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# **PHD THESIS**

**CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF CYSTIC  
ECHINOCOCCOSIS IN WESTERN ROMANIA**

## **ABSTRACT**

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**T i m i ș o a r a**

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## ABSTRACT

Cystic echinococcosis (CE) is a zoonotic parasitic disease caused by taenia *Echinococcus granulosus sensu lato*. The disease represents a public health problem in many parts of the world due to its potential to cause significant morbidity and mortality. High prevalence rates of CE are often seen in rural and pastoral areas, where dogs are allowed to roam freely, and parasitic eggs contaminate the environment.

Transmission in humans occurs through ingestion of water, food, soil contaminated with eggs or through close contact with dogs. The disease remains silent a long period of time and symptomatology usually appears due to the cyst growth or occurrence of complications.

Diagnosis of CE is based on imaging techniques (ultrasonography, radiography, computed tomography, and magnetic resonance). These methods are useful for establishing the location and stage of the cysts. Serologic assays are widely used as screening or confirmatory tests, in addition to imaging techniques. The goal of serologic testing is to identify specific IgG anti-*Echinococcus granulosus* antibodies.

The extent of CE throughout the population can be measured by retrospective studies, serological and molecular surveys. Retrospective studies give valuable information regarding the epidemiology, and highlight the challenges associated with the diagnosis and treatment of the disease. Serology surveys are vital tools to the surveillance and management of CE because they facilitate the detection of affected persons, monitor the spread of the infection, and the efficacy of control measures. Molecular surveys, on the other side, can provide information on the transmission dynamics of CE, the role of wild and domestic animals as intermediate hosts and the impact of human activities on parasite life cycle.

The importance of the studies regarding CE derives from the fact that an early diagnosis of hydatid cysts can prevent complications and improve patient's outcome. In addition, knowing the genotype responsible for CE can help prevent further transmission

of the infection. The research, conducted in this PhD thesis, aimed to investigate the epidemiology of CE in humans from Western Romania and the molecular distribution of *E. granulosus* genotypes/species implied in the human disease.

The content of the thesis is structured in three parts, the general part, the special part and the conclusions.

**The general part** presents the current state of knowledge regarding the studied topic. Aspects related to taxonomy, epidemiology, clinical presentations of CE and its diagnostic methods were addressed. Worldwide data on hospital-reported cases, seroprevalence of infection, and the molecular distribution of the species/genotypes responsible for human CE cases were presented.

**The special part** consists of five studies conducted in two counties from Western Romania: Arad and Timis. These studies included a serological investigation of the prevalence and potential risk factors for *Echinococcus granulosus* in blood donors, three retrospective investigations of hospitalized cases with hydatidosis, and finally the molecular identification of the species/ genotypes responsible for CE in hospitalized patients from Western Romania.

**The conclusions** include the most relevant aspects noticed in the five epidemiological studies and recommendations for the prevention and control of CE.

This research had three primary objectives:

- to estimate the seroprevalence of anti-*Echinococcus* antibodies and identify the risk factors associated to prevalence in healthy blood donors from Timis county;
- to assess the epidemiological features and clinical data on CE patients admitted to surgical clinics from referral hospitals in Timis and Arad counties;
- to determine the *Echinococcus* genotypes that are implied in human CE in Western Romania.

To accomplish these objectives, five studies were performed. For the first objective, a study on healthy blood donors was carried out. For the second objective, three studies

were performed: one retrospective study aimed to assess the adult cases of CE, one epidemiological study aimed to evaluate the pediatric cases of CE, and one study aimed to compare the adult and pediatric epidemiological features of the disease. For the third objective, a molecular study on samples collected from individuals surgically treated for CE was conducted.

### **Seroprevalence of anti-*Echinococcus granulosus* antibodies and Risk Factors associated with prevalence in Blood Donors from Western Romania**

The first study aimed to determine the seroprevalence of anti-*Echinococcus* antibodies and potential risk factors associated to prevalence in healthy blood donors from Western Romania. A cross-sectional study was conducted on 1347 consecutive healthy blood donors from Timiș county. The recruitment of participants occurred between 19 November - 21 December 2018 at the Regional Blood Transfusion Center in Timisoara. The individuals were tested for anti-*Echinococcus* IgG antibodies using ELISA immunoassay.

