Evidence of human neurocysticercosis in Slovenia

BARBARA ŠOBA[1], BOJANA BEOVIĆ[2], ZALA LUŽNIK[2], MIHA SKVARČ[1] *and* JERNEJ LOGAR[1]*

[1] *Faculty of Medicine, Institute of Microbiology and Immunology, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia*
[2] *Department of Infectious Diseases, University Medical Centre Ljubljana, Japljeva 2, 1000 Ljubljana, Slovenia*

(*Received 3 July 2013; revised 22 September 2013; accepted 13 October 2013; first published online 26 November 2013*)

SUMMARY

To assess the prevalence of *Taenia solium* cysticercosis in patients with neurological disorders in Slovenia, serum/cerebrospinal fluid (CSF) samples from 348 suspected patients were collected between the beginning of January 2001 and the end of December 2012 and analysed serologically for the presence of anti-*T. solium* IgG antibodies. Of 20 patients whose samples tested positive or equivocal by enzyme-linked immunosorbent assay (ELISA), samples of 7 patients were confirmed positive by Western blot (WB). The overall seroprevalence rate of *T. solium* infection in patients with neurological disorders included in the study was 2.0%. Serological results of positive patients corresponded to clinical and/or imaging findings concerning their brain cysts. Based on their personal data, it was ascertained that neurocysticercosis (NCC) positive patients had immigrated or came to Slovenia from the former Yugoslav republics. Since the disease is believed not to be endemic in Slovenia we assume that all of the NCC-positive patients had acquired the infection before immigration to Slovenia or visiting or being visited by their relatives infected with an adult *T. solium* parasite. The present results represent the first insight into the prevalence of NCC in patients with neurological disorders in Slovenia.

Key words: neurocysticercosis, seroprevalence, enzyme-linked immunosorbent assay, Western blot, Slovenia.

INTRODUCTION

Neurocysticercosis (NCC) is the most common parasitic disease of the nervous system in humans and is the main cause of adult-acquired epilepsy in areas in which infection with *Taenia solium*, also called the pork tapeworm, is endemic. The disease is acquired by accidental ingestion of tapeworm eggs shed in the feces of humans infected with an adult *T. solium* parasite. Once in the gastrointestinal tract, the eggs liberate hexacanth embryos or oncospheres. Oncospheres penetrate the intestinal wall and can migrate with the blood almost anywhere in the body, most often to the central nervous system where they transform into cysticerci and may cause NCC (Carpio, 2002; García *et al*. 2003; Flisser, 2013).

NCC is associated with significant morbidity and mortality. It has been suggested that several million people worldwide have an infection, while 50 000 of them die of it annually (Eddi *et al*. 2003). Epileptic seizures are the most common presentation of NCC occurring in up to 75% of infected patients. NCC can also be presented with intracranial hypertension, which develops in approximately 25% of cases, focal neurological deficits, meningitis, myeloradiculopathy, mental status changes, encephalitis, dizziness and visual disturbances (Sinha and Sharma, 2009).

NCC is endemic in Latin America, parts of Africa and Oceania and most of Asia (García *et al*. 2003). It is presumed that NCC continues to be endemic in Eastern Europe as well (Del Brutto, 2012*a*). Although transmission is absent or rare in most developed countries an increasing frequency of NCC in non-endemic areas due to international travel and migration has been observed (White, 1997; Nash and García, 2011; Del Brutto, 2012*a*). Slovenia is not considered an endemic region for the infection. However, due to frequent immigration from countries of the former Yugoslavia with autochthonous NCC (Doder *et al*. 2002; Talan-Hranilovic *et al*. 2002; Titlic *et al*. 2007; Meštrović *et al*. 2012), cases of NCC are expected in Slovenia. The aim of this study was to examine whether patients with neurological disorders in Slovenia are infected with the larvae of *T. solium*.

