

ORIGINAL ARTICLE

Medicine Science 2023;12(3):818-22

Research of cystic echinococcus serology in people living in rural areas and dealing with animals with the immunochromatographic method: A small-scale cross-sectional study

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Received 21 June 2023; Accepted 03 August 2023

Available online 21.08.2023 with doi: 10.5455/medscience.2023.06.097

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Abstract

Cystic echinococcus (CE) is a parasitic disease in humans and animals. Our study was performed with the aim of identifying the serology of hydatid cyst disease caused by the Echinococcus granulosus vector, causing a significant health problem in Eastern Anatolia and specifically in our province, among people living in rural areas and involved in animal husbandry. The population for this study comprised 75 volunteers living in rural regions of Erzurum practicing animal husbandry. Blood samples were stored at -40 °C until the time of the study. A survey form was completed to be able to identify the demographic data of participants and to reveal risk factors in terms of infectious vectors. After thawing blood samples at the time of the study, the immunochromatographic test procedure was implemented. The age distribution in the study group was 18 to 70 years, with a mean age of 46.8±15.97 years. The group comprised a total of 75 people, including 29 women (38.7%) and 46 men (61.3%). Of these 75 people with immunochromatographic screening performed, 72 were negative (96%) and 3 were weakly positive (0.5) (4%). It is notable that 4% CE seropositivity was found among individuals with no complaints. Linked to this seropositivity result, it is considered that creating a comprehensive CE screening program will be beneficial for individuals involved in animal husbandry.

Key words: Hydatid cyst, echinococcus granulosus, serology, immunochromatographic test

Introduction

Cystic echinococcus (CE) disease is a significant parasitic disease with distribution mostly in the liver but also in lungs and other organs, frequently observed in countries with intense agriculture and animal husbandry, generally transmitted to humans through dog feces [1]. It causes serious economic losses as well as the damages it causes to human health, especially in developing countries [2].

Hydatid cyst is found in every continent of the earth, apart from Antarctica. Echinococcus granulosus is found in all countries

located in the Mediterranean region, especially. Countries like China, Argentina and Peru, and countries located in the Central Asian and East African regions are accepted as endemic for CE [3]. Case reports have been made from all regions in Türkiye, while the disease is more intense in the Southeast Anatolia, Eastern Anatolia, Central Anatolia, Thrace and Marmara regions where animal husbandry is commonly performed [4].

The prevalence of the disease displays significant differences between rural and urban settlement areas. The incidence among those living in rural regions is reported to be 2-6 times higher. Additionally, it was reported that seropositivity increases with

CITATION

Yilmaz A, Orhan F, Uslu H, Ucar M. Research of cystic echinococcus serology in people living in rural areas and dealing with animals with the immunochromatographic method: A small-scale cross-sectional study. Med Science. 2023;12(3):818-22.



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age and this shows that infection continues throughout life [5].

In all countries around the world, it is necessary to create prevention and control programs to reduce CE disease. These programs must be planned and implemented together with neighboring countries. With these control implementations, success can be achieved in solving the problems caused by the disease, calculating the prevalence for the country in general and performing epidemiological research in cooperation with health institutions [6,7].

Diagnosis of CE is generally based on imaging methods; however, some lesions or masses identified with imaging techniques may be mistakenly identified as hydatid cyst. For this reason, in these situations, the use of an appropriate serological technique in addition to the imaging technique will contribute to accurate diagnosis [8]. Hydatid cyst kits used routinely in microbiology laboratories and sold commercially use pure or semi-purified antigens. Our study is based on the serological technique of the immunochromatographic method (ICT) and uses the principle of combining monoclonal or polyclonal antibodies developing in the body against *Echinococcus granulosus* antigens with these antigenic structures [9].

There is a need for accurate and rapid diagnostic tests with the aim of being able to make diagnoses in rural regions or endemic areas without, or with very limited, laboratory facilities. The immunochromatographic test is among rapid diagnostic tests using serological techniques. Immunochromatographic techniques may be used for diagnosis of hydatid cyst due to having advantages like high sensitivity and specificity, being economic, easy to study and providing rapid results [9,10].

In a literature scan, it appears most hydatid cyst serology research was completed on patients attending hospital or with some complaints. In our study, different to the literature, research was completed not on people suspected of having disease, but on those living in rural areas with high probability of disease incidence. For this purpose, our primary aim in our study is to investigate the serology of hydatid disease caused by *E. granulosus*, which causes an important public health problem in Eastern Anatolia and especially in our province, in a small-scale cross-sectional study using rapid diagnostic test in people living in rural areas.