The seroprevalence of anti-*Echinococcus* antibodies in the study group was 2.8% (38/1347). The seroprevalence rate was 3.7% (22/592) in females and 2.1% (16/755) in males. Anti-*Echinococcus* antibodies were detected in 2.3% (14/607) in age group 18–30 years, 3.6% (13/359) in age group 31–40 years, 2.9% (8/272) in age group 41–50 years and 2.8% (3/109) in age group 50–63 years. The seroprevalence rate was 3.1% (30/979) in urban area and 2.2% (8/368) in rural area. There were no significant associations between the presence of anti-*Echinococcus* antibodies and gender, place of residence, age, dog interaction, or sheep husbandry.

This was the first study in Europe to assess the seroprevalence of anti-*Echinococcus* antibodies in healthy blood donors. The results indicate that *Echinococcus* infection can be present in asymptomatic individuals.

## **Cystic Echinococcosis in Hospitalized Adults from Western Romania: 2007–2022**

The second study focused on the epidemiological characteristics of adults with CE from Arad and Timiș counties. The medical data charts of individuals hospitalized with CE between 1 January 2007 and 1 September 2022, in general and thoracic surgery referral centers from County Emergency Clinical Hospital Arad, Municipal Emergency Clinical Hospital Timisoara, and “Pius Brînzeu” County Emergency Clinical Hospital Timisoara were examined retrospectively.

Information regarding age, gender, place of residence, length of hospital stay, number of hospitalizations associated with CE, cyst location, number of cysts, investigations, presence of complications, and therapy were collected and statistically analyzed.

The study included 366 patients aged 18 - 90 years. Hospitalization rates were higher in individuals between 50 - 59 years (22.7%, 83/366), and people living in rural regions (61.5%, 225/366). Females accounted for 53% (194/366) of the cases. A descending trend ( $R^2 = 0.346$ ,  $p = 0.02$ ) in the number of cases was observed during the study period. In 82.5% (302/366) of the cases, the cysts were found in the liver. In lung and spleen involvement, males accounted for higher rates compared to females ( $p=0.01$ ).

Complications were present in 26.8% patients. The most frequently reported complications included biliary fistula, allergy, and cyst infection. The mean length of hospital stay for patients who developed complications was  $15.7 \pm 8.3$  days, which was higher than the mean length of hospital stay for patients without complications ( $11.5 \pm 7.3$  days) ( $p=0.001$ ). The length of hospital stay was  $>14$  days in 51% (50/98) of the patients with complications, compared to 25% (67/268) in patients without complications ( $p < 0.001$ ). Surgery was the treatment of choice in 81.4% (298/366) of the cases, followed by the PAIR technique in 14.5% (53/366) of the cases.

The results of this study indicate that this zoonotic disease remains a significant public health problem in Western Romania and people diagnosed with CE require hospitalization and extensive medical care. The disease was most frequently reported in rural inhabitants. In most of the cases liver was the most affected organ. Patients with lung hydatid cysts required a longer hospitalization compared to those with other localizations. Surgery was the therapy of choice in most of the cases.

## **Cystic Echinococcosis in Hospitalized Children from Western Romania: 2007 – 2022**

The third study examined the epidemiological aspects of CE in children hospitalized between 2007 - 2022 at the County Emergency Clinical Hospital from Arad and the Emergency Clinical Hospital for Children “Louis Turcanu” from Timisoara.

The medical data charts between 1 January 2007 and 1 September 1 2022 were retrospectively examined. Hospital databases and medical records were investigated. Data on age, gender, place of residence, length of hospital stay, cyst location, and presence of complications were gathered and analyzed.

The study included 60 patients aged between 3 and 17 years. The highest number of cases were observed in age group 11-17 years (65%, 39/60) and in patients from rural area (68.3%, 41/60). Boys accounted for 60% (36/60) of the total number of cases. During the studied period a descending trend in the number of cases was observed ( $p=0.04$ ,  $R^2=0.28$ ).

The most affected organ was the liver in 71.7% (43/60) of the cases, followed by lungs in 25% (15/60) of the cases. In pulmonary involvement, boys accounted for higher rates compared to girls ( $p=0.01$ ). Complications were present in 23.3% (14/60) of the cases and cyst superinfection was most frequently reported.

