The Prevalence of Human Cystic Echinococcosis in an Endemic Region of Adana: A Pilot Radiologic-Serologic Survey

Adana'nın Endemik Bir Bölgesinde İnsanlarda Kist Hidatik Yaygınlığı: Radyolojik-Serolojik Pilot Bir Çalışma

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Geliş Tarihi/*Received:* 10.10.2008
Kabul Tarihi/*Accepted:* 25.03.2009

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ABSTRACT Objective: Cystic echinococcosis (CE) is a zoonosis in which most transmission depends on domestic animal reservoir hosts, livestock husbandry and dog management practices as well as human behavior. CE remains silent for years before the enlarging cysts cause symptoms in the affected organs. Radiographic surveys using imaging techniques, especially portable ultrasound (US) scanners at community level, provide a relatively new mass screening approach for human surveillance in CE control programs. This study conducted in a district of Adana located in the southeastern part of Turkey, aimed to find out the prevalence of CE. **Material and Methods:** This study was conducted in two selected villages named Kürkçüler and Bağtepe, Adana in 2006. Samples were collected from 1272 participants who were screened by portable US and by chest x-ray and were analyzed for anti-*Echinococcus granulosus* antibodies by enzyme linked immunosorbent assay (ELISA) and Western blot (WB) tests. **Results:** Of the total participants 46 (3.6%) were diagnosed with CE by US. No cystic lesions were detected by chest x-ray films. Forty (3.1%) and 41 (3.2%) participants were positive for CE by ELISA and WB tests, respectively. Of the US-positive CE participants 54.3% were antibody seropositive by ELISA and 73.9% by WB. Sera samples taken from US-negative participants were 1.2% seropositive by ELISA and 0.6% by WB. **Conclusion:** In conclusion, the findings of this study suggest that US combined with radiological and serological surveys should be used for the diagnosis of CE in the field.

Key Words: Echinococcosis; epidemiology; radiology; serology

ÖZET Amaç: Kistik ekinokokkoz (KE) köpek besleme alışkanlığının bulunduğu, büyük ve küçükbaş evcil hayvan yetiştiriciliğinin yaygın olduğu ve bir arada bulunduğu insan topluluklarında görülen bir zoonozdur. KE yerleştiği organda büyüyüp semptom verene kadar yıllarca sessiz kalabilir. KE tanısı için görüntüleme yöntemlerinin uygulandığı çeşitli radyolojik incelemelerden özellikle taşınabilir ultrasonografi (US) ile kırsal alanlarda yaşayan topluluklarda tarama yapılması KE kontrol programı açısından önemli kolaylıklar sağlamaktadır. Bu çalışma Türkiye'nin güneydoğusunda yer alan Adana'da gerçekleştirilmiş olup, bu bölgede KE yaygınlığının saptanması amaçlanmıştır. **Gereç ve Yöntemler:** Bu çalışma 2006 yılında Adana iline bağlı Kürkçüler ve Bağtepe köylerinde yapılmıştır. Örnekler iki farklı köyden 1272 katılımcıdan elde edilmiştir. Katılımcılar, akciğer filmi ve taşınabilir ultrasonografi (USG) ile taranmış, anti-KE antikorları açısından ELISA ve Western Blot (WB) testleri ile incelenmişlerdir. **Bulgular:** USG yöntemi ile 46 (%3.6) kişide KE saptanmıştır. Akciğer grafilerinde herhangi bir lezyona rastlanmamıştır. Kırk (%3.1) katılımcıda ELISA ile, 41 (%3.2) katılımcıda WB yöntemi ile KE'ye karşı antikor pozitifliği saptanmıştır. USG incelemesi pozitif olanların %54.3'ü ELISA ile, %73.9'u WB ile seropozitif bulunmuştur. USG incelemesi negatif saptanan katılımcıların %1.2'si ELISA ile, %0.6'sı WB ile KE seropozitif bulunmuştur. **Sonuç:** Bu çalışmanın bulguları, saha koşullarında yapılan taramalarda USG temelli radyolojik ve serolojik yöntemlerin birlikte kullanılmasını önermektedir.

Anahtar Kelimeler: Kist hidatik; epidemiyoloji; radyoloji; seroloji

Turkiye Klinikleri J Med Sci 2009;29(4):890-4

Cystic echinococcosis (CE) caused by infection with the tapeworm *Echinococcus granulosus* is one of the most serious and geographically widespread of the parasitic zoonoses, yet very little is known about its public health importance and epidemiology within defined endemic communities.[1] Cyclical transmission of the parasite between domestic dogs and sheep in pastoral areas, usually associated with home slaughter, is responsible for maintaining the infection in most endemic regions.[2,3] This infection is a major health problem in all regions of Turkey, but it is particularly prevalent in rural areas where domestic livestock-raising is more common. The frequency of the infection has been quantified mainly by retrospective reviews of surgical records of hospitals.[4] Human CE community-based surveys have also demonstrated the importance of CE in Turkey.[5,6] In addition, the use of a portable US scanner and chest x-ray has recently become the most effective community based screening technique for CE.[7,8] Thus, we aimed to compare the people living in two different villages, Kürkçüler and Bağtepe in order to determine the prevalence of CE. Using portable US scanner, chest x-ray, enzyme linked immunosorbent assay (ELISA), and WB methods we investigated the prevalence of CE among people living in two villages of Adana located in the southeastern part of Turkey.

