

Public health significance of echinococcosis

Pejnović¹, A., B. Njari²

Conference paper

SUMMARY

Echinococcosis is a cosmopolitan zoonosis caused by infection with adult larval form of *Echinococcus granulosus*, a cause of unilocular echinococcosis (hydatidosis), or, less often, *Echinococcus multilocularis*, a cause of multilocular echinococcosis (alveolar echinococcosis). Humans act as intermediate hosts and are infected through direct contact with definitive hosts, namely parasite-infested dogs. Echinococcosis is a disease that is prevalent throughout the world, including Croatia. As such it is categorized as a disease of great significance for public health. A timely and valid laboratory diagnostic testing to determine its seroprevalence is therefore of clinical and epidemiological importance in order to successfully monitor, control and treat the disease. Although sometimes limited, serological testing is increasingly used and ought to be accepted and implemented should the disease be suspected. In view of the above, within this study we have analysed IgG subclass antibodies of 599 patient serum samples by using the ELISA assay during 2015 to determine the presence of *Echinococcus granulosus*. Obtained results indicated that 6 % of samples were positive, 11 % borderline and 83 % negative. All ELISA reactive samples were further confirmed using the Western blot (WB) assay. By applying these procedures we have established that 14/599 samples were positive and calculated the prevalence of 2.3 % in general population (4.25 % among male and 4.8 % among female patients). The analysis of results according to age showed that the majority of tested patients and the highest seroprevalence determined by WB procedure for general population pertained to the age group of 61 years and older (4.1 %).

Key words: *Echinococcus granulosus*, echinococcosis, prevalence

INTRODUCTION

Echinococcosis is a parasitic zoonosis caused by tapeworms, most commonly *Echinococcus granulosus*, which causes unilocular echinococcosis (hydatidosis), and, less often, *Echinococcus multilocularis*, a cause of multilocular echinococcosis (alveolar echinococcosis). The least prevalent tapeworm species found in Croatia is the *Echinococcus vogeli*, the causative agent of polycystic echinococcosis. The consequent diseases are globally distributed and thrive in moderate and subtropical climates, as well as in areas with developed livestock production (Richter, 1991). The World Health Organization (WHO) data indicate that human incidence rates for cystic echinococcosis in endemic areas can reach more than 50 per 100,000 persons (Anon., 2016 a). Prevalence levels as

high as 5-10 % may occur in parts of Argentina, Peru, East Africa, Central Asia and China, but vary for different years and areas. In 2009 they consequently amounted 8.1 % in Turkey, 5 % in North India and 1.4 % in Uruguay.

Seroprevalence may also vary within a country. For example, in 2012 a seroprevalence of 1.6 % was recorded in Central Iran, while seroprevalence of 6.7 % was recorded in Iran's western parts. An extensive research reporting a seroprevalence of 2.6 % was also conducted in Chile, in 2009. However, there are no precise data on the seroprevalence in the USA, the lack of which can be attributed to greater migrations of population from endemic zones and subsequent settlements in other parts of the country. According to results of research conducted in Croatia that were at our disposal, the seroprevalence of

¹ Anamarija Pejnović, Division of Microbiology, Croatian Institute of Public Health, Zagreb

² Full professor tenure Bela Njari, PhD, University of Zagreb, Faculty of Veterinary Medicine, Department of Hygiene, Technology and Food Safety, Zagreb

Corresponding author: anamarija.pejnovic@hzjz.hr

E. granulosus in patients who developed a severe form of cystic liver disease amounted to 3.9 %, whereby due seroprevalence was higher among male (4.6 %) than among female patients (2.9 %) (Anon., 2016a; Tabain et al., 2011).

Public health aspect

Echinococcosis is as a cosmopolitan zoonosis caused by the above mentioned tapeworms, i.e. their adult larval form, namely cestodes of the genus *Echinococcus*, pertaining to the family *Taeniidae*. Each of the three mentioned species of tapeworm that are of significance for medicine is distinguished by characteristic features and leads to a specific clinical picture. In addition, each requires a different host. Dogs (and certain wild canids) are the definitive hosts of *E. granulosus*, while humans and certain animals (sheep, cattle, pigs) act as intermediate hosts during the larval stage. Though humans also act as intermediate hosts during the larval stage of *E. multilocularis*, foxes and small forest rodents are considered its definitive hosts. Dogs, and even certain rodents, are the definitive hosts of *E. vogeli*, while certain other mammals, in addition to humans, act as its intermediate hosts (carriers). We can therefore reasonably conclude that humans always act as intermediate, but almost never as definitive hosts of tapeworm species *E. granulosus* (Mlinarić-Galović et al., 2003).

