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Original Article

Evaluation of Echinococcosis Pre-Diagnosis Patients Admitted to the National Parasitology Reference Laboratory of Turkey from 2014-2019

***Banuçiçek Yücesan [1], Cahit Babür [2], Selçuk Kılıç [3], Asiye Uğraş Dikmen [4]**

1. *Department of Control of Zoonotic Disease, School of Health Sciences, University of Çankırı Karatekin, Cankiri, Turkey*
2. *Department of National Parasitology Reference Laboratory, Public Health General Directotare of Turkey, Ankara, Turkey*
3. *Department of Medical Chemical Biological Radiological and Nuclear Defense, Institute of Gulhane Health Sciences, University of Health Sciences, Ankara, Turkey.*
4. *Department of Public Health, School of Medicine, University of Gazi, Ankara, Turkey*

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***Correspondence**
Email:
yucesanbanu@yahoo.com

Abstract

Background: Echinococcosis is a common parasite with zoonotic character created by a small cestode, *Echinococcus* spp., and is an important public health problem in Turkey as well as all over the world. We aimed to investigate antibodies in serum samples of suspected Echinococcosis patients sent to the National Parasitology Reference Laboratories of the General Directorate of Public Health.

Methods: Serum samples of 2390 patients sent to our laboratory between January 1, 2014 and May 01, 2019, evaluated by ELISA, Indirect Hemagglutination Test (IHA) and Western Blot (WB) methods are presented. Our laboratory is the national reference laboratory. All kinds of tests requested from suspected patients can be performed

Results: Overall, 1199 (50.2%) of 2390 serum samples were female and 1191 (49.8%) were male. It was observed that 178 (14.9%) of men and 210 (17.5%) of women were seropositive. There was no statistical difference between the sexes in terms of seropositivity. Of all samples, 1941 (81.2%) were negative, 388 (16.2%) were positive, and 61 (2.6%) were borderline. Results determined as borderline are considered suspicious and a recommendation is made to repeat the test after 15 days. A statistical difference was found in the distribution of seropositivity by years. While seropositivity was lowest in 2014, it was found to be highest in 2018 and 2019.

Conclusion: Despite all the precautions taken, it is seen that echinococcosis still continues to exist in Turkey as a zoonotic disease. Hence, CE has been involved in Turkey Zoonotic Diseases Action Plan (2019-2023) and decided to carry out studies for the protection and prevention of the disease.

Introduction

Echinococcosis is a parasite with a zoonotic character created by the small cestode *Echinococcus* spp. Echinococcosis is common all over the World. In addition, it is among the most important parasitic diseases encountered in Mediterranean countries and creates serious problems in terms of public health and economics (1). *Echinococcus* spp. consists of eight known species: *E. granulosus*, *E. multilocularis*, *E. oligarthra*, *E. vogeli, E. equinus, E. ortlepp*, *E. shiquicus* and *E. canadensis* cluster. It is considered that *E. canadensis* cluster can be separated into different species with future studies (1-3). *E. granulosus* causes cystic echinococcosis disease, *E. multilocularis* alveolar echinococcosis disease, *E. vogeli* and *E. oligarthrus* cause polycystic echinococcosis disease (2).

Nowadays morphological and biological characteristics have begun to be evaluated in detail with genetic and phylogenetic analyzes carried out with molecular studies and the lineage diversity of the parasite is tried to be explained (4). Echinococcosis is a multidisciplinary disease, and in 2020 the World Association of Echinococcosis has agreed on three disease names: Cystic echinococcosis (CE), Alveolar echinococcosis (AE) and Neotropical echinococcosis (NE) (5). The main host for echinococcosis is carnivores and the intermediate host is mammals such as humans, sheep, goats, and cattle etc. Parasite eggs taken by the intermediate host develop in organs such as the liver, and, to a lesser extent, the lungs, brain, heart, and bone. Cystic and alveolar echinococcosis can create an asymptomatic period that can last for years until the parasite's cysts develop and clinical symptoms appear (1,6-7). Cystic echinococcosis has been a significant public health problem all over the world since 1950. For this reason, it was included among 17 diseases neglected by the WHO in 2013 (8). The WHO has speeded up its efforts to control and eliminate this zoonotic disease. For this purpose, the WHO, World Animal Health Organization (OIE) and United Nations Food and Agriculture Organization (FAO) have issued joint statements (8). In this context, CE was included in the list of notifiable infectious diseases in 2005.

