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Seroprevalence of *Taenia solium* infections in Croatian patients presenting with epilepsy

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Abstract

Epilepsy is one of the most common neurological disorders, while neurocysticercosis caused by *T. solium* infection of the central nervous system currently represents the leading cause of secondary epilepsy in Central and South America, East and South Asia, and sub-Saharan Africa. As a result of increased migration from these endemic regions, neurocysticercosis and subsequent epilepsy are becoming a growing public health problem in developed countries, as well. In order to determine the prevalence of *T. solium* infection in patients with epilepsy in Croatia, a retrospective serological study was conducted. A total of 770 serum samples were tested for the presence of *T. solium* IgG antibodies using commercial qualitative enzyme immunoassay (*Taenia solium* IgG-ELISA, Bioactiva Diagnostica GmbH). Western blot technique (LBDIO Diagnostics) was used as a confirmatory test for the diagnosis. The overall seroprevalence rate of *T. solium* infection in patients with clinically proven epilepsy was 1.5%. Although the results have shown that infection with this tapeworm is rare in Croatia, this study hopes to increase awareness about the importance of preventive measures and benefits of accurate and timely diagnosis. Intervention measures for infection control are crucial, namely sanitation improvement, control of domestic pig-breeding, detailed meat inspection, detection and treatment of tapeworm carriers, hand washing and health education.

Introduction

Taenia solium, also called the pork tapeworm, is a parasite belonging to the Taeniidae family, which can cause taeniasis and cysticercosis in humans. Taeniasis can be found strictly in the human host, after ingestion of raw or undercooked pork meat contaminated with the larval stage (*Cysticercus cellulosae*) of this tapeworm. The activity of proteolytic enzymes in the stomach liberates the larvae which develop into adult worms in the small intestine. Body of a fully developed tapeworm can be from two to seven meters long, consisting of several hundred proglottids with huge number of infective eggs (Flisser, 1994). As adult worms do not cause substantial damage to the intestine, they can persist there for years, usually without any symptoms (Schantz *et al.*, 1998). What causes concern regarding *T. solium* infection is human cysticercosis, which results either from the ingestion of parasitic eggs passed in human stool directly, or after faecal-oral transmission from food or water contaminated with eggs.

Whilst outside of the central nervous system (CNS) tapeworm larvae cause no major symptoms, the ones in the brain (neurocysticercosis) are associated with significant morbidity and mortality (Garcia *et al.*, 2003). Neurocysticercosis is considered the most common parasitic infection of the CNS and the main cause of epilepsy in the developing countries (Roman *et al.*, 2000). Epileptic seizures are usually the only manifestation of this disease, and can be found in 50-80% of patients with parenchymal brain cysts or calcifications (Chopra *et al.*, 1981, Del Bruto *et al.*, 1992, Commision on Tropical Diseases, 1994). Although cysticercosis is endemic to Asia, Africa and Latin America (Willingham & Engels, 2006), it has been diagnosed more frequently in developed countries too (Schantz *et al.*, 1998, White, 2000).

According to references, 50 million people worldwide are estimated to have *T. solium* infection, while each year 50 thousand die of cysticercosis (Eddi, 2003). Data on the prevalence of this infection in Europe is scarcely available and nonexistent when it comes to Croatian population.

The primary goal of this research was to establish the prevalence of *T. solium* infection in Croatian patients with clinically manifest epilepsy.

Material and methods

Between 2005 and 2009, a total of 770 serum samples were collected from patients aged 3-76 with a clinical diagnosis of epilepsy. All were referred to the Croatian National Institute of Public Health with a clinical diagnosis of epilepsy and no other available clinical or diagnostic data. This study was approved by the Ethical Committee of the Croatian National Institute of Public Health.

Collection and analysis of serum samples

Serum samples were obtained from patients in rural and urban areas of Croatian mainland. Serological analysis was performed at the Parasitology Department of the Croatian National Institute of Public Health (CNIPH). For the determination of IgG-class antibodies against *T. solium*, a commercial qualitative enzyme immunoassay (*Taenia solium* IgG-ELISA, Bioactiva Diagnostica GmbH, Germany) was used, according to manufacturer's recommendations. Manufacturer also stated a diagnostic sensitivity of 93.8% and diagnostic specificity of > 95%.

As cross reaction can occur in ELISA test, positive samples were confirmed using western blot method. For that purpose, *Cysticercosis* Western Blot (WB) IgG test (LDBIO diagnostics, Lyon, France) was used. According to WB manufacturer's instructions, there are six bands especially chosen for their specificity for Cysticercosis antibodies: 6-8 kDa, 12 kDa, 23-26 kDa, 39 kDa, 45 kDa and 50-55 kDa. The presence of a minimum of two well-defined bands of the above six on the strip is indicative of the existence of IgG-class antibodies in the tested sample. Manufacturer also stated a diagnostic sensitivity of 96.1% and diagnostic specificity of up to 100%.

Data analysis was performed using chi-square test. A *p* value less than 0.05 was considered to be significant.

