients (3 to 18 years old) treated at the neurology Department of the Hospital Infantil de México, Ministry of Health, Mexico. Patients entered the hospital because they had seizures at the time of the study. The patients were on anticonvulsive treatment scheme with monthly control on the levels of anti-convulsant (valproic acid, phenobarbital or carbamazepine) in serum; also, the patients had a complete medical record and were available for review. Magnetic resonance imaging (MRI) or computed tomographic scans (CT scans) were performed according to the needs of each individual.

Healthy children

We examined 40 serum samples from clinically healthy children (60% girls, 40% boys with an age range 5 to 18 years) who attended a health centre in Mexico City for medical examination on their physical condition. The parent or custodian answered a brief clinical-epidemiological and socio-demographic questionnaire in order to identify risk factors associated with the transmission of *Taenia solium.*

Serum samples

A total of 3 ml of blood was taken and the serum sample was removed. Sera with haemolysis, lipemia or insufficiency (volume < 0.050 ml) were excluded. In all cases, the consent of the parent or custodian was obtained for the collection of samples and the determination of antibodies. The Research Committee of the Institute for Epidemiological Diagnosis and Reference, Ministry of Health, Mexico approved the research protocol.

Determination of antibodies

Determination of antibodies by ELISA was carried out using the AccuDiag™ Cysticercosis IgG (*Taenia solium*) ELISA Kit (Diagnostic Automation Inc., Woodland

Hills, CA, USA), which uses the *T. solium* cyst fluid as antigen and the values of sensitivity and specificity diagnostic reported by the manufacturer were of 88 and 96%, respectively. Serum samples were analysed at the same time by Western blot, which uses cyst glycoproteins as antigen. Sensitivity values range between 59% (a single cyst) and 95% (multiple cysts), while the value of specificity is 100% (Aguilar-Rebolledo *et al*., 2002; Proaño *et al*., 2002).

Statistical analysis

Absorbance values for ELISA were obtained at 450 nm; according with the manufacturer, a sample was positive when absorbance was equal to or greater than 0.3 OD units and a sample was negative when absorbance was less than 0.3 OD units. In Western blot, a positive sample recognize at least one of seven diagnostic glycoproteins (50, 39–42, 24, 21, 18, 14 and 13 kDa). Data statistical analysis was done using IBM SPSS Statistics (IBM Corporation, Armonk, NY, USA) and, the Chi square test was used to search for association between epidemiological variables using the OpenEpi software (Centers for Disease Control and Prevention, USA).

RESULTS

Socio-economic characteristics of the population

Table 1 shows the socio-economic characteristics of the population. The group of patients complied with 16 girls and 25 boys with an average age of 7.1 ± 3.8 years. There was no family history of clinical importance in 14 patients. However, febrile seizures (n = 2) or epilepsy (n = 2) were found, while in the rest of the children (n = 14) diabetes mellitus type 2, high blood pressure, smoking or alcoholism were found. With respect to the residence, 30 patients came from urban areas (19 from State of Mexico and 11 from Mexico City) and 11 from rural areas (1 from Guanajuato, 2 from Guerrero, 1 from Hidalgo, 1 from Morelos, 2 from Oaxaca, 1 from Puebla, 1 from San Luis Potosí and 2 from Veracruz). Poverty was the most repeatedly economic

Table 1. Socio-economic characteristics of the paediatric population in study

	Patients with seizures (N = 41)	Healthy Children (N = 40)	*p* (test[1])	Risk Ratio (Confidence Intervals)
Gender				
Female	39% (n = 16)	60% (n = 24)	0.0591 (Chi square)	0.66 (0.4176 – 1.031)
Male	61% (n = 25)	40% (n = 16)		
Age (average ± sd[2])	7.0 ± 3.7	10.3 ± 4.2	0.4666 (F) 0.0004 (Student's t)	
Socio-economic level				
Low[3]	93% (n = 38)	80% (n = 32)	0.9576 (Chi square)	1.99 (0.7406 – 5.349)
Medium	7% (n = 3)	20% (n = 8)		
Residence				
Rural	27% (n = 11)	0%	0.0003 Mid p - exact	2.33 (1.78 – 3.058)
Urban	73% (n = 30)	100%		
Overcrowding[4]				
Yes	63% (n = 26)	0%	0.0001 Mid p - exact	3.67 (2.382 – 5.645)
No	37% (n = 15)	100%		

[1] two-tails
[2] standard deviation
[3] include poverty
[4] three or more habitants per room.

stratum (n = 22), followed by the low (n = 16) and medium (n = 3) strata. Analysis of data shows that 26 patients were living in overcrowded conditions. With respect to housing, 73% of patients had cement housing, but 17% had blade ceiling and walls of cardboard/sheet. Only 10% of the studied patients had housing of mixed materials (sheet roof, ground, walls of cardboard floor). The healthy group clustered 24 girls and 16 boys; the average age was 10.3 ± 4.2 years. There was no history of seizures in the family; however, 12 donors presented diabetes mellitus type 2, high blood pressure, smoking or alcoholism. All donors resided in Mexico City (urban environment), with low socio-economic level. Dwellings were of cement without overcrowding.