A patient with a pre-diagnosis of echinococcosis can be diagnosed by showing cysts with imaging methods such as computed tomography (CT), ultrasonography and magnetic resonance imaging (MRI). However, when there is uncertainty, difficulty in making a diagnosis, confirm the diagnosis or to follow the treatment, laboratory analyzes can be used (9-11). The condition of echinococ cysts determines the antibody reaction that occurs in the body. Since intact cysts do not leak antigen, they also block the formation of antibody response. However, antibody response can be detected more easily in leaky cysts (10). For laboratory diagnosis of CE and AE, antibody, antigen, cytokine detection, parasite detection (microscopy; parasite detection in cyst fluid taken by interventional radiological methods, detection of parasite DNA by molecular methods, etc.) are performed. Nowadays, for the definitive diagnosis of echinococcosis, one or more of these methods are used in combination. The disease may need to be supported by different laboratory analyzes due to its location in different organs, heterogeneity and complex host-parasite relationships. Therefore, many analysis methods such as Enzyme-Linked Immuno Sorbent Assay (ELISA), Indirect Haemaggutination test (IHA), Indirect Fluorescent Antibody test (IFA), immunochromatography, Western Blot (WB) and Polymerase Chain Reaction (PCR) have

been developed and used in laboratories (10,11).

We aimed to document the researches made from serum samples sent to the National Parasitology Reference Laboratories of the Ministry of Health, General Directorate of Public Health (NPRL, GDPH) between 2014-2019 with suspicion of echinococcosis.

Materials and Methods

Serum samples sent to NPRL, GDPH - with the suspicion of echinococcosis from different hospitals and laboratories in various provinces between 01 January 2014 and 01 May 2019 were examined. *E. granulosus* IgG antibodies with were investigated by IHA (Fumouze Laboratoires, Fransa; Behring, Almanya), ELISA-IgG (Novalisa *Echinococcus* IgG, NovaTec, Germany) and Western Blot (WB) (Euroimmun, Germany). These tests were studied with commercial kits in line with the recommendations of the manufacturers. All of these tests are methods that can be applied in the national reference laboratory. However, it is not applied to all patients. Only requested tests are performed on suspicious patients. In line with the Health Implementation Communiqué in Turkey, only the desired tests can be studied from an economic point of view, there is the opportunity to apply more than one test at the limit values where there is much doubt or in line with the demands of the institutions.

Statistical analysis

The analyzes of the study were made in SPSS 23.0 statistical program (IBM Corp., Armonk, NY, USA). Descriptive analyzes are presented with frequency and percentage distribution. The suitability of the quantitative data to the normal distribution was evaluated and parametric tests were used (student-t test, ANOVA). The comparison of qualitative data was made with the chi-square test. $P<0.05$ was considered statistically significant.

Ethics approval

This study was carried out with the approval of Gazi University ethics commission, dated 08022021 and numbered E.23479.

Results

A total of 2390 samples were analysed over a six-years period (2014-2019). Table 1 shows the distribution of samples coming to our laboratory by years. The two provinces with the highest number of examples are Ankara and Kırıkkale.

Table 1: Distribution of samples by years (n=2390)

Years	*Number*	*Percent (%)*
2014	119	5.1
2015	408	17.1
2016	423	17.7
2017	510	21.3
2018	497	20.7
2019 (until May)	433	18.1
Total	2390	100

Of the 2390 serum samples included in the study, 1199 (50.2%) were female and 1191 (49.8%) were male. When the gender distribution of the samples from 2014-2019 was examined, it revealed that there was no statistical difference (x2= 9.23 P=0.11). The mean age was 26.4±15.1. There was no difference in age distribution over the years. Table 2 points out the distribution of males and females and their positivity rates of the samples. There was no

statistical difference between the sexes in terms of seropositivity (x2= 3.13 *P*=0.20). Of all samples, 1941 (81.2%) was negative, 388 (16.2%) positive, and 61 (2.6%) as borderline (Table 2).

Table 2: Distribution of sample results by gender

Tests	*Positive*	*Borderline*	*Negative*	*Total*
Men	178 (14.9%)	29 (2.4%)	984 (82.6%)	1191
Women	210 (17.5%)	32 (2.7%)	957 (79.8%)	1199
Total	388	61	1941	2390

A statistical difference was found in the distribution of positivity by years (x2= 19.6, *P*=0.01). While the positivity was the lowest in 2014, the highest was in 2018 and 2019. The borderline was seen at most in 2019 (Table 3). The positive, negative and borderline results of IHA, ELISA and WB tests studied in suspicious patients are given in Table 4 in detail.

