Cystic echinococcosis in Central Anatolia, Turkey

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ABSTRACT

Objective: Human cystic echinococcosis (CE) caused by infection with a larval stage of *Echinococcus granulosus* is a serious public health problem in Turkey. Echinococcosis is a zoonotic disease; dogs and livestock are important hosts in transmission. The aim of this study is to evaluate the rate of CE in Kayseri Rural Area, Central Anatolia, Turkey.

Methods: At the present study, we planned to evaluate the rate of CE in Kayseri rural area in Central Anatolia between 2000 and 2002. We investigated 2,242 subjects using enzyme-linked immunosorbent assay (ELISA) and indirect fluorescence antibody (IFA), and we examined the seropositivity by using Western blotting (WB).

Results: The seropositivity rate was 2.7% by ELISA and IFA. We retested seropositive serum samples and 200 seronegative sera by WB. Seropositive serum samples were studied using abdominal ultrasound and chest x-ray to confirmed the presence of hydatid cyst and we found 10 (0.5%) different localized cysts.

Conclusion: The results of our study indicate that Kayseri rural area has a high endemicity of human CE.

Saudi Med J 2006; Vol. 27 (2): 205-209

Hydatid cysts of *Echinococcus granulosus* (*E. granulosus*) develop in internal organs (mainly liver and lungs) of humans and other intermediate hosts as unilocular fluid-filled bladders. The definitive hosts of *E. granulosus* are carnivores such as dogs and wolves, which are infected by ingestion of offal containing hydatid cysts with viable protoscoleces. After ingestion, the protoscoleces evaginate, attach to the canine intestinal mucosa, and develop into adult stages. Sexual maturity (length of 3-6 mm) is reached 4-5 weeks later. Eggs or gravid proglottids are the shed in the feces. Following ingestion by human or ungulate intermediate host (sheep, goats, pigs, cattle, horses, and camels) an oncosphere larva is released from the egg. The larvae then penetrate into the lamina propria and are transported passively through blood or lymph vessels to the liver, lungs, or other organs, where the oncosphere larvae develop into hydatid cysts (metacestode larvae). It has parasite-derived layers: an inner nucleated germinal layer, and an outer acellular laminated layer surrounded by a host-derived fibrous capsule. Brood capsules and protoscoleces bud from the germinal membrane. The range of intermediate host species depends on the infecting strain of *E. granulosus*, regional or local differences in the availability of the various intermediate host species, and other factors.[1] Since the life cycle relies on carnivores eating infected herbivores, humans are usually a "dead-end" for the parasite.[2] Cystic echinococcosis (CE) is an important parasitic disease caused by the dog tapeworm *Echinococcus granulosus*. This is extremely widespread in eastern

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Received 17th August 2005. Accepted for publication in final form 20th November 2005.

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and southern Europe, the Middle East, North Africa and South America.[3] Human CE is a serious public health problem in Turkey, in health and economic terms. The Kayseri district of Central Anatolia has a high number of surgical cases of CE in Turkey, but there were a few clinical and community investigations reported. Some surgical reports of hospitals were performed to evaluate the condition of CE. Therefore, we have decided to evaluate the CE among people living in Kayseri rural area. The present epidemiological study was conducted from September 2001 until July 2003, using the enzyme linked immunosorbent assay (ELISA), indirect fluorescence antibody (IFA) and then Western blotting (WB) to determine the anti-*E.granulosus* IgG antibodies.