MATERIALS AND METHODS

Between 1 January 2001, and the end of December 2012, 348 patients suspected of having NCC were examined serologically for the presence of anti-*T. solium* IgG antibodies at the Laboratory of Parasitology, Institute of Microbiology and Immunology, Faculty of Medicine Ljubljana. The suspicion of NCC was based on clinical data and/or imaging findings by ultrasound scanning or computerized

* Corresponding author: Faculty of Medicine, Institute of Microbiology and Immunology, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia. E-mail: jernej.logar@mf.uni-lj.si

Parasitology (2014), **141**, 547–553.
doi:10.1017/S0031182013001947

tomography of brain lesions. The requirement for cysticercosis serology was left to the discretional criteria of the attending clinician. A total of 421 serum samples and 110 cerebrospinal fluid (CSF) samples were collected from patients of both genders and different ages. Sera and CSFs were first analysed by commercial qualitative enzyme-linked immunosorbent assay (ELISA) (*T. solium* IgG ELISA, NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany; Cysticercosis (*T. solium*) – Serologie, Biotrin International, Lyon, France). ELISA positive or equivocal samples were confirmed by Western blot (WB) (Cysticercosis WB IgG, LDBIO Diagnostics, Lyon, France). According to the manufacturer's criteria, six bands on the membrane strip (6–8, 12, 23–26, 39, 45 and 50–55 kDa) bearing electrophoretically separated *T. solium* larval antigens have been especially chosen for their specificity for cysticercosis antibodies. When serum samples are tested for the presence of antibodies to *T. solium* larvae antigens, the presence on the strip of at least two well-defined bands among the six mentioned above is indicative of cysticercosis/NCC while in the case of CSF samples, the presence on the strip of one well-defined band among the six mentioned above is enough. The presence on the strip of the 6–8 kDa band has been described as indicative of active cysticercosis/NCC (Simac *et al*. 1995). While, according to the manufacturer, a weak cross-reactivity of anti-*Echinococcus* antibodies with some of *T. solium* larvae antigens is possible, all WB confirmed cysticercosis positive samples were also tested for the presence of anti-*Echinococcus* IgG antibodies using the *Echinococcus* WB IgG (LDBIO Diagnostics, Lyon, France).

RESULTS

Of 421 serum samples and 110 CSF samples corresponding to 348 patients with neurological disorders, 39 sera from 20 patients and 7 CSF samples from 4 patients were tested positive or equivocal by ELISA for the presence of anti-*T. solium* IgG antibodies. Of the ELISA positive or equivocal samples 17 sera from 7 patients and 7 CSF samples from 4 patients were tested positive by WB. Altogether, 7/348 (2.0%) patients tested positive by WB (Table 1). Demographic and clinical data of patients confirmed to be NCC positive by WB and, for comparison, of patients tested positive or equivocal by ELISA but negative by WB are presented in Tables 1 and 2, respectively.

The mean age of NCC positive patients was 44.1 years. All of them were immigrants or came to Slovenia from countries of the former Yugoslavia. According to the manufacturer's criteria for the interpretation of WB results 6 patients suffered from active NCC, since their samples were positive for 6–8 kDa band and 1 patient had inactive NCC (Table 1, Fig. 1). All WB positive serum and CSF samples were negative for echinococcosis according to the *Echinococcus* WB assay, indicating that cysticercosis-positive WB results were not the reflection of anti-*Echinococcus* IgG antibodies in patients' samples.

The mean age of patients tested positive or equivocal by ELISA but negative by WB was 41.5 years. Slovenia is the country of origin of 10/13 of these patients. Two were immigrants from countries of the former Yugoslavia and one was an immigrant from Africa. Clinical data and/or definitive diagnosis of 10/13 patients were successfully acquired. For nine of them NCC could be excluded on the basis of other definitive diagnosis but no aetiology was confirmed for the remaining one.

DISCUSSION

NCC is still endemic in Eastern Europe. Moreover, its prevalence is increasing in some non-endemic Western European countries so that the disease may become a public health problem in these countries in the next few years (Del Brutto, 2012*a*). Indeed, in his review of the Western European literature on NCC over the past 40 years Del Brutto identified 779 cases of NCC. Of these, only 28 were diagnosed before 1985. This rise in prevalence is mainly due to immigration of carriers from endemic areas and tourists coming back from endemic countries. Portugal and Spain with 384 and 228 NCC patients, respectively, are the countries with the largest number of reported cases from 1970 to 2011, followed by France, the UK, Italy and Germany (Del Brutto, 2012*a*). Recently, a systematic review on epidemiology and management of cysticercosis and *T. solium* in Europe from 1990 to 2011 was published by The COHEMI (COordinating resources to assess and improve HEalth status of MIgrants from Latin America) Project Study Group. Like Del Brutto, the authors find that imported cases of cysticercosis, of which 74.7% were diagnosed in migrants and 17.6% in European travellers, show an increasing trend (Zammarchi *et al*. 2013).

