Original Article

Seroprevalence of human cystic echinococcosis and risk factors in animal breeders in rural communities in Denizli, Turkey

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Abstract

Introduction: Cystic echinococcosis (CE) is a serious public health problem in sheep-raising regions of Turkey. The aim of this study was to determine the prevalence and associated risk factors of echinococcosis in rural regions of Denizli in Turkey.

Methodology: This study was undertaken in four townships in Denizli County between May 2009 and July 2009. Family members were interviewed to assess possible risk factors for infection and tested for anti-*E. granulosus* antibodies by enzyme-linked immunosorbent assay (ELISA).

Results: Of the 1,133 individuals included in the study, 78 (6.9%) were found to be anti-EG seropositive. Multivariate analysis showed that the 30–39 year age group (odds ratio [OR]: 3.29; 95% confidence interval [CI]: 1.30 ± 8.33; p = 0.01), the ≥ 60 year group (OR: 4.08; 95% CI: 1.57 ± 10.61; p = 0.004), and the group that reported sometimes or never getting veterinary care for their animals (OR: 1.75; 95% CI: 1.05 ± 2.93; p = 0.032) had higher rates of seropositivity. Multivariate analysis showed that education was not significantly associated with seropositivity. Furthermore, no significant correlation with location, occupation, dog ownership or contact with dogs, or with cattle and/or sheep/goat ownership was found. Regular veterinary care and education had significant effects on lowering the prevalence of CE.

Conclusions: Our results suggest that preventive measures, such as regular veterinary care for animals and educative and supportive activities oriented to the people working in farming and animal husbandry should be taken to decrease the prevalence of human CE in Turkey.

Key words: cystic echinococcosis; ELISA; epidemiology; risk factors; Turkey.

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Introduction

Echinococcosis is a chronic disease in humans that is caused by the cestode *Echinococcus,* which includes *E. granulosus, E. multilocularis, E. vogeli*, and *E. oligarthrus*. *E. granulosus* and *E. multilocularis* are the most common, causing cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively [1]. The life cycle of *Echinococcus* includes a definitive host (dogs or related species) and an intermediate host (sheep, goats, or swine) [2]. Humans are incidental intermediate hosts; they do not play a role in the transmission cycle [2,3]. *E. granulosus* is distributed worldwide, and it occurs on all continents, including Turkey [1]. Infection with *E. granulosus* is estimated in 2% to 6% of endemic populations, and the annual incidence in Europe is on the rise in some areas [4]. Risk factors include an agricultural or stock-raising lifestyle, low socioeconomic status, climate, bad hygiene, illegal or uncontrolled slaughter, and uncontrolled dog populations [4-7]. The outcome of infection in livestock and humans is cyst development in the liver (50%–70%), lungs (20%–30%), or other organ systems, but cysts may be found in any organ of the body [4-7].

Cystic echinococcosis is one of the most important parasitic zoonoses in all regions of Turkey, resulting in high economic losses both in the public health sector and in the livestock industry. In Turkey, 24.5% of the population lives in rural areas, where most people are farmers or animal breeders [8]. Reliable data on the prevalence and incidence of echinococcosis in Turkey have not been compiled. According to the data released by the Ministry of Health, 59,808 cases (3,518/year) and 939 deaths (55/year) were reported

between 1987 and 2004 in Turkey [9]. Although high prevalence rates of human and animal echinococcosis have been documented in different regions of Turkey, no studies have been conducted to determine the prevalence and risk factors for CE in the Denizli region.

The aim of this study was to determine the prevalence and associated risk factors of echinococcosis in rural regions of Denizli in Turkey.

Methodology

Study area

This study was conducted over a period of three months, between May 2009 and July 2009. Denizli is located in south-western Anatolia and covers 11,868 km^2. The population of the province was 926,362 according to the 2009 National Census and annual health statistics findings, with 31.2% of the population living in rural areas. Occupations in these rural areas are mainly farming and animal raising.