To fully grasp due public health significance, one only needs to consider the life cycle of *E. granulosus*.

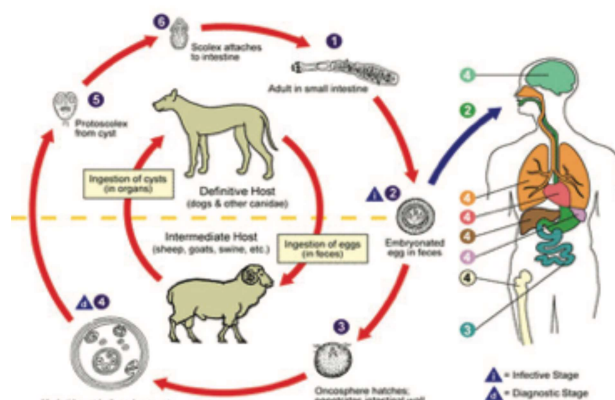


Image 1 *E. granulosus* life cycle*

Source: <http://www.cdc.gov/dpdx/echinococcosis/>

Humans therefore become infected by ingesting echinococcus eggs with food, primarily vegetables, and water that was contaminated with feces of dogs that are considered definitive hosts, or through putting dirty hands into the mouth, often after direct contact with a definitive host (dog). It is worth noting that, in contrast to some previous opinions, the incidence of echinococcosis is, due to its widespread distribution, currently considered on the rise, especially since it is not subject to seasonal changes

(Anon., 2016b). Sources of infection are the cyst-containing internal organs of animals that are ingested by dogs as raw food. Infected persons were thus generally in direct contact with such entrails fed to the dogs. Several strains have been identified within the species (approximately 10). Strains that have the most impact in Croatia include the dog-sheep and the dog-pig strains (Stojević, 2010). The dog-sheep strain (the so-called “shepherd strain”) is mentioned as the most dangerous. Begovac et al. (2006) indicated that hydatid cysts can be localized in the liver (in approximately 70 % of cases), lungs (in approximately 20 % of cases) and other organs (in approximately 10 % of cases) of affected patients. The majority of cases points to longer incubation periods before symptoms become apparent. Symptoms vary and manifest on a case per case basis (fatigue, pain, blood panel, excessive sweating, fever, cough, shortness of breath, swelling, etc.). In more severe cases they can be potentially lethal. A once inevitable surgical procedure (Anon., 2016c) of removing the cyst has been successfully replaced by either chemotherapy (Albendazole 15 mg/kg 2xD 1 to 6 months) or a combination of treatment and ultrasound-guided cyst aspiration, namely the PAIR (puncture-aspiration-injection-reaspiration) procedure. All current and emerging treatment options are subject to audit by the WHO (Moro and Schantz, 2009).

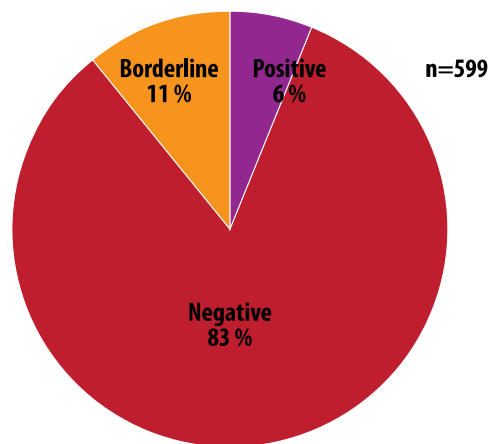
Out of available treatment options, serological testing is currently predominately used to diagnose infectious diseases, specifically the immunofluorescence (IF) assay, the enzyme-linked immunosorbent assay (ELISA) and a confirmatory Western blot (WB) test. Even though their sensitivity ranges from 80 to 100 %, i.e. from 88 to 96 % for the echinococcosis of the liver, they are less susceptible to pulmonary (50-56 %) and other forms of the disease (25-56 %). The need for caution in diagnosing the disease mandates a necessity to always confirm a screening test by due WB test, although, due to a reduced specificity of such testing, even negative findings do not exclude the disease with absolute certainty.