In Slovenia, *T. solium* taeniasis was virtually eliminated by adequate swine husbandry and improved meat inspection and therefore the country is not considered endemic for cysticercosis. Nevertheless, it has to be stressed that Slovenia played an important role in international migrations in the last two decades, and so imported cases of NCC are to be expected. The most important were the southeast to northwest migrations from the former Yugoslavia (made up of six republics: Bosnia and Herzegovina, Croatia, Macedonia, Montenegro, Serbia, Slovenia), especially from Bosnia and Herzegovina. There is no internationally published data on endemicity of human cysticercosis in Bosnia and Herzegovina. However, from 1991, when Slovenia declared its

https://doi.org/10.1017/S0031182013001947 Published online by Cambridge University Press

Table 1. Demographic and clinical data of patients with neurocysticercosis

Patient	Gender	Age (years)	Year of established diagnosis	Country of origin	Samples confirmed positive	Neurological manifestation; Pathology	Active/inactive NCC
1	M[a]	51	2001	Former Yugoslav republic	Serum	NS[d]	Active
2	F[b]	41	2001	Bosnia and Herzegovina	Serum, CSF[c]	Headache, chronic meningitis; diffuse granular parenchymal calcifications (cerebral and cerebellar)	Active
3	M	41	2003	Former Yugoslav republic	Serum[e]	Epilepsy, headache, mental changes, hand tremor; right temporopolar cyst	Active
4	F	47	2004	Bosnia and Herzegovina	Serum, CSF	Headache, dizziness, chronic meningitis; subarachnoid and frontobasal cyst	Active
5	F	40	2006	Bosnia and Herzegovina	Serum[e]	Epilepsy; Parenchymal calcifications	Inactive
6	F	58	2010	Bosnia and Herzegovina	Serum, CSF	Headache, mental changes, basal meningitis; cyst above planum sphenoidale, small frontotemporal cysts, hydrocephalus, arachnoiditis	Active
7[f]	M	31	2012	Serbia	Serum, CSF	Mental changes; hydrocephalus, intraventricular cyst	Active

[a]M = male; [b]F = female; [c]CSF = cerebrospinal fluid; [d]NS = not specified; [e]CSF negative by WB; [f]Not a resident of Slovenia.

independence from Yugoslavia, until the end of 2000, five cases of NCC were detected in Slovenia, three of the patients being immigrants from Bosnia and Herzegovina (unpublished data), suggesting that this country could be endemic for the disease. In Croatia, Slovenia's neighbouring country, seven autochthonous cases of NCC were described by Talan-Hranilovic *et al*. between 1988 and 2000 (Talan-Hranilovic *et al*. 2002) and an additional case was reported by Titlic *et al*. (2007). Apart from that, 11 out of 770 (1.77%) patients with epilepsy were found to be seropositive for NCC in a prevalence study conducted in Croatia from 2005 to 2009 (Meštrović *et al*. 2012). Serbia, another ex-Yugoslav republic, is also endemic for the disease with 78 autochthonous cases from 1990 to 2011 (Doder *et al*. 2002; Zammarchi *et al*. 2013). There are no data on endemicity of cysticercosis in Macedonia and Montenegro. In Slovenia's other neighbouring countries, Austria, Hungary and Italy, almost all of the reported cysticercosis cases were imported (Zammarchi *et al*. 2013). Moreover, immigrations to Slovenia from these and other European and world countries are scarce.