This study was undertaken in four townships in Denizli County, where there were more animal breeders than in other towns. Lists of animal breeders who lived in these towns were obtained from the Denizli Provincial Directorate of Agriculture. According to the lists from the selected regions, 8,148 families raised animals. To accept a 5% prevalence of hydatid cyst, with a 95% confidence interval, 2% deviation, the minimum sample size was calculated as 440 families. A systematic sampling method proportional to size was used, and 44 separate settlements were identified in the four townships. Accordingly, twenty-seven settlements in the town of Çivril, seven settlements in Buldan, six settlements in Honaz, and four settlements in Bozkurt were included. Ten families were selected randomly from each settlement; the minimum sample size created a list of 440 families. In every settlement, one family was selected as a reserve family.

Questionnaires

Individuals ≥ 18 years of age were included in the study. After receiving individual written permission, a questionnaire was administered to obtain basic epidemiological and individual information regarding known CE risk factors. Age, sex, educational level, residence location, dog ownership, and handling of domestic dogs were recorded. The questionnaire was administered in face-to-face interviews by an assistant doctor from the Department of Public Health. Only one investigator administered the surveys, in order to prevent inter-observer differences.

Collection of blood samples and ELISA

Approximately 10 mL venous blood samples were taken from each family member. All blood samples were transferred to the laboratory on ice on the same day of collection and separated after centrifugation at 1500 gms for 5 minutes. Serum samples were collected and stored at -20°C or -70°C until tested for anti-*E. granulosus* antibodies (anti-EG) by enzyme-linked immunosorbent assay (ELISA).

All serum samples were tested for IgG antibodies to *E. granulosus* by microplate ELISA. An ELISA for immunoglobulin G (IgG) was used to detect anti-*E. granulosus* antibodies. Seropositive persons were called to the hospital for further (radiological) examination.

Antigens

Hydatid cyst fluid (HCF) was aspirated under sterile conditions from hydatid cysts obtained from sheep slaughtered at local abattoirs. The hydatid fluid was centrifuged at 1500xg at 4°C for 15 minutes to separate protoscoleces and other solid agents. The protein concentration in the supernatant was measured with a Bausch & Lomb spectrophotometer (Spec 21) and stored at -20°C until use.

Enzyme-linked immunosorbent assay

A conventional ELISA was used, according to the method described by Engvall and Perlmann [10]. The flat-bottomed wells of polystyrene microtiter plates (EIA microtitration plate 96 flat bottom Lot No: 805202. Linbro, McLean, USA) were coated by overnight incubation at 4°C with 100 µL of HCF antigen (5 µg of protein per mL). The plates were washed three times in PBS (pH 7.2) and stored at 4°C until use. The antigen-coated plates were left for blocking with 150 µL 0.5% casein buffer (CB) at room temperature for one hour, after which an additional washing was performed immediately. The test sera were doubly diluted in 40 mL CB + 10 µL Tween-20 starting from 1:64 (630 µL CB + 10 µL sera) to 1:16.000; 100 µL diluted sera were added to each well. In addition, sera from uninfected humans were added to every plate for negative controls. The plates were incubated at 37°C for one hour. After washing by CB, 100 µL anti-human IgG peroxidase conjugate antibody (Sigma, Immunochemical, Cat No: SA-8667, St. Louis, USA) was added to each well and incubated at 37°C for one hour. After incubation and washing with CB, 100 µL substrate solutions (ABTS tablet [Sigma] + H2O2 in citrate phosphate buffer) was added to all

of the wells. The enzyme substrate reaction was allowed to proceed for 60 minutes at room temperature, and the optical density (OD) at 405 nm (OD405) of each well was determined by using the ELISA plate reader (Titertek, Multiskan Plus MK II, Helsinki, Finland). Cutoff values were determined as the mean plus three standard deviations of the OD observed with normal human controls [11].

Ethical approval for the study was given by the Ethics Committee of Pamukkale University of Medical Sciences (26 May 2009, B.30.2.PAU.0.01.00.00.400-3/125).

Statistical Analysis

Statistical Package for Social Science (SPSS) version 17.0 was used to analyze the data. A Chi-square test was used to determine the significance in prevalence according to the variables. Odds ratios for risk factors analysis were calculated by univariate and multivariate logistic regression models. Only independent variables with p values less than 0.30 based on bivariate analysis were included in the multivariate model. The differences among the groups were considered significant at values of $p < 0.05$.