Table 3: Distribution of sample results by years

Tests	*Positive*	*Borderline*	*Negative*	*Total*
2014	19 (16.0%)	-	100 (84.0%)	119
2015	45 (11.0%)	8 (2.0%)	355 (87.0%)	408
2016	61 (14.4%)	9 (2.1%)	353 (83.5%)	423
2017	56 (11.0%)	12 (2.4%)	442 (86.6%)	510
2018	89 (17.9%)	2 (0.4%)	406 (81.7%)	497
2019 (until May)	118 (27.3%)	30 (6.9%)	285 (65.8%)	433
Total	388	61	1941	2390

Table 4: Distribution of sample results according to the tests performed

Test	*Positive N(%)*	*Borderline N(%)*	*Negative N(%)*	*Total*
IHA	373 (16.6)	50 (2.2)	1815 (81.1)	2238
ELISA	51 (17.8)	17 (5.9)	218 (76.2)	286
WB	47 (15.6)	4 (1.3)	250 (83.0)	301

One hundred sixteen (116) of the samples were studied with IHA+ELISA+WB. Twenty eight of these samples (24.1%) were positive in all three tests. Fifty-three of the samples were tested with dual test (IHA+WB) and 39 (73.5%) were positive in both tests. Forty-four of the samples were tested with the dual test (IHA+ELISA) and 43 (97.7%) were positive. Thirty-four of the samples were tested with the double test (WB+ELISA) and 31 (91.1%) were positive.

In Table 5, the positivity compliance rates of the tests studied together are available. The positivity percentages are given in the above paragraph.

Table 5: Positivity concordance rates of the tests tried

Test	*IHA*	*ELISA*	*WB*
IHA	-	43	39
ELISA	43	-	31
WB	39	31	-

Discussion

Echinococcus spp. is an important parasite with worldwide distribution except Antarctica. *E. granusosus* and *E. multilocularis* types of parasites, which are thought to be zoonotic in all species in the genus, pose much attention because they infect humans more frequently (3,7). Prey and predator relationship exist in the transmission of the parasite. Wildlife plays an important role in the distribution of this parasite (12,13). Canids are definitive hosts for these tapeworms and contain adult forms. Intermediate hosts are organisms that disperse larval cysts (14). For this reason, the rate of spread of these diseases is seen to be high in countries where animal husbandry is high.

It is seen that it is more common in Eastern and Southeastern Anatolia regions where animal husbandry is common in Turkey. When the data of the Ministry of Health between the years 2008-2019 are evaluated, it is seen that the number of cases has increased over the years (15). In our study, a statistical difference was found in the distribution of positivity by years (x2= 19.6, p=0.01). The positivity was lowest in 2014 and highest in 2018 and 2019. As a result, in this study, it revealed that the positivity increased in the following years.

Since echinococcosis is a disease that is frequently diagnosed clinically and radiologically, seroepidemiological field studies in Turkey are limited. There has been an increasing in the number of serological studies in our country. In our study, it is observed that there has been an increase in the number of samples coming to our laboratory over the years. Serological studies for echinococcosis in different regions of our country can be performed using different techniques (ELISA, IHA, WB, and IFAT) (15). In our country, CE seropositivity varies between 0.53% and 45% with the use of different socioeconomic conditions and different diagnostic methods (16-31). In previous studies conducted in our laboratory, the seropositivity rate was 35.5% between 2003 and 2005 (32), 15% from 2009-2013 (29). In this present study, 16.2% (2014-2019) seropositivity was detected. This value shows that the seroprevalence of echinococcosis, which has a decreasing trend over the years, is similar to the previous period. It is thought that the increase in the level of knowledge of our people about the disease with the measures taken in recent years can be counted among the reasons for this. Echinococcosis distribution was found unequal in Europe and widespread in Middle Eastern countries. In North Africa, rates were found to be quite high, with the exception of Egypt. East Africa is a highly endemic region (15). The prevalence of CE infection varies considerably in different geographic regions. The reported prevalence may also depend on the techniques used for diagnosis, such as radiology, laboratory etc.

The prevalence of CE is reported to be more frequent in men in areas where the most common risk factor is exposure to infected domesticated ruminants (33-35). This is due to the fact that men are more in close contact with farm animals.