The overall seroprevalence rate of NCC in the present study which included patients with neurological disorders from the beginning of 2001 until the end of 2012 was 2.0%. Seven patients were found to be infected. All of them were immigrants or came to Slovenia from countries of the former Yugoslavia (Table 1). However, all but one of seven positive patients were residents of Slovenia at the time their samples were collected and the study was conducted. The youngest, a 31-year-old seropositive patient from Serbia (Patient 7, Table 1) came to Slovenia for medical consultation concerning his serious neurological condition. He was not a resident of Slovenia at that time. However, medical treatment at University Medical Centre Ljubljana, Slovenia, where NCC was diagnosed, had been recommended to him by his relatives, who had immigrated to Slovenia years before. Although Serbia is endemic for cysticercosis, he was believed to have acquired the infection while a shepherd in Bosnia and Herzegovina. Excluding Patient 7 for not being a resident of Slovenia, the incidence of NCC in Slovenia in the period from 2001 to 2012 was estimated to be $0.30/10^5$ inhabitants, with a mean annual incidence of 0.025 cases per 10^5 inhabitants.

The diagnosis of NCC relies on the correlation of clinical features with the results of neuroimaging (Bale Jr., 2000). Serology mostly has a confirmatory role and should be used in conjunction with neuroimaging (García *et al*. 2003, 2012). Of serological techniques ELISA and WB are most commonly used (Carpio, 2002). In the present study, samples from 20/348 patients were tested positive or equivocal by ELISA. This gives the seroprevalence rate of 5.7% using ELISA. However, the seroprevalence dropped

https://doi.org/10.1017/S0031182013001947 Published online by Cambridge University Press

Table 2. Demographic and clinical data of patients tested positive or equivocal by ELISA but negative by Western blot (WB) for the presence of anti-*T. solium* IgG antibodies

Patient	Gender	Age (years)	Country of origin	Samples positive/ equivocal by ELISA and negative by WB	Clinical manifestation; Pathology	Definitive diagnosis
8	F[b]	52	Slovenia	Serum	Encephalitis, left facial paraesthesia, ptosis, diplopia, headache; diffuse degenerative deformations and stenotic changes in spinal channel	Echinococcosis
9	M[a]	20	Slovenia	Serum	Headache, nausea; hydrocephalus, cystic intracranial formation which was surgically removed	Medulloblastoma
10	M	4	Slovenia	Serum	N/A[c]	N/A
11	F	33	Slovenia	Serum	Dizziness, retrobulbar pain, fever, peripheral eosinophilia	Sinusitis
12	F	36	Slovenia	Serum	Weakness, headache	Sleep disorder
13	F	50	Slovenia	Serum[d]	Headache, dizziness, visual impairment, arthralgia	Viral meningitis
14	M	47	Former Yugoslav republic	Serum	Ear pain, tinnitus, hypoacusis	Spinal channel echinococcosis
15	F	56	Slovenia	Serum[d]	Headache, facial paresis, vertigo	Lyme borreliosis
16	M	35	Slovenia	Serum	N/A	N/A
17	M	26	Nigeria	Serum	N/A	Pneumonia, pericarditis
18	M	80	Slovenia	Serum	N/A	Echinococcosis
19	M	53	Slovenia	Serum[d]	N/A	N/A
20	F	48	Serbia	Serum[d]	Headache, calcifications in the brain	No aetiology confirmed, stable

[a]M = male; [b]F = female; [c]N/A = data not available; [d]CSF negative by ELISA and by WB.

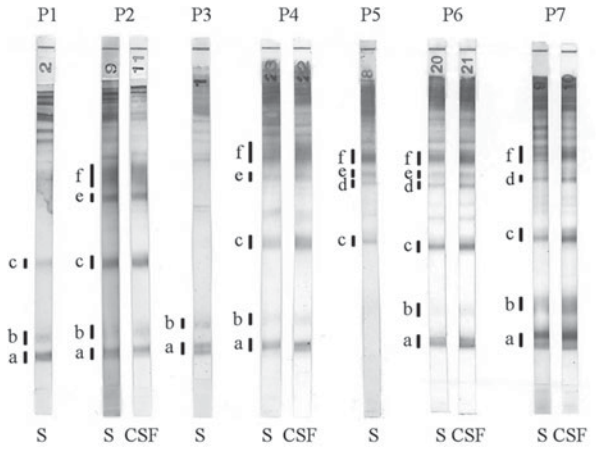

Fig. 1. Positive results of Western blot (WB) assay of serum (S) and cerebrospinal fluid (CSF) samples from seven patients (P1–P7) with neurocysticercosis. Cysticercosis specific bands are: a (6–8 kDa), b (12 kDa), c (23–26 kDa), d (39 kDa), e (45 kDa) and f (50–55 kDa). Band a is indicative of active cysticercosis.