Despite the downward trend in the number of cases with CE in children, the disease remains a severe public health concern. The presence of CE in children shows

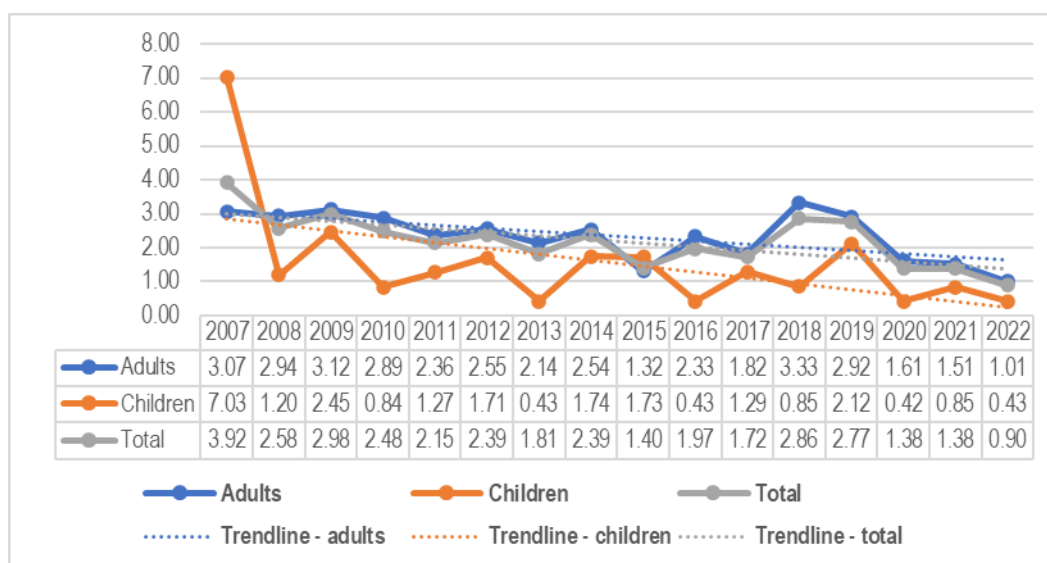


that they were exposed to the parasite during childhood. A higher number of cases was observed in age group 11-17 years, in boys, and children from rural area. Liver was the most affected organ, followed by lung. In lung involvement, boys were more affected than girls.

## **Cystic Echinococcosis in Hospitalized Children and Adult Patients from Western Romania: 2007–2022**

The aim of the fourth study was to compare the demographic and epidemiological characteristics of CE in children and adults from Western Romania, using the hospital data recorded between 2007 and 2022 .

This research included 426 individuals with CE (3-90 years, mean age=41.9), of which 14.1% (60/426) were children and 85.9% (366/426) were adults. There was a decline in the number of cases during the study period, from 47 cases in 2007 to 11 cases in 2022 ( $p=0.004$ ). Overall, a decreasing trend of the CE incidence was also noted during the study period ( $R^2=0.47$ ,  $p=0.003$ ). The incidence trends in adults decreased from  $3.07/10^5$  adult inhabitants in 2007 to  $1.01/10^5$  adult inhabitants in 2022 ( $R^2=0.38$ ,  $p=0.009$ ). In children, the CE incidence decreased from  $7.03/10^5$  in 2007 to  $0.42/10^5$  in 2022 ( $R^2=0.27$ ,  $p=0.03$ ) (Figure 1).



**Figure 1. Incidence of CE in children and adults from Western Romania between 2007 and 2022.**

Three out of four cases (92.3%, 393/426) had a single organ involvement. Children (16.7%, 10/60) had a higher rate of multiple organ involvement than adults (6.3%, 23/366;  $p = 0.005$ ). The most affected organ was the liver in both children and adults (81%, 345/426). However, children (25%, 15/60,) were more likely to develop lung involvement than adults (13.1%, 48/366) ( $p=0.02$ ). The majority of patients (74.9%, 319/426) had only one hospitalization. However, children had a higher rate of multiple hospitalizations (40%, 24/60) compared to adults (22.7%, 83/366,  $p = 0.004$ ).

CE is a severe zoonotic disease that affects people of all ages. Rates of infection were higher in pediatric and adult inhabitants from rural area. Liver was the most affected organ in both children and adults, but in lung involvement children were more affected. Rate of multiple organ involvement was higher in children compared to adults. Multiple hospitalizations were more frequently reported in children than in adults. Although the number of cases decreased during the studied period, patients with CE required hospitalization and specialized medical care suggesting that the disease remains a public health concern in Western Romania.