Results

A total of 1,133 individuals (603 women and 530 men, 44.9 ± 15.05 years of age; minimum 18, maximum 90) living in the villages of Buldan, Honaz, Civril, and Bozkurt in Denizli were included in the study. All of the persons included in this study worked in farming and animal husbandry. Of the 1,133 individuals, 78 (6.9%) were found to be anti-EG seropositive. The age and gender distributions of seropositive patients are shown in Table 1. The seropositivity rate of females (8.1%) was higher than that of males (5.5%) ($p = 0.08$); females were 1.53 times more likely to be seropositive than were males (OR = 1.53; 95% CI = 0.93–2.52).

The regions with the highest prevalence rates of CE were Bozkurt (9.8%) and Honaz (8.3%); the lowest prevalence rates were in Çivril (6.6%) and Buldan (5.3%) ($p = 0.49$).

The mean ages of the seropositive and seronegative participants were 48.3 ± 16 and 44.61 ± 15 years, respectively ($p = 0.035$). The highest prevalence rates by age group were in the 30–39 year age group (9.5%) and in the ≥ 60 year age group (10.3%) ($p = 0.02$) (Table 1).

Table 1. Univariate analysis of CE seropositivity by gender, location, age, occupation, education

		ELISA IgG		Univariate analysis	
	N	**Positive**	**%**	**Odds ratio (95% CI*)**	**p**
Gender					0.08
Male	530	29	5.5	Reference	
Female	603	49	8.1	1.53 (0.93 ± 2.52)	
Location					0.49
Buldan	169	9	5.3	Reference	
Honaz	157	13	8.3	1.6 (0.62 ± 4.21)	
Çivril	715	47	6.6	1.25 (0.58 ± 2.80)	
Bozkurt	92	9	9.8	1.93 (0.67 ± 5.54)	
Age (years)					**0.02**
18-29	194	6	3.1	Reference	
30-39	241	23	9.5	**3.31 (1.24 ± 9.27)**	
40-49	292	16	5.5	1.82 (0.65 ± 5.30)	
50-59	212	13	6.1	2.05 (0.71 ± 6.18)	
≥60	194	20	10.3	**3.06 (1.33 ± 10.28)**	
Occupation					0.08
Farmers	1065	77	7.2	5.2 (0.77 ± 10.2)	
Others	68	1	1.5	Reference	
Education					**0.028**
Illiterate	157	18	11.5	**1.98 (1.09 ± 3.55)	
Primary school	867	56	6.5	Reference	
Secondary school, college and above	109	4	3.7		
Totally	**1,133**	**78**	**6.9**		

*CI: confidence interval **Comparison was made between two groups (illiterate and the others)

Table 2. Univariate analysis of CE seropositivity by veterinary control, dog ownership or exposure, and some characteristics of livestock

		ELISA IgG		Univariate analysis	
	N	Positive	%	Odds ratio (95% CI*)	p
Veterinary control animals					**0.04**
Regular	137	8	5.8	Reference	
Frequently	739	45	6.1		
Sometimes or never	257	25	9.7	**1.67(0.99 ± 2.82)	
Owned animals					0.89
Cattle	763	52	6.8	***1.43(0.31 ± 8.22)	
Sheep/goat	38	2	5.3	Reference	
Both cattle and sheep/goat	332	24	7.2		
Dog owner					
Yes	734	51	6.9	1.03 (0.62 ± 1.72)	0.91
No	399	27	6.8	Reference	
Dog exposure					
Yes	557	43	7.7	1.29 (0.8 ± 2.11)	0.28
No	576	35	6.1	Reference	

*CI: confidence interval ** Comparison was made between two groups (regular control and others)
*** Comparison was made between two groups (cattle and others)

Table 3. Multiple logistic regression analysis of CE seropositivity by gender, location, age, occupation, veterinary control, and some characteristics of livestock