to 2% (7/348 patients) when the seropositivity of ELISA-positive or equivocal patients was confirmed by WB. Other authors had also reported lower specificity of ELISAs and recommended the use of WB as a confirmatory test in the laboratory diagnosis of cysticercosis (Gekeler *et al*. 2002). WB is reported to have sensitivity of 98% and specificity approaching 100% for patients with two or more cystic or enhancing cerebral lesions (Tsang *et al*. 1989). Therefore, the use of ELISA as a screening test and immunoblot as a confirmatory test contribute considerably to the diagnosis of the disease (Gekeler *et al*. 2002). However, it has to be stressed that the sensitivity of WB is much lower (50% or even less) in patients with a single cerebral cyst or in those with calcifications alone (Wilson *et al*. 1991; Rajshekhar and Oommen, 1997; Singh *et al*. 2010). In light of this information it is possible that some patients with NCC might have been missed by serology in our study. To check the clinical status for patients tested positive or equivocal by ELISA but negative by WB we tried to collect their demographic and clinical data retrospectively from their clinicians. Clinical data and/or definitive diagnosis of 10/13 patients were successfully acquired (Table 2). For all but one of these 10 patients NCC could be excluded on the basis of other definitive diagnoses. For a 48-year-old female (Patient 20, Table 2) with headache and calcifications in her brain who immigrated to Slovenia from Serbia no aetiology was confirmed and therefore NCC could not be ruled out. We do not have the data on definitive diagnosis for serologically negative patients. Our laboratory is the only one performing serology on cysticercosis in Slovenia. Had cysticercosis been among the mandatory reportable diseases we could verify the accuracy of our test results by comparing the list of reported cases at the national Institute of Public Health with the list of patients tested serologically for cysticercosis.

As reported by Fleury *et al*. (2010) the prevalence of NCC is age-dependent. Namely, in population-based epidemiological studies performed in Mexico, the prevalence of NCC was found to increase with age and reach a maximum in subjects 46–55 years old (Sarti-Gutierrez *et al*. 1988; Sarti *et al*. 1992; Fleury *et al*. 2003, 2006). In a study conducted in Mbulu District, Tanzania, the highest prevalence rate was detected in the age group of 16–45 years (Mwang'onde *et al*. 2012). Moreover, the mortality rate due to cysticercosis was the highest between the ages of 15 and 54 with a mean age of 40.5 years in a study by Sorvillo *et al*. conducted in the USA (Sorvillo *et al*. 2007). Seropositive patients in our study were from 31 to 58 years old, with a mean age of 44.1 years, which is in agreement with the above-mentioned studies.

Entering the central nervous system (CNS), cysticerci of the parasite are viable and as such they evoke minimal or no inflammatory responses in the surrounding tissues. After a variable time the parasite degenerates. Most symptoms in NCC are the direct result of the inflammatory process that accompanies its degeneration. This stage is called an active stage of NCC (Bale Jr., 2000; Moskowitz and Mendelsohn, 2010; Del Brutto, 2012*b*). The degenerative process ends with the transformation of the parasite into a calcified nodule. This inactive stage of NCC is associated with decreased inflammation. However, on the basis of recent data it has been hypothesized that calcifications may experience periodic morphological changes which may expose trapped parasitic antigens to the host immune system and result in recurrent seizures, focal neurological deficits or recurrent episodes of headache in some patients (Nash *et al*. 2008; Ooi *et al*. 2011; Del Brutto and Del Brutto, 2012; Gupta *et al*. 2012; Rathore and Radhakrishnan, 2012; Del Brutto, 2013). All but one of seven patients who were confirmed to be seropositive for cysticercosis in our study had active cysticercosis according to WB (Fig. 1). In these six patients active NCC was presented clinically as well (Table 1). For the patient with inactive NCC (Patient 5) presented by parenchymal calcifications and epilepsy, the 6–8 kDa band on the WB strip, described as indicative of active cysticercosis/NCC, was absent (Fig. 1). Moreover, this patient's CSF was negative for the presence of anti-*T. solium* antibodies.