Results

Of 770 analyzed serum samples, 396 (51%) were from male and 374 (49%) from female patients. In 23 (3%) serum samples from patients with epilepsy, IgG antibodies to *T. solium* were detected by ELISA: 52% among male and 48% among female patients. Of 23 positive serum samples, 11 (1.5%) were confirmed by WB. Using WB, the seroprevalence rate was established at 1.77% (7/396) for male and 1.07% (4/374) for female patients, with no statistically significant difference (p = 0.609).

The seroprevalence rate in patients residing in urban regions was 1.4% (7/484), identical to the seroprevalence rate in patients residing in rural regions (4/286).

In a group of patients under 18, a positive serum was found in only one subject, which accounts for the seroprevalence rate of 0.4% (1/249), compared to 1.92% (10/521) of positive subjects in a group of patients over 18. Although seropositivity differed between the two evaluated age groups, this difference was not statistically significant (p = 0.181).

Discussion

Epilepsy is one of the most common and challenging neurologic disorders, while neurocysticercosis caused by *T. solium* infection of the CNS currently represents the leading cause of secondary (acquired) epilepsy in adults in Central and South America, East and South Asia, and sub-Saharan Africa (Scott *et al.*, 2001, Raccurt *et al.*, 2009). As a result of increased migration from these endemic regions,

neurocysticercosis and subsequent epilepsy are considered one of the growing public health problems in developed countries, too (Schantz *et al.*, 1998, White, 2000, Carpio, 2002).

The overall seroprevalence rate of *T. solium* infection, as reported hereby, was 3% using ELISA and 1.5% when confirmed with WB. Although WB assay is generally recommended for immunodiagnostic of neurocysticercosis, ELISA is more commonly used in epidemiological studies as it is technically simpler and cheaper (Carpio, 2002). Gekeler and co-authors concluded that using ELISA as a screening method and immunoblot as a confirmatory test considerably improves diagnosis of this disease (Gekeler *et al.*, 2002). In order to determine the value of immunodiagnosis for neurocysticercosis, the authors tested 222 serum and cerebrospinal fluid samples from patients with neurocysticercosis and healthy subjects. The sensitivities of ELISA and immunoblot test in neurocysticercosis patients were almost identical (80% and 81.7%, respectively). The overall specificity of ELISA was only 75.3% due to frequent false-positive results, but the specificity of immunoblot test was clearly higher (99.4%). Even though WB is confirmed as a superior method by a majority of studies, some authors suggest considering ELISA as the better method for the serological confirmation of neurocysticercosis in children, as it ensures higher specificity and diagnostic efficacy than the dot-blot assay in the examined population (Mandal *et al.*, 2006).

Seroprevalence of this infection in the group of patients over 18 was not significantly higher in comparison with the group of patients under 18 (where only one case was serologically confirmed). Furthermore, no statistically significant difference was found in the infection rate in terms of gender or residence. All patients from whom sera were collected and tested were residents of Croatia at the time. It must be noted that the presence of cysticercal antibodies only suggests that the patients have acquired the infection, not necessarily in Croatia.

Several problems need to be addressed. Tests which have been used are mainly geared to detect infection with the larval stage of cysticercosis, so their validity with the adult stage is less well known. Furthermore, a positive serological test for cysticercosis may only indicate that the patient has had contact

with *T. solium* antigens in various forms. Cysticercosis can also be situated in another organ (such as skeletal muscle), or may already be resolved with circulating antibodies (Serrano Ocana *et al.*, 2009). In patients with neurocysticercosis, clinical presentation and the results of neuroimaging procedures vary extensively and often do not facilitate a definite diagnosis (Gekeler *et al.*, 2002).

Studies based on hospital series have reported that 50% of the 100 patients studied in Mexico have neurocysticercosis as the main cause of late-onset epilepsy (Medina *et al.*, 1990). In a large series studied in Colombia and Peru where EITB method was used, cysticercosis was proven as the cause of epilepsy in 14% and 12% of the cases, respectively (Garcia *et al.*, 1993, Palacio *et al.*, 1998). It must be noted that there are very few studies on the neurocysticercosis in Europe. Seizures were the most common inaugural sign in 29 patients with neurocysticercosis diagnosed in south-eastern France between 1988 and 1999 (Rousseau *et al.*, 1999). A recent study in an Indian community suggested that the region-specific prevalence rates of epilepsy in India are partly dependent on the prevalence of neurocysticercosis in the given community (Goel *et al.*, 2011).

This study provides important and previously unknown data on the prevalence of *T. solium* infection in Croatian patients with epilepsy. Although infection with this tapeworm is rare in Croatia, this study will, hopefully, increase the awareness of the importance of preventive measures and benefits of accurate and timely diagnosis among physicians and public health workers. Intervention measures for infection control are crucial, namely sanitation improvement, control of domestic pig-breeding, detailed meat inspection, detection and treatment of tapeworm carriers, hand washing and health education.

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