		Multivariate analysis	
Variables	**N**	**Odds ratio (95% CI*)**	**p**
Gender			
Male	530	Reference	
Female	603	1.54 (0.94 ± 2.50)	0.086
Location			
Buldan	169	Reference	0.29
Honaz	157	1.55 (0.63 ± 3.81)	0.35
Çivril	715	1.20 (0.56 ± 2.57)	0.65
Bozkurt	92	2.35 (0.882 ± 6.27)	0.09
Age (years)			
18-29	194	Reference	0.014
30-39	**241**	**3.29 (1.30 ± 8.33)**	**0.012**
40-49	292	1.73 (0.66 ± 4.54)	0.27
50-59	212	2.01(0.74 ± 5.45)	0.17
≥60	**194**	**4.08 (1.57 ± 10.61)**	**0.004**
Occupation			
Farmers	1,065	4.11 (0.55 ± 30.5)	0.17
Others	68	Reference	
Veterinary control animals			
Regular or frequently	876	Reference	
Sometimes or never	**257**	**1.75 (1.05 ± 2.93)**	**0.032**
Owned animals			
Sheep/goat	38	Reference	
Cattle	763	0.45 (0.10 ± 2.06)	0.31
Both cattle and sheep/goat	332	1.10 (0.64 ± 1.90)	0.74
Dog exposure			
Yes	557	1.38 (0.85 ± 2.25)	0.20
No	576	Reference	

Univariate analysis showed that participants in the 30–39 year age group and in the ≥ 60 year age group were approximately three times more likely to be seropositive than those in the other older age groups (OR = 3.31; 95% CI = 1.24 ± 9.27, OR = 3.06; 95% CI = 1.33 ± 10.28, respectively).

When education level was examined, it was determined that the rate of seropositivity was 11.5% in illiterate people; statistically significant differences were found between seropositivity and education level (p = 0.028) (Table 1). Univariate analysis showed that illiterate people were approximately two times more likely to be seropositive than were other people (OR = 1.98; 95% CI = 1.09 ± 3.55).

Seropositivity for CE showed no significant correlation with location, occupation, dog ownership, or contact with dogs (Tables 1 and 2). In addition, no statistically significant differences were found between seropositivity and cattle and/or sheep/goat ownership (Table 2). Seropositivity was found to be significantly lower (5.8%) in those who had regular veterinary care for their animals (p = 0.04) (Table 2). Univariate analysis showed that those who sometimes or never had veterinary care for their animals were 1.67 times more likely to be seropositive than those who had regular veterinary care for their animals (OR = 1.67; 95% CI = 0.99 ± 2.82).

Multivariate analysis showed that those in the 30–39 year age group, the ≥ 60 year age group, and those who sometimes or never had veterinary care for their animals had higher rates of seropositivity than did participants in other variable groups (Table 3).

Seropositive persons were invited to the hospital for further examination (*e.g.*, radiological examination), but only 26 of the 78 seropositive individuals showed up for further examination; their radiological findings (ultrasound and chest X-ray) were negative.

Discussion

Although CE is one of the emerging zoonotic diseases and an endemic disease in most parts of Turkey, little is known about the epidemiology of the disease and its public health importance in Turkey. This study was the first community-based survey of hydatic disease in Denizli. Reports of CE in Turkey are derived primarily from the records of general surgery clinics [6,12]. The annual surgical cases of CE in Turkey are reported to be 0.8–2 per 100,000 population [11]; however, epidemiologic studies have reported higher prevalence rates: 291–6,884 per 100,000 population [7,13]. The seroprevalence rate of CE is 2.7%–14.6% in different areas of the country [7,13-17]. In our study, the seropositivity rate was detected as 6.9% (6,884/100,000) in four towns in Denizli. Different studies in other countries have shown 3%–13.8% CE seroprevalence [18-21].

The seropositivity rate in our study was higher than that found in some earlier reports from our region, but lower than that found in other reports [7,9,13,17]. Seropositive persons were called to the hospital for further radiological examinations; however, 52 of the 78 did not show up. A total of 26 of the 78 seropositive individuals did come to the hospital for further examination, but their radiological examination (ultrasound and chest X-ray) results were negative. The 26 seropositive individuals with negative ultrasounds and chest X-rays could be explained by aborted infection, undetectably small cysts, or false-positive reactions. The use of serological tests in community screening can have a number of benefits. Serologic tests are the most widely used method, one that is applicable, low-cost, not time consuming, and easy to perform on large numbers of serum samples. The presence of a specific antibody alone does not confirm diagnosis, as individuals may be seropositive for a number of reasons, such as previous exposure to the parasite without progressive disease or cross-reactivity with other conditions. False-positive results occur because of cross-reactions with helminth species (such as *Taenia*, *Fasciola*, *Schistosoma*, and *Toxocara*) or non-infectious conditions, such as cancer, pregnancy, or autoimmune diseases [21,22]. Furthermore, small cysts in the very early stages are not easily detectable by radiological examination [21].