This study provides important data on the prevalence of *T. solium* cysticercosis in patients with neurological disorders in Slovenia. Although the results showed that NCC cases are rare in Slovenia and are particularly due to immigrations from endemic countries, a search for a possible adult tapeworm carrier in the NCC patient's immediate environment or for an infestation by adult tapeworm in NCC patient himself should be considered.

https://doi.org/10.1017/S0031182013001947 Published online by Cambridge University Press

Namely, several studies have demonstrated that the presence of a tapeworm carrier in the household is the main risk factor for NCC (Sarti-Gutierrez *et al*. 1988; Flisser, 2002; Lescano *et al*. 2007). Moreover, even though the study by The COHEMI Project Study Group suggests that the risk of spreading cysticercosis in Europe from asymptomatic tapeworm carriers coming from abroad is quite low, as only five cases of *T. solium* immigrant carriers were found, its authors agree that the possibility of spreading cysticercosis this way cannot be ruled out (Zammarchi *et al*. 2013). To conclude, clinicians in Slovenia should include NCC in the differential diagnosis for each immigrant patient and returning traveller with CNS involvement. Moreover, the accuracy of epidemiological information which is currently inadequate due to the lack of specific surveillance systems should be improved. As proposed by The COHEMI Project Study Group this could be achieved by including cysticercosis among the mandatory reportable diseases in Europe.

FINANCIAL SUPPORT

The work was supported by Grant No. 0381-029, P3-0083, from the Slovenian Research Agency.

REFERENCES

Bale, J. F., Jr. (2000). Cysticercosis. *Current Treatment Options in Neurology* **2**, 355–360.

Carpio, A. (2002). Neurocysticercosis: an update. *Lancet Infectious Diseases* **2**, 751–762.

Del Brutto, O. H. (2012*a*). Neurocysticercosis in Western Europe: a re-emerging disease? *Acta Neurologica Belgica* **112**, 335–343. doi: 10.1007/s13760-012-0068-3.

Del Brutto, O. H. (2012*b*). Neurocysticercosis: a review. *Scientific World Journal* 159821. doi: 10.1100/2012/159821.

Del Brutto, O. H. (2013). Neurocysticercosis: new thoughts on controversial issues. *Current Opinion in Neurology* **26**, 289–294. doi: 10.1097/WCO.0b013e32836027fa.

Del Brutto, O. H. and Del Brutto, V. J. (2012). Calcified neurocysticercosis among patients with primary headache. *Cephalalgia* **32**, 250–254. doi: 10.1177/0333102411433043.

Doder, R., Madle-Samardzija, N., Canak, G., Vukadinov, J., Turkulov, V. and Sević, S. (2002). [Neurocysticercosis – 5 years' experience at the Clinic for Infectious Diseases.] *Medicinski Pregled* **55**, 523–527.

Eddi, C., Nari, A. and Amanfu, W. (2003). *Taenia solium* cysticercosis/taeniosis: potential linkage with FAO activities; FAO support possibilities. *Acta Tropica* **87**, 145–148.

Fleury, A., Gomez, T., Alvarez, I., Meza, D., Huerta, M., Chavarria, A., Carrillo Mezo, R. A., Lloyd, C., Dessein, A., Preux, P. M., Dumas, M., Larralde, C., Sciutto, E. and Fragoso, G. (2003). High prevalence of calcified silent neurocysticercosis in a rural village of Mexico. *Neuroepidemiology* **22**, 139–145.

Fleury, A., Morales, J., Bobes, R. J., Dumas, M., Yánez, O., Piña, J., Carrillo-Mezo, R., Martínez, J. J., Fragoso, G., Dessein, A., Larralde, C. and Sciutto, E. (2006). An epidemiological study of familial neurocysticercosis in an endemic Mexican community. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **100**, 551–558.