Methods. The study area was located at the Central Anatolia of Turkey. This area consists of rural regions in Central Anatolia. Twenty-one villages were randomly selected from districts of Kayseri between 2000 and 2002. Farming and animal raising are the most important activities of the population. Domestic animals commonly found were dogs, cats, chickens, ship, goat and cattle. Informations concerning residence history, occupation, dog ownership and feeding practices, and if animals were slaughtered at home were obtained from the sample studies. The study population for investigation of anti-E.granulosus antibodies consisted of 2242 resident subjects (1027 males and 1215 females) aged between 10-90 years (mean 40.6±16.3 years). Approximately 5 ml venous blood samples were taken from the patient's brachial vein and separated after centrifugation at 1000 gms for 10 min and stored at -70^{0}C until the analysis. Serum samples from surgically confirmed CE patients from Turkey were used as positive antibody controls and healthy Turkish people as negative controls. Parasitized viscera were obtained from recently slaughtered animals and were transported immediately to the laboratory under appropriate sanitary conditions. Hydatid cyst fluid (HCF) was aspirated from fertile cysts by syringe which was clear in appearance and collected in a cylinder or bottle and was left to allow coarse particles such as protoscoleces, brood capsules and so forth. Then the fluid was decanted and transferred to centrifuge tubes and centrifuged at 3000 x g for 15 minutes. The supernatant fluid was then frozen at -70°C until use. Protoscoleces obtained from fertile HCF were used as an antigen for IFA. They were washed 3 times for 5 min in phosphate-buffered saline (PBS) pH 7.2 (centrifugation at 1000 x gms) and were resuspended in PBS and applied in 5 μl drops to the covered Teflon and 10-well microscope slides. Preparations were air dried overnight at room temperature and individually wrapped and stored at -20°C until use.[4]

Immunodiagnostic tests used were ELISA and IFA techniques as initial screening tests to estimate the prevalence of CE in male within the rural areas. The standard micro-ELISA was used according to the method described by Engel and Perlmann.[5] Briefly, wells of polystyrene microtitration plates (Linberg EIA microtitration plate 96 flat bottom) were coated by overnight incubation at +4°C with 100 μl of HCF antigen (5 μg of protein per ml). The plates were washed 3 times in PBS (pH 7.2) and stored at +4^{0}C until use. The antigen coated plates were left for blocking with 0.5% casein buffer (CB) at room temperature for one hour. After, an additional washing used immediately. The test sera were doubly diluted in 40 ml CB+10 μl Tween-20 starting from 1:64 to 1:16.000. 100 μl-diluted sera were added to each well. Plates were incubated at 37°C for one hour. After washing by CB, 100 μl anti-human IgG-peroxidase conjugate (Sigma Immunochemical, cat no: SA-8667) was added to each well and incubated at 37°C for one hour. After incubation and washing with CB, 100 μl substrate solution (ABTS tablet-Sigma + H_2O_2 in citrate phosphate buffer) was added to all of the wells. The enzyme substrate reaction was allowed to proceed for 45 min at room temperature, and the color intensity was measured at 405 NM wavelength directly in the wells with an automated ELISA reader (Anthos-2000). Positive and negative serum samples were included in each plate along with substrate and buffer blanks. Titers of 1:128 or higher were considered as positive. Indirect fluorescence antibody was carried out using the method described by Bongort et al.[6] Briefly, the serum samples were doubly diluted in PBS starting from 1:8. Eight μl-diluted samples were dropped into each well of the covered Teflon microscope slides. Slides were incubated for 30 min at 37^{0}C and washed with PBS twice. Eight μl fluorescein-labeled anti–human immunoglobulin-G (conjugate) into each reaction field. They were all incubated for 30 min at 37^{0}C and washed with PBS twice. Places were embedded glycerol/PBS onto a cover glass drops of 5 μl was added to per reaction field and read under the fluorescence microscope (Nicon E-600). It was accepted as negative if the protoscoleces were completely dark and if it was green fluorescence light we accepted it as positive titter (**Figures 1a & 1b**).We considered positive if the titter was ≥1:16. We used Western blotting analysis for retesting the seropositive serum samples and 200 seronegative sera. We purchased the WB kits from EUROIMMUN commercial manufacturer. This technique was performed as manufacturer's instructions.