### **Identification of *Echinococcus granulosus sensu lato* (s.l.) Genotypes Responsible for Human Cystic Echinococcosis in Western Romania**

The fifth study focused on the molecular epidemiology of human hydatidosis in Western Romania. The research comprised individuals who underwent surgery for CE in hospitals from Timis county between 2019 -2022. During surgery, hydatid fluid and/or cystic membranes were extracted from liver, lung, peritoneal, pancreatic, splenic, and osseous cysts and molecularly analyzed. Mitochondrial NADH dehydrogenase 1 (nad1) gene was amplified using primers JB11 (5'-AGATTCGTAAGGGGCCTAATA-3') and JB12 (5'-ACCACTAACTAATTCACTTTC-3').

Samples were taken from 26 individuals with age ranging from 20 to 84 years; 65.4% (17/26) of the individuals were from rural areas and 65.4% (17/26) of the individuals

were females. In the tested samples, *Echinococcus granulosus* G1-3 complex and *Echinococcus canadensis* G7 were detected. Sequence analysis indicated that *E. canadensis* G7 was present in 84.6% (22/26) of the samples. *Echinococcus granulosus* G1-G3 complex was identified in 15.4% (4/26) of the samples: G1 was identified in 2 cases and G3 also in 2 cases.

This is the first research to emphasize the significance of *Echinococcus canadensis* G7, in humans from Western Romania. These findings suggest a major role of the pig in maintaining the life cycle of *E. granulosus* in Western Romania.

## CONCLUSIONS AND PERSONAL CONTRIBUTIONS

There is limited information in the scientific literature regarding the epidemiological situation of CE in Romania. This PhD thesis includes the first scientific paper in Europe to assess the seroprevalence of *Echinococcus* infection in healthy blood donors. Furthermore, the epidemiological features of CE in children and adults from Western Romania were assessed and genotypes of *Echinococcus* responsible of infection were investigated in patients surgically treated for CE. All the objectives of the present thesis were accomplished, resulting in a comprehensive and important piece of research.

The current research presents new and essential data on the epidemiology of *E. granulosus* in Western Romania. The main conclusions resulted from the present research are as follows:

1. The seroprevalence of anti-*Echinococcus* antibodies in blood donors was assessed for the first time in Europe and Romania.
2. The 2.8% prevalence in blood donors suggests that this zoonotic infection may be found in healthy, asymptomatic individuals.
3. In the adult population, CE was most frequently noted in rural residents.

4. In adults, single organ involvement was observed in most of the cases and liver was the most affected organ.
5. In adults with pulmonary and renal CE, rates of infection were higher in men compared to women.
6. Adult patients with lung involvement required a longer hospitalization compared to those with other localizations.
7. The presence of complications in adults led to a prolonged hospital stay.
8. In children, the disease was more prevalent in boys, in residents from rural areas and in the age group 11-17 years.
9. A single organ involvement was noted in most of the pediatric cases and liver was the most affected organ.
10. In children with pulmonary CE, rates of infection were higher in boys compared to girls.
11. In pulmonary CE, higher rates were reported in children compared to adults.
12. Children had more frequently multiple organ involvement compared to adults.
13. Higher rates of multiple hospital presentations were registered in children compared to adults.
14. There was a decrease in the incidence and number of CE cases in children and adults between 2007 and 2022
15. Although a descending trend in the incidence and number of cases was observed in both children and adults, patients diagnosed with CE needed hospitalization and prolonged medical treatment.
16. The most frequent genotype in humans with CE from Western Romania was *Echinococcus canadensis* G7.

The findings of this research may serve as a foundation for further investigations and highlights the need for effective methods for preventing the spread of this zoonosis. Public health strategies should be put into practice and constantly improved. Surveillance programs are essential for understanding the epidemiological picture of this disease, taking action in risk regions, and establishing priorities. Data are also required to assess the results of control activities and track the progress of the implemented control measures.

The findings of the research presented in this PhD thesis offer an overview of the extent of *Echinococcus* infection in Western Romania. Moreover, the present research has an international impact as it enhances the knowledge on the epidemiological characteristics of CE, reveals the existence of anti-*Echinococcus* antibodies among asymptomatic healthy blood donors, and presents the major role of *Echinococcus canadensis* G7 in human infection. The epidemiological data of the thesis will be useful to the national and international scientific community for future studies and for the development of case management strategies and prevention programs. Control measures for CE must be implemented to reduce the prevalence and the burden of the disease in Romania.