Fleury, A., Escobar, A., Fragoso, G., Sciutto, E. and Larralde, C. (2010). Clinical heterogeneity of human neurocysticercosis results from complex interactions among parasite, host and environmental factors. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **104**, 243–250. doi: 10.1016/j.trstmh.2010.01.005.

Flisser, A. (2002). Risk factors and control measures for taeniosis/cysticercosis. In *Cestode Zoonoses: Echinococcosis and Cysticercosis, an Emergent and Global Problem*, Vol. 341. (ed. Craig, P. and Pawlowski, Z.), pp. 335–342. IOS Press, NATO Science Series, Amsterdam, the Netherlands.

Flisser, A. (2013). *Taenia solium, Taenia saginata* and *Taenia asiatica*. In *Guide to Foodborne Pathogens* (ed. Labbé, R. G. and García, S.), pp. 317–328. John Wiley & Sons, Chichester, UK. doi: 10.1002/9781118684856.fmatter.

García, H. H., Gonzalez, A. E., Evans, C. A. and Gilman, R. H.; for the Cysticercosis Working Group in Peru (2003). *Taenia solium* cysticercosis. *Lancet* **362**, 547–556.

Garcia, H. H., Rodriguez, S., Gilman, R. H., Gonzalez, A. E. and Tsang, V. C.; for the Cysticercosis Working Group in Peru (2012). Neurocysticercosis: is serology useful in the absence of brain imaging? *Tropical Medicine and International Health* **17**, 1014–1018. doi: 10.1111/j.1365-3156.2012.03037.x.

Gekeler, F., Eichenlaub, S., Mendoza, E. G., Sotelo, J., Hoelscher, M. and Löscher, T. (2002). Sensitivity and specificity of ELISA and immunoblot for diagnosing neurocysticercosis. *European Journal of Clinical Microbiology and Infectious Diseases* **21**, 227–229.

Gupta, R. K., Awasthi, R., Rathore, R. K., Verma, A., Sahoo, P., Paliwal, V. K., Prasad, K. N., Pandey, C. M. and Narayana, P. A. (2012). Understanding epileptogenesis in calcified neurocysticercosis with perfusion MRI. *Neurology* **78**, 618–625. doi: 10.1212/WNL.0b013e318248deae.

Lescano, A. G., Garcia, H. H., Gilman, R. H., Guezala, M. C., Tsang, V. C., Gavidia, C. M., Rodriguez, S., Moulton, L. H., Green, J. A. and Gonzalez, A. E.; Cysticercosis Working Group in Peru (2007). Swine cysticercosis hotspots surrounding *Taenia solium* tapeworm carriers. *American Journal of Tropical Medicine Hygiene* **76**, 376–383.

Meštrović, T., Sviben, M., Vilibić-Čavlek, T., Ljubin-Sternak, S., Tabain, I. and Mlinarić-Galinović, G. (2012). Seroprevalence of *Taenia solium* infections in Croatian patients presenting with epilepsy. *Journal of Helminthology* **86**, 259–262. doi: 10.1017/S0022149X11000253.

Moskowitz, J. and Mendelsohn, G. (2010). Neurocysticercosis. *Archives of Pathology and Laboratory Medicine* **134**, 1560–1563. doi: 10.1043/2008-0756-RS.1.

Mwang'onde, B. J., Nkwengulila, G. and Chacha, M. (2012). The serological survey for human cysticercosis prevalence in Mbulu district, Tanzania. *Advances in Infectious Diseases* **2**, 62–66. doi: 10.4236/aid.2012.23009.

Nash, T. E. and García, H. H. (2011). Diagnosis and treatment of neurocysticercosis. *Nature Reviews Neurology* **7**, 584–594. doi: 10.1038/nrneurol.2011.135.

Nash, T. E., Pretell, E. J., Lescano, A. G., Bustos, J. A., Gilman, R. H., Gonzalez, A. E. and Garcia, H. H.; Cysticercosis Working Group in Peru (2008). Perilesional brain oedema and seizure activity in patients with calcified neurocysticercosis: a prospective cohort and nested case-control study. *Lancet Neurology* **7**, 1099–1105. doi: 10.1016/S1474-4422(08)70243-6.