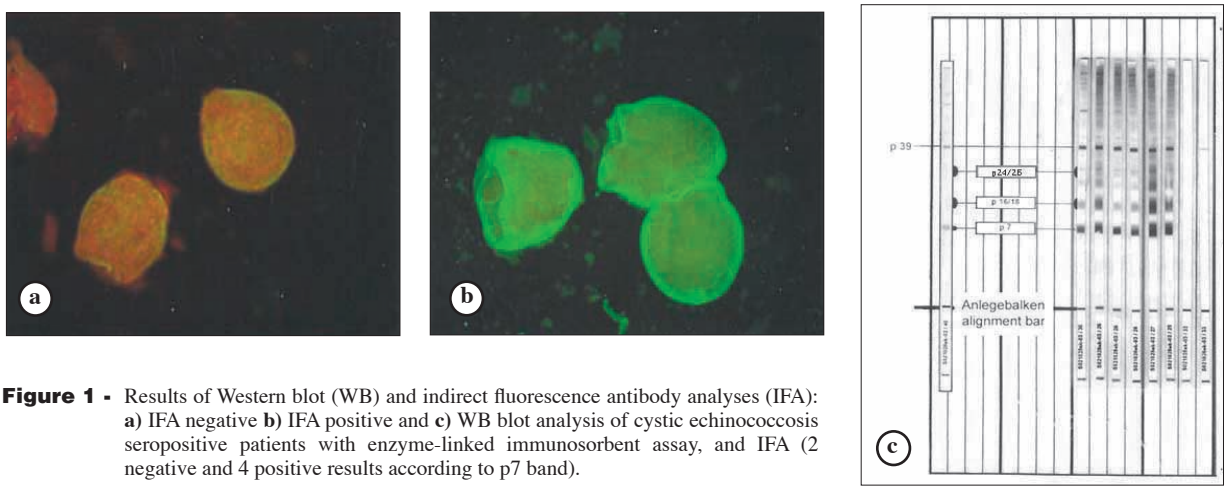

Figure 1 - Results of Western blot (WB) and indirect fluorescence antibody analyses (IFA): **a)** IFA negative **b)** IFA positive and **c)** WB blot analysis of cystic echinococcosis seropositive patients with enzyme-linked immunosorbent assay, and IFA (2 negative and 4 positive results according to p7 band).

Table 1 - Study areas and results.

Region	Population screened	Immunodiagnostic tests results		Radiological observations
		ELISA-IFA	WB	
Akcakaya	51	1	0	-
Basakpinar	84	0	0	-
Develi	296	15	4	3 operated liver cysts, 1 liver + lungs cysts
Dokuzpinar	78	1	0	-
Ebic	25	0	0	-
Erciyes	46	0	0	-
Gelbula	143	5	2	1 operated kidney cyst
Gomurgen	118	5	2	1 liver cyst
Gurpinar	266	8	1	-
Hacilar	50	6	2	1 lungs cyst
Han yeri	140	0	0	-
Incesu	22	0	0	-
Kayaonu	39	0	0	-
Kepez	37	0	0	-
Mahzemin	66	0	0	-
Resadiye	91	2	1	-
Suksun	170	4	3	1 liver cyst
Tuzhisar	266	13	5	1 liver cyst, 1 spleen cyst
Yaziyurdu	21	0	0	-
Yesilhisar	86	0	0	-
Zincidere	147	1	1	-
Total	**2242**	**61**	**21**	3 operated liver cysts, 1 operated kidney cyst,
(%)		**(2.7)**	**(0.9)**	3 liver cysts, 1 liver + lungs cyst,
				1 lungs cyst, 1 spleen cyst
				10 (0.5)

ELISA - enzyme-linked immunosorbent assay, IFA - indirect fluorescence antibody, WB - Western blotting

Statistical analysis was performed with SPSS software package (Version 9.0 for Windows). Chi-square test was used and $p<0.05$ was accepted as statistically significant.