MATERIAL AND METHODS

This study was conducted in two selected villages named Kürkçüler and Bağtepe, Adana, during 2006. A total of 1272 persons living in these villages were included in the study. The number of participants from Kürkçüler was 675 (381 females, 294 males) and from Bağtepe was 597 (341 females and 256 males). The participants aged between 5 and 87 years (Mean±SD; 44.4±18.6 in Kürkçüler and 43.6±19.1 in Bağtepe). They were examined for CE by a portable US (SonoSite 180/plus, hand-carried US, Washington, USA) and chest x-ray (Definium AMX 700, GE Healthcare, San Diego, USA) in the field.

About 5 mL of venous blood samples were collected from all participants. After centrifugation, the sera samples were stored at -20°C, and anti-*Echinococcus granulosus* antibodies were determined by Echinococcosis ELISA and WB (Euroimmune, Germany), according to the protocols established by the manufacturer. A central dispensary building/primary health care center was determined in each of the two villages, and the village people who were convenient were involved in the study. US screening was accomplished by using a portable real time US with a 3.5-mHz sector probe. Individuals were scanned standing to facilitate rapid screening from the front and the back. The liver, pancreas, spleen, and kidneys were carefully examined and any pathologic cystic images were recorded. Cysts due to CE were differentiated from other cystic lesions if one or more of the characteristic diagnostic criteria of CE were present, namely, a laminated membrane and/or daughter cysts. All CE cysts were classified according to their size, morphology, and echotomographic appearance.[9] For indeterminate results, the patients were reevaluated using an advanced US scanner (Logic 7, General Electric Medical Systems Europe, France) with a 3.5-7.5 MHz multifrequency convex probe. Diagnosed patients were provided a treatment consultation at the University hospital clinic.

The study protocol was approved by the local Ethics Committee, and a written informed consent was obtained from all participants. A brief questionnaire was completed and physical examinations were performed for all participants and information on the kept domestic livestock and dogs were collected. The data were also used to obtain details of the relationship between humans and dogs, to quantify the human:dog ratio, and to determine retrospectively the number of previous surgical operations for CE in the population examined.

STATISTICAL ANALYSIS

SPSS 16.0 was used for analysis. Two independent samples t-test was applied to compare ages between villages. The Chi-square test was used to compare screening tests between villages. Mc

Nemar test and Kappa statistics were used to get accuracy of ELISA and WB compared to Ultrasonography. Odds ratio (OR) and its 95% confidence interval (CI) of developing CE were calculated.

RESULTS

A total of 1.272 people -722 (56.8%) females, 550 (43.2%)- were screened for CE by imaging and serological methods. No cystic lesions were detected on chest x-rays. Forty-six (3.6%) [Kürkçüler 39 (5.8%), Bağtepe 7 (1.2%) p< 0.001] participants had newly diagnosed abdominal cysts that were eventually designated as CE by US. Of these participants, 40 (3.1%) [Kürkçüler 36 (5.3%), Bağtepe 4 (0.7%), p< 0.001] were positive for CE by ELISA and 41(3.2%) [Kürkçüler 34 (5.0%), Bağtepe 7 (1.2%), p< 0.001] were positive by WB (Table 1).

Of the 46 US-positive CE participants 25 (54.3%) were antibody seropositive and 15 (1.2%) of the 1226 US-negative participants were antibody seropositive by ELISA p= 0.405. Similarly, 34 (73.9%) of US-positive CE participants and 7 (0.6%) of US-negative participants were antibody seropositive by WB p= 0.359 (Table 2).

During re-evaluation of 46 US positive individuals, the cysts were localized only in the liver. All patients exhibited hepatic cysts, 84.8% of which involved the right lobe. The diameters of the CE ranged from 1.5 cm to 6 cm with an average diameter of 2.9 cm. Age-specific prevalence rates for both sexes combined was highest for participants aged 31-44 years (32.6%). The female seropositivity rate (4.3%) was higher than that for males (2.7%) but this difference was not significant (p= 0.172). The kept dog population in Kürkçüler and Bağtepe was 77.8% and 21.6%, respectively (p< 0.001). In addition, US test result in kept dog and non kept dog population was 5.0% and 2.1%, respectively (p< 0.01) (Table 3).

OR of developing CE was 2.47 [95% CI (2.29-4.74), p= 0.006] times higher for kept dogs compared to non kept dogs householders.