MATERIALS AND METHODS

In the process of determining the specific IgG antibodies to *Echinococcus granulosus*, the Croatian Institute of Public Health, Department of Parasitology – National Reference Laboratory for Human Parasites has during 2015 collected 599 patient serum samples. The determination of IgG antibodies to *E. granulosus* from serum samples was performed by applying the ELISA technique (Enzyme-Linked Immunosorbent Assay), i.e. by using the commercial ELSA kit (NovaTec Immundiagnostica GmbH, Germany). The spectrophotometric microplate reader used was the Bio-Tek ELx800 G (Dialab, USA). All ELISA reactive samples were further confirmed using the commercial Western blot (WB) assay (LDBIO Diagnostics, France).

RESULTS AND DISCUSSION

The results of our research conducted by using the ELISA procedure indicated 34 (6 %) positive findings of IgG antibodies to *E. granulosus* from a total of 599 analysed patient serum samples collected during 2015, as well as 65 (11 %) borderline and 500 (83 %) negative samples (Figure 1). A sampling distribution (Figure 2) demonstrates that rural areas produced a greater number of positive findings (56 %) than their urban counterparts (44 %). The representation of *E. granulosus* findings by counties, per 100,000 inhabitants (Figure 3) shows that, compared to Croatian southern counties, with the exception of the Zadar County, the majority of positive samples came from continental Croatia. It also indicates that for some counties no samples positive to *E. granulosus* were found.



Positive = 34; Borderline = 65; Negative = 500 samples.

Figure 1: Representation of the overall results of 599 serum samples analysed for the presence of *Echinococcus granulosus* by ELISA method in 2015

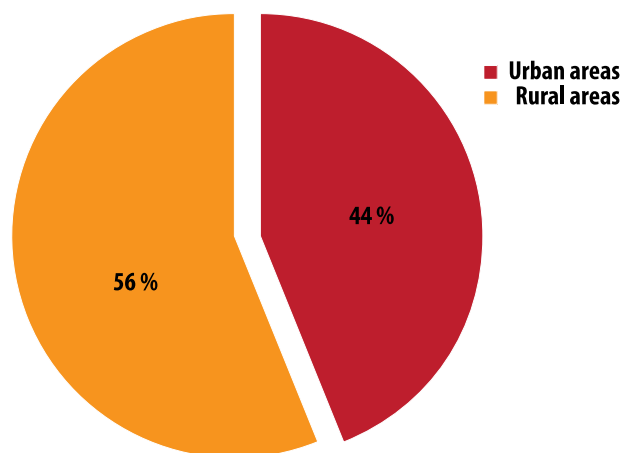


Figure 2: Representation of 2015 findings positive to *E. granulosus* in urban and rural areas

Seroprevalence by age and gender (Figure 4), according to ELISA assay, points to a large number of female serum samples (7.6 %) positive to *E. granulosus* in the age group of 61 years and older. Compared to female patients, there were more male positive samples (16.7 %) in the age

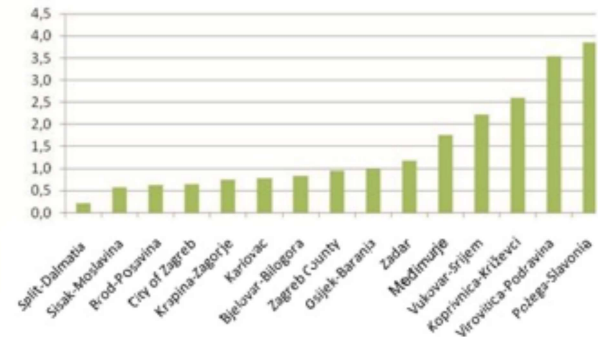


Figure 3: Representation of 2015 findings positive to *E. granulosus* by county (per 100,000 inhabitants)

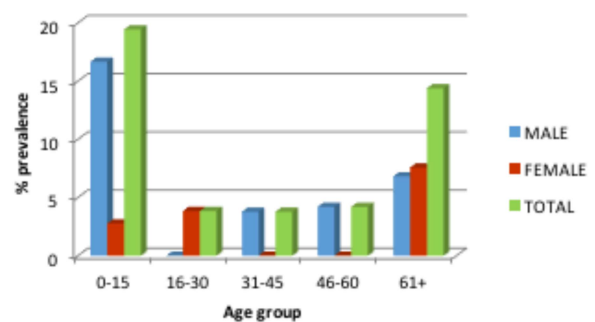


Figure 4: Distribution of 2015 ELSA reactive samples to *E. granulosus* according to age group