Cystic echinococcosis can affect people of almost all ages, from below 1 year of age to over 75 years, and both sexes [1]. In general, CE infection increases with age [23]. The highest numbers of CE cases were recorded by age groups: 21–30 years in Kenya and 21–40 years in Libya [1] .Bai *et al*. reported that the seropositivity rate for CE increased significantly with increasing age [24]. In our study, the prevalence for CE in the < 30 years age groups was markedly lower than that in the older age groups. Prevalence reached a peak in the 30–39 and > 60 age groups. Univariate analysis showed that seropositive prevalence was approximately three times higher in those age groups than in the 18–29 age groups. In addition, multivariate analysis showed that the same age groups had higher rates of seropositivity than did the other ones.

In this study, there were no statistically significant differences between the seropositive and seronegative

groups in terms of location of residence. Regarding the prevalence of CE in the four townships screened, the highest and lowest prevalence rates were detected in Bozkurt and Buldan, respectively. There were no statistically significant differences between townships. All individuals included in the study lived in rural areas; therefore, no differences were expected in terms of seropositivity.

Educational status showed statistically significant differences. Univariate analysis showed that illiterate people were approximately two times more likely to be seropositive than were those with a higher educational status ($p = 0.03$). However, multivariate analysis showed that education was not found to be significantly associated with seropositivity.

In both univariate and multivariate analysis, cystic echinococcosis seropositivity showed no significant correlation with occupation, but the prevalence for CE in farmers (7.2%) was higher than in the other occupational groups (1.5%) ($p = 0.08$).

Our investigation, like previous surveys, showed that more females than males were infected. Seropositivity was higher in females (8.1%) than in males (5.5%) ($p = 0.08$). This may be due to specific activities performed by women, such as feeding dogs and cleaning stables, where women are in more contact with risk factors than men are, in addition to farming and herding. As such, there may be more opportunities for women to be exposed to environments contaminated by *Echinococcus* spp. eggs, resulting in the higher prevalence we observed in females. The United Kingdom, the Middle East, and North Africa have reported higher numbers of affected women [1,25,26]. However, this is in contrast to studies conducted by Cohen *et al*. and Qaqish *et al*., wherein such associations were not observed [27,28].

Dog ownership and contact with dogs were not found to be significantly associated with seropositivity in this survey. This is in agreement with some studies [19,21,29,30]. Nonetheless, other studies have found dog ownership to be a significant risk factor for CE [31,32]. Due to cultural and religious beliefs, Muslim families in the present survey kept dogs far away from their residences and avoided direct contact with them. In addition, cattle and/or sheep/goat ownership were not found to be significantly associated with seropositivity. However, univariate and multivariate analysis showed that seropositive rates were lower in animals receiving regular veterinary care ($p < 0.05$).

The present study has some limitations. One is the absence of a true standard that would enable evaluation of alternative diagnostic tests; we could not evaluate with a second serological test. However, the ELISA we used is one of the most sensitive serological tests for the diagnosis of hydatid disease, and is inexpensive and relatively easy to use. Additionally, this test can be use for large-scale screening of populations in which hydatidosis is endemic. The second limitation is that additional radiological evaluations could only be made in a small number of patients. Another one of the limitations is that only animal breeders who were present and who volunteered to participate in the study were examined, so the EC prevalence rate may have been overestimated.

Conclusions

We found a high prevalence of CE among animal breeders in the studied rural areas. Regular veterinary care and education had significant effects on lowering the prevalence of CE. Our results suggest that preventive measures, such as regular veterinary care for animals and educative and supportive measures oriented to the people working in farming and animal husbandry should be taken in order to decrease the prevalence of human CE in Turkey.

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