TABLE 1: US and serological (ELISA and WB) screening test results by two villages for cystic echinococcosis.

	Kürkçüler		Bağtepe		p
	n	%	n	%	
US					<0.001
+	39	5.8	7	1.2	
-	636	94.2	590	98.8	
ELISA					<0.001
+	36	5.3	4	0.7	
-	639	94.7	593	99.3	
WB					<0.001
+	34	5	7	1.2	
-	641	95	590	98.8	

US: Ultrasound, ELISA: Enzyme linked immunosorbent assay, WB: Western blot.

TABLE 2: Comparison of US and serological test results in people surveyed for CE.

	US				
	+		-		
	n	%	n	%	p McNemar
ELISA					0.405
+	25	54.3	15	1.2	
-	21	45.7	1211	98.8	
WB					0.359
+	34	73.9	7	0.6	
-	12	26.1	1219	99.4	

CE: Cystic echinococcosis, US: Ultrasound, WB: Western blot, ELISA: Enzyme linked immunosorbent assay.

TABLE 3: Kept dogs for Kürkçüler and Bağtepe villages and US results.

	Kept Dogs				
	+		-		
	n	%	n	%	p
US					<0.01
+	33	5	13	2.1	
-	621	95	605	97.9	
Villages					<0.001
Kürkçüler	525	77.8	150	22.2	
Bağtepe	129	21.6	468	78.4	

US: Ultrasound.

DISCUSSION

The diagnosis of CE is primarily based on imaging and serological methods. In a growing body of re-

search, both serology and US were used[7,8,10-12] and chest radiography might also be applied.[5] US is usually found to be superior to serology for the diagnosis of hepatic CE while chest X-ray is accepted as the best way for screening lung lesions.[13] In addition, US allows early diagnosis of the infection before the immune response is detectable serologically and contrary to serology, it immediately determines the site, size, position and condition of liver cysts. The differential diagnosis between univesicular CE and simple hepatic cysts, however may not be possible merely by US and serologic tests may be required.[5]

Conducting a research on 630 primary school children in Manisa Turkey, Özkol et al reported that 8.9% and 10.1% were seropositive for antibody to *Echinococcus* by ELISA and by indirect hemagglutination (IHA).[5] In another study carried out in Kayseri, 61 (2.7%) of 2242 blood samples tested by ELISA and indirect fluorecent andtibody (IFA) were seropositive.[6] In our study, however, US and chest x-rays were used together with ELISA and WB. US, in this study, was more acceptable than the other methods in field studies and more effective in providing information about the cyst nature. The results of serologic tests may be difficult to interpret, especially when the seroprevalence is high in the community and the detected antibody levels are low.[14] In some community-based studies, where serological assays and US were performed together, the rate of seropositive participants was higher than those detected by US.[7] Although the high seropositivity levels could bee attributed to extra-abdominal or abortive *Echinococcus* infections, many of them were likely to be false-positives due to cross-reactions with other parasitic infections. In our study we did not find any extra-abdominal CE infections. The use of two different serological tests may increase the sensitivity, yet, false negative results still may not be eliminated as in our ELISA (six false negative) and WB (five false negative) results. In addition, we did not detect any significant difference between ELISA and WB positivity rates, which were 3.1 and 3.2%, respectively. According to the Mc Nemar test results, WB seropositivity rate was higher than that of ELISA, but was statistically insignificant ($p= 0.359$) compared to the gold standard US method. Besides, Kappa values for agreement between US and ELISA were 0.567, $p< 0.001$, and 0.774, $p< 0.001$, respectively. The use of WB and US is valuable for epidemiological surveys in CE diagnosis. Thus, the detection of specific and non-specific reactions may vary due to the type of serological tests used. ELISA with crude hydatid cyst fluid antigen was reported to lack specificity. This suggests that a follow-up study of positive participants by both WB and ELISA may be useful when assessing the value of serological tests in mass-screening studies.

Both radiological and serological positivity rate in the Kürkçüler village was significantly higher as compared to the Bağtepe village (Table 1). This significant difference in the positivity rates might have arisen from the comparatively higher water contamination status and CE endemicity. All six members of one family had CE in Kürkçüler. This was associated with the contamination of their drill water supply. In addition, the comparison of dog owning revealed that the kept dog population in Kürkçüler was 77.8% and in Bağtepe it was 21.6% ($p< 0.001$). Thus, there appeared to be a close relation between kept dog and CE (Table 3).

In conclusion, the results of this study suggest that radiological and serological surveys should be made for the diagnosis of CE in the field. To our knowledge, very few studies to date have reported using US and chest films together with ELISA and WB for epidemiological survey of CE. Thus, larger epidemiological studies are required. In addition, since the incidence varies largely in different areas of Turkey, reliable information is necessary on the in-country geographical distribution.

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