Material and Methods

In this study, blood samples taken from 75 volunteer participants living in rural regions and dealing with animals in counties linked to Erzurum province were stored at -40°C in the Atatürk University Faculty of Medicine Microbiology laboratory until study. Additionally, participants completed a survey form to determine demographic data (name, surname, age, sex, place of residence) and to reveal risk factors in terms of infectious vectors. After thawing blood samples stored at -40°C until study, the immunochromatographic test (VIRAPID® HYDATIDOSIS/VIRCELL) method was performed in accordance with the manufacturer's instructions. Results were read within 30 minutes.

Assessment: Formation of only a control line on the cassette

was accepted as negative reaction (0), formation of a second line apart from the control line was accepted as positivity and graded according to the color assessment scale for the line with positive reactions of 0.5 (low positive), 1, 2, and 3 (Figure 1).

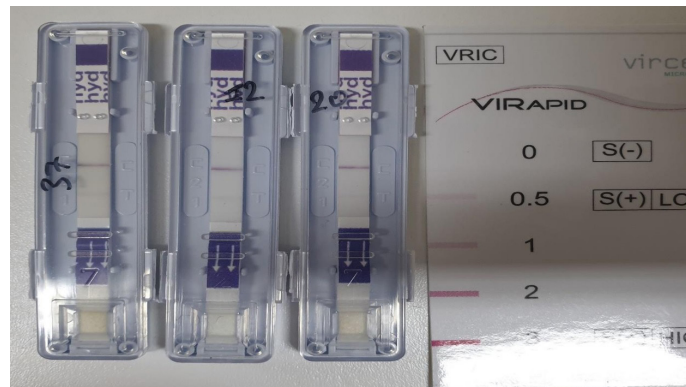


Figure 1. Weak positive samples according to immunochromatographic test

Statistical analysis

Analysis of data obtained in the research used the SPSS (Statistical Package for Social Sciences) 24.0 program. Additionally, for comparison of numerical data with two-way variables, the independent variables chi-square test was used. The obtained results were assessed at 95% ($p < 0.05$) significance level.

Ethical considerations

Ethics committee approval: This was obtained from Atatürk University, Faculty of Medicine, Clinical Research Ethics Committee with decision number 105 dated 31.03.2022.

The research was supported by Atatürk University scientific research project unit as project TAB-2022-10851.

Results

The ages in the study group varied from 18-79 years with a mean age of 46.8 ± 15.97 years. The study group included 29 women (38.7%) and 46 men (61.3%) for a total of 75 participants. Screening with the immunochromatographic method found 72 of these 75 participants were negative (96%), while 3 were positive (4%, weak positive (0.5)) (Figure 1).

The distribution of participants in the study according to county in Erzurum is shown in Table 1. In our study, the counties of residence for individuals positive for CE serology and numbers of positive individuals were 1 person aged 55 years in Hınıs county, 1 person aged 36 years in Çat county and 1 person aged 49 years in Oltu county.

When possible risk factors for CE serology were researched among the participants, results related to responses given to questions about consuming raw meat or not, having pets, hunting, and type and species of animals farmed are shown in Table 2.

The positive results obtained from serum samples analyzed with the immunochromatographic method is shown in Figure 1.

Table 1. Number and percentages according to county for participants

Town	The number of participants	Percentile rate%
Köprüköy	4	5.3
Karayazı	7	9.3
Çat	6	8.0
Oltu	5	6.7
Pasinler	2	2.7
Tekman	7	9.3
Hınıs	6	8.0
Horasan	5	6.7
Aşkale	8	10.7
Olur	3	4.0
Tortum	3	4.0
İspir	3	4.0
Şenkaya	7	9.3
Aziziye	9	12.0
Total	75	100.0

Table 2. Cystic Echinococcosis serology results according to various risk factors

	Pozitivity	Negative	P value
Eating raw meat			
Yes	1 (%14.3)	6 (%85.7)	0.145
No	2 (%2.9)	66 (%97.1)	
Keeping pets			
Yes	1 (%4.3)	22 (%95.7)	0.919
No	2 (%3.8)	50 (%96.2)	
Hunting activity			
Yes	1 (%25.0)	3 (%75)	0.154
No	2 (%2.8)	69 (%97.2)	
Type of livestock made			
Cattle breeding	2 (%3.2)	62 (%96.8)	0.351
Sheep and goat farming	1 (9.1)	10 (%90.9)	
Gender			
Male	2 (%4.3)	44 (%95.7)	0.146
Woman	1 (%3.4)	28 (%96.6)	

Discussion

Hydatic cyst disease continues to be a zoonotic parasitic infection that may cause serious economic harm due to injury to a variety of tissues and organs in animals, in addition to affecting public health in the world and in our country [8]. CE disease has higher incidence in countries with weak socioeconomic status, inadequate health standards and more common animal husbandry and is reported to be observed more frequently in occupational groups like farmers, hunters, shepherds and veterinarians [11].