Ooi, W. W., Wijemanne, S., Thomas, C. B., Quezado, M., Brown, C. R. and Nash, T. E. (2011). A calcified *Taenia solium* granuloma associated with recurrent perilesional edema causing refractory seizures: histopathological features. *American Journal of Tropical Medicine and Hygiene* **85**, 460–463. doi: 10.4269/ajtmh.2011.11-0221.

Rajshekhar, V. and Oommen, A. (1997). Serological studies using ELISA and EITB in patients with solitary cysticercus granuloma and seizures. *Neurological Infections and Epidemiology* **2**, 177–180.

Rathore, C. and Radhakrishnan, K. (2012). What causes seizures in patients with calcified neurocysticercal lesions? *Neurology* **78**, 612–613. doi: 10.1212/WNL.0b013e318248df75.

Sarti, E., Schantz, P. M., Plancarte, A., Wilson, M., Gutierrez, I. O., Lopez, A. S., Roberts, J. and Flisser, A. (1992). Prevalence and risk factors for *Taenia solium* taeniasis and cysticercosis in humans and pigs in a village in Morelos, Mexico. *American Journal of Tropical Medicine and Hygiene* **46**, 677–685.

Sarti-Gutierrez, E. J., Schantz, P. M., Lara-Aguilera, R., Gomez Dandoy, H. and Flisser, A. (1988). *Taenia solium* taeniasis and cysticercosis in a Mexican village. *Tropical Medicine and Parasitology* **39**, 194–198.

Simac, C., Michel, P., Andriantsimahavandy, A., Esterre, P. and Michault, A. (1995). Use of enzyme-linked immunosorbent assay and enzyme-linked immunoelectrotransfer blot for the diagnosis and monitoring of neurocysticercosis. *Parasitology Research* **81**, 132–136.

Singh, G., Rajshekhar, V., Murthy, J. M., Prabhakar, S., Modi, M., Khandelwal, N. and Garcia, H. H. (2010). A diagnostic and therapeutic

scheme for a solitary cysticercus granuloma. *Neurology* **75**, 2236–2245. doi: 10.1212/WNL.0b013e31820202dc.

Sinha, S. and Sharma, B. S. (2009). Neurocysticercosis: a review of current status and management. *Journal of Clinical Neuroscience* **16**, 867–876. doi: 10.1016/j.jocn.2008.10.030.

Sorvillo, F. J., DeGiorgio, C. and Waterman, S. H. (2007). Deaths from cysticercosis, United States. *Emerging Infectious Diseases* **13**, 230–235.

Talan-Hranilovic, J., Sajko, T., Negovetic, L., Lupret, V. and Kalousek, M. (2002). Cerebral cysticercosis and echinococcosis: a preoperative diagnostic dilemma. *Archives of Medical Research* **33**, 590–594.

Titlic, M., Tonkic, A., Jukic, I., Lahman-Doric, M., Kolic, K., Buca, A., Milas, I. and Dikanovic, M. (2007). Neurocysticercosis – non-specific clinical and neuroradiological presentation. *Bratislavske lekarske listy* **108**, 414–416.

Tsang, V. C., Brand, J. A. and Boyer, A. E. (1989). An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *Journal of Infectious Diseases* **159**, 50–59.

White, A. C., Jr. (1997). Neurocysticercosis: a major cause of neurological disease worldwide. *Clinical Infectious Diseases* **24**, 101–113.

Wilson, M., Bryan, R. T., Fried, J. A., Ware, D. A., Schantz, P. M., Pilcher, J. B. and Tsang, V. C. (1991). Clinical evaluation of the cysticercosis enzyme-linked immunoelectrotransfer blot in patients with neurocysticercosis. *Journal of Infectious Diseases* **164**, 1007–1009.

Zammarchi, L., Strohmeyer, M., Bartalesi, F., Bruno, E., Muñoz, J., Buonfrate, D., Nicoletti, A., García, H. H., Pozio, E., Bartoloni, A.; COHEMI Project Study Group (2013). Epidemiology and management of cysticercosis and *Taenia solium* taeniasis in Europe, systematic review 1990–2011. *PLoS ONE* **8**, e69537. doi: 10.1371/journal.pone.0069537.