Clinical features of the pediatric population with seizures

In general, 76% of the patients show generalized seizures; in particular, 14 patients showed tonic-clonic seizures, 6 patients with clonic seizures and 11 with absence, myoclonic or atonic. Partial seizures were observed in 10 patients (24%), 7 of them with simple crisis and 3 with complex. Table 2 shows that 93% of the patients (n = 38) had neuroimaging studies. MRI in 14 patients, CT scan in 4 and, both studies in 20. Electroencephalogram (EEG) was made in 18 patients of the group with MRI and CT scan. The presence of a cyst of unknown origin was reported in five patients, a tumor in one, a hyperintense image in 3 and multiple periventricular nodules in another one. Table 3 shows the clinical diagnosis of the 41 patients with seizures, in general, 24 patients reported idiopathic epilepsy, 12 patients reported neurological disorders (skull-brain trauma, encephalitis and tuberculosis sclerosis, among others) and five reported the presence of a cyst.

Serological data

The determination of antibodies to *Taenia solium* cysticerci is show in Table 3. The frequency of positive by ELISA was the 12.19% (n = 5) in paediatric patients with seizures, while no positives were detected in the healthy children group. The positive samples were classified in two groups; the former was integrated by 2 serum samples from children whose diagnosis was seizures in study, since we could not determine the cause of the crisis but a cyst was detected in the imaging studies. The remaining positive samples corresponded to patients with Down syndrome (n = 1), Lennox Gastaut syndrome (n = 1) and hippocampal sclerosis (n = 1). No positive samples were detected by Western blot in the patients neither in the healthy donors. In patients with seizures, the determination of antibodies by ELISA was associated with the observation of cysts by MRI (p = 0.047; Chi square test), but found no association with the socio-economic characteristics of the patients, the clinical family history or the clinical background.

Table 2. Diagnosis by medical imaging scans in patients with seizures

	Patients with image (N = 38)	Patients without image (N = 3)
Imaging scans	14 with MRI[1] 4 with CT scans[2] 20 with MRI + CT scans[3]	Not done
Results	28 with no structures 5 with a cyst 1 with a tumor 3 with hyperintense images 1 with periventricular nodules	–

[1] Nuclear magnetic resonance (MRI).
[2] Computed tomography (CT) scans.
[3] Includes 18 patients with electroencephalogram.

Table 3. Antibodies to *Taenia solium* cysticerci in serum samples of paediatric patients with seizures of unknown origin

		Patients with seizures (N = 41)		Healthy Children (N = 40)	
		Positive	Negative	Positive	Negative
ELISA		n = 5 (12%)	n = 36 (88%)	–	n = 40 (100%)
Western blot		–	n = 41 (100%)	–	n = 40 (100%)
Clinical diagnosis	Cyst	n = 2	n = 3		
	Sclerosis hippocampal	n = 1	n = 1		
	Lennox Gastaut syndrome	n = 1	n = 1		
	Down syndrome	n = 1	–		
	West syndrome		n = 1		
	Traumatic brain injury		n = 1		
	Supracranial tumor		n = 1		
	Encephalitis		n = 4		
	Idiopathic Epilepsy		n = 24		

DISCUSSION

Neurocysticercosis is a parasitic disease associated with the appearance of seizures at the third decade of life. Here, we found that 5/41 (12%) of pediatric patients with seizures were positive for antibodies against the *Taenia solium* cysticerci. However, it is worth highlighting that 3/5 came from patients with non-infectious, neurological disorders (Down syndrome, Lennox-Gastaut syndrome and hippocampal sclerosis). Previously, it has been reported that in Down syndrome children less than 6 years of age, the levels of serum immunoglobulins did not differ from healthy controls, but after that age, considerable hyper-IgG and -IgA were found (Nespoli *et al*., 1993), also in Lennox-Gastaut syndrome children, it has been reported a consisting of elevated IgG and IgM concentrations (Carvalho *et al*., 2014). Although it has been documented on the presence of hippocampal sclerosis in association with neurocysticercosis (Singla *et al*., 2007), in this work, the causality between neurocysticercosis and hippocampal sclerosis cannot be definitively proved, since no evidence of cysts was obtained during the imaging scan. Thus, it may be possible that the reactivity of the samples could be from the three patients with other neurological disorders who may be associated with a non-specific cross-reactivity.