There is a need for multidimensional programs to ensure eradication in our region and country by controlling CE disease, a threat to public health. In this way, one of the efforts required

is to determine the prevalence rates of the vector in society and in animals in the regions where the disease is endemic. Studies performed with this aim will provide significant contributions to this effort.

Most serological studies in the literature using laboratory diagnosis of hydatid disease appear to be performed with patients attending hospital and with some clinical complaints. Our study, different to the literature, was completed with people living in rural areas with high probability of disease incidence, and not with individuals suspected of having the disease. Additionally, due to the late emergence of clinical findings of disease and the long and costly treatment duration, it is clear that screening these people will provide significant social benefit and economic advantage by ensuring early diagnosis of even one person.

The Virapid Hydatidosis kit, containing a semi-purified form of hydatid cyst fluid enriched with antigen 5 and antigen B used in our study and in the study by Hernandez-Gonzalez et al. [12], was identified to have sensitivity of 83% and specificity of 93%. The same study identified positivity in 27 out of 47 echinococcus samples (63%) used for cross reaction control. In studies using this kit in our country, the sensitivity of the kit was identified as 86.2-96.8% with specificity of 87.5-100% [13-15].

Programs about early diagnosis and treatment of hydatid cyst are not found in several countries where the disease is observed more frequently. As a result, the true prevalence of the disease is not fully known both in society and in individuals in the same environment as the patient. When the literature is researched, the most comprehensive screening study appears to have been performed in Argentina. In this research, radiological screening was performed for 42.374 individuals and according to the research results, asymptomatic cyst cases were encountered in 192 people (0.44%) [16].

In our close geography, Dabaghzadeh et al. [17] chose 680 asymptomatic individuals with the random sampling method from 6 villages in 7 counties in Alborz province in the center of Iran. They found 3.4% positivity rate in this sero-epidemiological research performed to identify the hydatid cyst positivity rate. Eilbigi et al. [18] identified hydatid cyst in 11 patients (5.1%) among 214 radiologically-screened individuals sharing the same living area with hydatid cyst patients treated in a state hospital in Afghanistan. They found 22 patients (10.2%) were serologically positive. During similar studies in Türkiye, Yılmaz et al. [19] found 5.7% seropositivity in Meat and Fish Institution employees, while Durmaz et al. [20] found 5.6% seropositivity in individuals working with animals.

In our study researching the antibody response against the CE vector *Echinococcus granulosus* among individuals working with animals, without any clinical complaints and living in an endemic region, the positivity rate was 4%. In fact, though every region has its own unique cultural and geographical features, results reached for asymptomatic individuals are similar to results from countries in our close geography and to results from

other studies in Türkiye.

Several studies researched the correlation between sex and CE. Some of these studies reported high seropositivity in women, while some reported high seropositivity in men. The study by Dabaghzadeh et al. [17] reported infection was more common in women than in men. Contrary to this, several studies report seropositivity is higher in men compared to women [21,22]. In our study, similar to the study in Türkiye by Kılıç et al. [23], no correlation of positivity with sex was identified.

Due to the late emergence of clinical findings of CE disease, long duration of treatment and high costs, it was considered that early identification of the disease among even one of the people with screening planned in this research would provide both significant social benefits and economic benefits. As a result of this study, CE serology was positive in three people, revealing the importance of performing this study. Additionally, individuals found to be positive at the end of the research were referred to a health institution for advanced studies.

In our study, the positivity rate was high among individuals answering yes to the question of whether they ate raw meat, compared to individuals answering no to this question. Again, seropositivity was higher among individuals answering yes to the question about hunting activity, compared to those answering no. However, when the positivity rates between the groups were statistically assessed, no significant difference was identified between the results ($p>0.05$).

Limitations of the study

A limitation of this study is the need to research a large population due to the broad scope of the geographic area of Erzurum province. However, budget and time restraints prevented us from performing more comprehensive research.

Conclusion

As a result of this study, the easy immunochromatographic method was assessed as beneficial for rapid diagnosis of CE with the aim of screening in endemic regions where laboratory conditions are not suitable. It is notable that 4% CE seropositivity was obtained for individuals without any complaints. Linked to this seropositivity result, it is thought that creating comprehensive CE screening programs for individuals dealing with animals will be beneficial. It is considered that the results obtained in this research will lead the way for performing more comprehensive studies related to the topic.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical Approval

This was obtained from Atatürk University, Faculty of Medicine, Clinical Research Ethics Committee with decision number 105 dated 31.03.2022.

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