Tablica 1: Seroprevalence of *Echinococcus granulosus* relative to age and gender, as confirmed by Western blot method (confirmatory test) employed in Croatia in 2015

Age group (years)	Male No. of positive / No. of tested (%)	Female No. of positive / No. of tested (%)	Total No. of positive / No. of tested (%)
0-15	0 / 46 (0)	0 / 35 (0)	0/81 (0)
16-30	0 / 17 (0)	1 / 21 (4,8)	1/38 (2,6)
31-45	1 / 37 (2,7)	0 / 44 (0)	1/81 (1,2)
46-60	2 / 47 (4,2)	0 / 110 (0)	2/157 (1,3)
61 +	3 / 90 (3,3)	7 / 152 (2,9)	10/242 (4,1)
Ukupno Σ	6 / 237 (2,5)	8 / 362 (2,2)	14/599 (2,3)

group of 0-15 years. The accurate indicator of final prevalence rate determined ensuing due WB assay confirmation indicated the highest prevalence rate for male patients of 4.2 %, pertaining to the age group of 46-60 years, and the highest prevalence rate for female patients of 4.8 %, pertaining to the age group of 16-30 years, while the highest prevalence rate for general population amounted to 4.1 % and pertained to the age group of 61 years and older.

In order to accurately represent the prevalence of *E. granulosus*, all ELISA reactive samples were further confirmed using the above mentioned WB assay. The WB test accordingly confirmed 14 positive samples (2.3 %) from the total number of 34 positive samples (6 %), i.e. 33 positive samples (5.7 %; 1 outside the WB), as echinococcus invasion (infection). This is consistent with previous research of several Croatian authors according to whom the rate of seroprevalence in Croatia amounts to 3.9 % (Tabain et al., 2011), an increase from previously recorded seroprevalence rate of 2.6 % (Morović, 1997). In addition, there are reports of the seroprevalence rate of 2.9 % recorded for Croatia in the period from 2007 to 2014 (Anon., 2016d). Such results can, however, generally speaking, be considered good since due prevalence rate recorded for Croatia is still much lower than both the prevalence rate recorded for Turkey (6.9 %) and prevalence rates referred to as average in official WHO reports (5-10 %). The trend of echinococcosis movements in Croatia was discussed in greater detail in the Annual Report of the Croatian Food Agency (Anon., 2016e), according to which the incidence of echinococcosis had been on the decline from 2005 (25 cases) to 2010 (10 patients), followed by an upward trend observed in 2014 (20 cases).

Moreover, the significance of echinococcosis is emphasised in a separate chapter of the White Paper on Food Safety (Brussels, January, 2000) which states that Mediterranean countries should pay echinococcosis special attention, as well as implement appropriate monitoring systems and prevention programmes (Hadžiosmanović and Kozačinski, 2004). The Directive 2003/99/EC also considers echinococcosis a priority within the control of parasitic diseases due to a number of epidemics caused by echinococci in European countries, and the considerable biological potential the parasite displays when we take into the account a relatively high mortality from certain forms of the disease (Garcia, 2001).

As a disease, echinococcosis can be most efficiently prevented by interrupting the chain of infection, especially when a mature pathogen (parasite) is located in dog's intestines. In contrast to countries where it is on the constant rise, the situation in Croatia can be considered satisfactory. Croatian islands that were previously considered endemic areas report almost no incidence of echinococcosis. In Croatia dogs do not usually roam outside their owner's yard, predominately consume commercial dog food and are subject to regular mandatory veterinary examinations (dehelminthization). And finally, it bears mentioning that the European Centre for Disease Prevention and Control's National Reference Laboratory for Human Parasites, acting within the Croatian Institute of Public Health, is the only Croatian laboratory authorized to administer serological tests for Echinococcus granulosus in the field of human medicine.

Instead of a conclusion, we will say that in order to successfully combat this zoonosis it is necessary to continuously strive to reduce the number of stray dogs, continue the implementation of systematic dog dehelminthization, dispose of dead animals and their entrails in an acceptable and appropriate manner, as well as perform and supervise slaughter activities in approved facilities. It is of utmost importance to promote lifelong learning about the disease at all levels, especially regarding the life of pets (dogs) and the practice to properly wash fruits and vegetables before consumption. Furthermore, it is important to prevent dogs from accessing raw animal entrails and avoid feeding them entrails that have not been heat-treated, as well as to protect water-wells and other sources of water from possible contamination with dog feces.

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