The two remaining positive samples were found to be associated with a single cyst of unknown origin. According to a previously proposed cysticercosis diagnostic criteria (del-Brutto *et al*., 2001; Carpio *et al*., 2016), these clinical cases could be classified as probable neurocysticercosis. Three minor diagnostic criteria (positive serology, active seizures and compatible image) are associated to an epidemiological criteria. (Mexico is internationally considered an endemic area for neurocysticercosis). Thus, the probable frequency of neurocysticercosis in the patients studied with seizures was 4.9% (2/41 patients), while the frequency of seropositivity by ELISA was 12.2% (5/41 patients).

Since the determination of seroprevalence against an infectious agent is an indirect measure to estimate its circulation, searching for antibodies in children is especially significant. However, scarce data of cysticercosis in children from open populations are available. As an example, a study of 18 necropsies of preteens, which was performed in Mexico in 1987, reported a prevalence of 0.5%, which is far below to those reported for adults (Ridaura-Sanz, 1987). Recent data regarding antibodies against the cysticercus include a prospective study of 112 paediatric patients with neurocysticercosis, which showed that the most common symptoms were seizures and

intracranial hypertension; but the presence of antibodies was only determined in 30 cerebrospinal fluid by the complement fixation reaction. The reaction was positive in 3/11 patients with the image of one parasite in a degenerative form, while 14/19 patients with multiple degenerative cysts were positive (Antoniuk *et al.*, 2006). Recently, Zammarchi *et al.* (2016) reported in a retrospective study that there is a correlation between the type of cerebral lesion and the positivity of Western blot on serum in patients with neurocysticercosis. The frequency of positivity was lower in patients with single cerebral calcification (30%) than in patients with multiple cerebral calcifications (38.5%). The frequency of patients with single non-calcified lesion was 45.5% while, in patients with multiple non-calcified lesions it was 63.2%.

Numerous studies have shown that the neurocysticercosis in children is associated with the presence of a single cyst (Singhi & Singhi, 2009) and, considering this diagnostic criteria for neurocysticercosis (del-Brutto *et al.*, 2001; Carpio *et al.*, 2016), the data reported in this study showed that the probable frequency of neurocysticercosis in the patients studied with seizures was of 4.9% (2/41 patients). No surprise that the positive outcome of these samples has not been corroborated by Western blot, since previously has been reported that the diagnostic sensitivity value is of 59 to 65% when there is a single cyst (Aguilar-Rebolledo *et al.*, 2002; Proaño *et al.*, 2002).

Different strategies are in development to increase the standard of health in the general population as well as several campaigns for the prevention and control on the transmission of *Taenia solium* (Flisser & Correa, 2010). Here, we indirectly provide evidence to document the reduction in the transmission of cysticercosis in Mexico, at least in the urban areas. However, it is widely known that the prevalence and the actual incidence of neurocysticercosis are difficult to determine, since the signs and symptoms are heterogeneous and the diagnosis requires immunological and imageneological studies that are not available in the population at risk (Fleury *et al.*, 2010).

The absence of antibodies in the paediatric population is a clear factor that indicates a restricted contact with the parasite. However, it is important for the creation of an algorithm of diagnosis to conclude the study of clinical cases, as demonstrated in this study, where the presence of antibodies was determined in 2/5 patients with seizures but with the presence of of a cyst. At the end of this study, the clinical records of the patients with cerebral cyst were reviewed again but no additional data were found. This finding could re-orient the clinical diagnosis in similar cases. Thus, the differential diagnosis with other pathologies such as tumours, tuberculoma, toxoplasmatic abscess, pyogenic abscess and meningoencephalitis among others, is important. The inclusion of cysticercosis diagnosis in an algorithm for differential testing probes could help establish a conclusive diagnosis in patients with seizures.

In conclusion, here we report a frequency of antibodies of 12% (5/41) in patients with seizures but, according the diagnostic criteria for neurocysticercosis previously proposed by del-Brutto *et al.* (2001) and recently by Carpio *et al.* (2016), the probable frequency of neurocysticercosis in patients with seizures was 4.9% (2/41). Data reported here suggest the necessity to establish an algorithm to confirm or exclude neurocysticercosis in paediatric patients with seizures. The absence of antibodies in the paediatric population supports the evidence to demonstrate the interruption of *Taenia solium* transmission.

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