In many seroprevalence studies in Turkey, the disease is more common in women than in men (23-32, 36-38). It is thought that the high rate in women may be due to the fact that they are more exposed to parasite eggs because they deal with agricultural work more than men and take care of dogs and animals more. In contrast, there was no statistically significant difference in prevalence between

male and female donors in this study. In our study, 50.2% of the serum samples were female and 49.8% were male. Although the seropositivity rate was 17.5% in women and 14.9% in men, there was no statistical difference between the sexes in terms of seropositivity (x2= 3.13 *P*=0.20).

The seroprevalence of CE infection demonstrates a trend for increasing with age, due to longer exposure time in industrial and farming activities and persistence of IgG antibodies (7,39). It has been reported in different studies that CE cases can be encountered at any age with high incidence in middle age (19,40). The mean age is 26.4±15.1 in our study. There was no difference in age distribution over the years.

In Turkey, from 163 serum samples, IHA, IFA and ELISA were positive in 51%, 53% and 42.95%, respectively (37). Karaman et al. evaluated CE in Kars using IHA and IFA tests and reported that they determined 34.6% seropositivity with both methods (16). Seventy percent of 465 patients in İzmir were positive with ELISA and 14% with IHA, and they found only 12% of the patients positive with both tests (19). The results of our study are in good agreement with previous studies.

In addition, when the positivity compatibility rates of the tests performed with this study are evaluated and the tests studied together are evaluated, IHA and WB are 73.5% positive, IHA and ELISA are 97.7% positive, and WB and ELISA are 91.1% positive. These tests are useful in supporting the diagnosis of CE for screening, diagnosis and postoperative patient follow-up. The use of a single test may be insufficient in the serological diagnosis of CE from time to time. It is more reliable to use at least two tests together in the studies of our reference laboratory. The IHA test is used more frequently in our laboratory for reasons such as ease of application in routine screenings, determination of antibody titers before and after treatment, facilitating patient follow-up, and less cost and equipment requirement.

The diagnosis of CE is mainly based on radiological and microbiological tests. The ELISA and IHA remains the most common method used to detect IgG antibodies against *Echinococ* spp. The ELISA is highly sensitive, easy to perform, has a great potential adaptability for automatization, can be applied to epidemiological surveys and is valuable for diagnosis of CE (7,11).

CE ELISA demonstrated a sensitivity, specificity, between 72%-96.7% and 92.6-100%, respectively, for IgG, when tested against the WB reference method. The ELISA is purported to be more sensitive than IHA for the diagnosis of CE (7,11,41-45). Furthermore, The lack of standard antigen, variation in the quality of anti-human globin preparations, and the use of various end points make interpretation of ELISA result difficult. Specificity of the ELISA can be increased with confirmation by WB (7,11).

The reasons for the preference of IHA tests are that the test time is short, less need for expert personnel, easy to interpret and economical. Various authors worldwide have obtained variable results for the IHA test, ranging from 34.9% to 88% for sensitivity (46-50), and results ranging from 44% to 70% for specificity (46,48,50). In general, ELISA and IHA results are compatible with each other, but different results have also been obtained. This is due to the use of different antigens in the kits and the different sensitivity of the tests (24,32,45). For this reason, more reliable results will be obtained by studying the two tests together and confirming the positivity with WB. Western blot results in better sensitivity than the ELISA and the IHA techniques (48). In our country, Sarı et al. investigated the sensitivity and specificity of ELISA, IFAT and IHA methods in patients with CE. As a result, they reported that the sensitivity of the ELISA method was 87.5%, the specificity was 100%, the sensitivity of the IHA method was 90%, the specificity was 97.5%, the sensitivity of the IFAT method was 82.5%, and the specificity was 100% (45). While Bilge et al. reported the

IHA test as 100% specific and 74.6% sensitive (47), Akısü et al. determined the sensitivity and specificity of the test as 96.7% and 82.2%, respectively (51).

Within the scope of the Zoonotic Diseases Action Plan for CE in Turkey (2019-2023), studies are carried out to increase the efficiency of health services within the framework of one health for the control of the disease (52).

Conclusion

The prevalence of CE is still an important problem in Turkey due to the insufficient compliance with hygienic conditions in areas with high animal husbandry and the high number of stray animals. We firmly believe that raising awareness of the public on this issue and taking protection and control measures can provide control of the disease in terms of public health.

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Conflicts of interest

The authors have no conflict of interest to declare.

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