Results. Twenty-one villages were randomly selected from the districts of Kayseri. Of the 2242 residents who were screened firstly by IFA and ELISA tests, 61 (2.7%) samples produce positive reactions to one or 2 serological tests. These serum samples were also examined by WB and found positive in 21 (0.9%) with 7 (or 8) kDa. band (**Figure 1c**). They were informed to undergo radiological examination (x-ray and ultrasonography). We discovered that 4 patients (2 females, aged 44 and 53 years; and 2 males, aged 48 and 61 years) undergone surgery due to CE (3 liver CE, one kidney CE) and one patient died due to congestive heart failure. Fifteen positive WB individuals were admitted to the hospital for further examinations. During radiological examination, 6 of 15 WB positive individuals had different localized cysts. Six (0.3%) asymptomatic (4 females, aged 10, 38, 52 and 54 years; and 2 males, aged 38 and 69 years) patients with CE were detected among rural residents screened serologically by ELISA, IFA, WB and then by abdominal ultrasound and chest x-ray. In one case, the cysts were localized in multiple organs (liver and lung). There were no cyst found in ELISA and IFA positive but 25 people WB negative. Results of the investigations were shown in **Table 1**. The female seropositivity rate (0.5%) was found to be greater than males (0.5%) but the difference was not statistically significant ($p>0.05$). Rates of ownership of dog in the seropositive and the investigated community were 38% and 35.2%, but the difference was not statistically significant ($p>0.05$).

Discussion. *Echinococcus granulosus* has a worldwide geographical distribution. It is found on all continents, with highest prevalence in parts of Eurasia (especially Mediterranean countries, the Russian Federation and adjacent independent states, and China), North and East Africa, Australia, and South America.[7] There is clear evidence for the emergence or re-emergence of human cystic echinococcosis in parts of China, Central Asia, Eastern Europe, and Israel.[7,8] Communities involved in sheep farming harbor the highest rates of infection, showing the public health importance of the sheep-dog cycle and the sheep strain of *E. granulosus* in transmission to people.[9,10] The numbers of surgically confirmed CE patients were 5.964 between 1984 and 1986, and 21.303 between 1987 and 1994 in Turkey. The average number of new cases of hydatid disease is approximately 2000-2500 per year. As these are surgically confirmed cases, the real number of patients was unknown. *Echinococcosis* was not among the diseases, which must be reported to Ministry of Health in Turkey until recent; thus the data is unreliable.[11] According to the hospital records, 349 CE cases were surgically proved in Kayseri between 1994 and 1998.[12] The use of sero-epidemiological methods to the study distribution, intensity and epidemiological factors affecting the prevalence of certain parasitic diseases has been widely accepted.[13,14] Limited epidemiological studies disclosed higher prevalence rates in other parts of Turkey; 585 per 100,000 population among 684 subjects[15] and 291 per 100,000 population among 2.055 subjects.[11] In the present study, we found that the prevalence of CE was 446 per 100,000 in rural area of Kayseri. All the above stated data clearly show that *Echinococcosis* is not limited to certain regions, in contrast it may be accepted a widespread problem in Turkey. According to our data, serologic results were different compared with radiological observations. Some reasons may be cause this condition: a) Serologic techniques may be showed false-negative results due to circulating immune complexes and false-positive data due to cross-reactivity with other parasitic infections. b) Some cysts could not detected by radiological examination due to extra hepatic and extra pulmonary localization. We did not carry out any investigation in other body zones by radiological methods. c) Some cysts were smaller in size that could not be detected by the radiological methods.

In conclusion, the current, extensive, sero-epidemiology-based study confirms the high endemicity of human CE in rural Kayseri in Central Anatolia with an overall prevalence of 0.5% for abdominal and pulmonary CE. If hydatid cysts in other sites in the human body are included, such as cerebral, bone locations, then the prevalence would be higher. The seropositive rate for anti-E.granulosus antibodies was high (2.7% with ELISA and IFA, 0.9% with WB) in the population screened. It is clear that CE is a major public health problem in Kayseri region. It has not yet performed any *Echinococci* control program in Turkey. Extensive studies are required to investigate the epidemiology of the disease in Kayseri and also all around Turkey to constitute a control program as well as in the other countries especially in the Middle East.[16,17] Therefore, serious consideration should be made regarding the implementation of suitable regional or national hydatid control program.

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