

Research Note

Detection of *Echinococcus granulosus* coproantigens in dogs from Antakya Province, Turkey

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Summary

Seventy-nine owned dogs (47 males and 32 females) from Antakya province were examined for *E. granulosus* by coproantigen ELISA (CHEKIT®-ECHINOTEST) between April to July in 2006. The estimated prevalence of *E. granulosus* infection was 8.86 % (7/79). Mean age of the dogs with *E. granulosus* was 2.3 years ranging from one to five years. There was no statistically significant difference regarding the prevalence between male and female dogs. A significant difference was detected between unrestrained (or free roaming) and indoor or chained dogs ($p < 0.05$). Although the prevalence of the *E. granulosus* infection in owned dogs in Antakya province is low, the incidence was quite high in the unrestrained and undewormed dogs. Therefore a preventive control programme in the dogs should be initiated in this region.

Key words: *Echinococcus granulosus*; dog; coproantigen ELISA; Antakya; Turkey

Introduction

Echinococcus granulosus infection is one of the most important zoonotic diseases, and it is a serious public health problem in various regions of the world, including Turkey (WHO/OIE, 2001). The parasite has a global distribution, but is particularly prevalent in rural areas, where generally it circulates between the dog, as the definitive host, and the sheep, which serve as intermediate host yielding fertile cysts and playing an important role in the transmission of the disease (Buishi *et al.*, 2005). In relation to seroepidemiologic studies in Turkey, the rate of echinococcosis in human population is 291/100,000 (Altintas, 1998). In people living in villages and working in agriculture and stock raising, a high seropositivity rate about 14.6 % was detected (Cetinkaya *et al.*, 2005). The estimated surgical case rate of cystic echinococcosis was also reported to be 0.87 – 6.6/100,000 (Ozkol *et al.*, 2005). Canine echinococcosis is

common in all parts of Turkey more widely distributed in the East and Southeast Anatolia regions (Umur, 2003). The prevalence of *E. granulosus* infection in dogs determined by necropsy and/or microscopic examination occurred between 0.9 and 44.0 %, and varies widely with geographical location (Table 1).

Table 1. Prevalence of *E. granulosus* in dogs in different geographical location of Turkey

Cities	Infection rate (%)	Authors
Elazig	18.86	Guralp <i>et al.</i> , 1977
Ankara	44.0	Doganay, 1983
Bursa	36.0	Tinar <i>et al.</i> , 1989
Izmir	5.5	Uner, 1989
Sivas	16.0	Saygi <i>et al.</i> , 1990
Ankara	0.9	Aycicegi, 1997
Kayseri	24.0	Sahin <i>et al.</i> , 1993
Kars	40.5	Umur & Arslan, 1997
Konya	28.3	Aydenizoz, 1997
Izmir (coproantigen ELISA)	15.0	Yolasigmaz <i>et al.</i> , 2001

Traditional parasitological diagnosis of canine echinococcosis, which is faecal examination or arecoline purgation, is either non-specific, of relatively low sensitivity or hazardous tests, and requires trained personnel (Allan *et al.*, 1992; Deplazes *et al.*, 1992). The utilization of sensitive diagnostic techniques for detection of echinococcosis in dogs is essential for diagnosis and control. Use of a newly developed coproantigen ELISA test would be conducive to determine the risk of canine echinococcosis in endemic areas (Lopera *et al.*, 2003).

Although, high prevalence of human and animal echino-

cocciosis has been well documented in different regions of Turkey, there are no studies carried out to determine prevalence and the risk factors for canine echinococcosis in the Antakya region. Stray dogs are an important problem, and they have free access to infective organs as a consequence of uncontrolled slaughter of animals except the slaughterhouse. Moreover, hydatid cysts were determined to be 48.75 % in slaughtered cows in Antakya province (Durgut *et al.*, 2003). Therefore, the aim of the present study was to determine the prevalence of *E. granulosus* in dogs from Antakya province using coproantigen ELISA test.

Materials and Methods

Study area and sampling

The study was carried out between April and July in 2006, in Antakya, which is the seat of the Hatay province in southern Turkey. Faecal samples of 79 owned dogs, comprising 47 males and 32 females, were examined using coprological examinations and coproantigen detection by enzyme linked immunosorbent assay (ELISA). Faecal samples were taken from each dog either rectally or collected immediately after defecation. Samples were placed in 5 % phosphate-buffered saline formalin solution and kept at +4 °C during the sampling, and stored at -18 °C until ELISA testing.

Questionnaire

A questionnaire was distributed to owners to collect information about the dog's age, sex and breed, as well as to have sufficient data on possible risk factors for canine echinococcosis, including the dog deworming, nature of dog food, and how dogs were restrained.

Coprological examination and coproantigen ELISA

In order to determine the presence of helminth in the dog, parasite eggs were isolated from the faecal samples by sedimentation and flotation techniques according to Ritchie, (1948) and Sloss *et al.*, (1994), respectively. The samples were also examined by ELISA for the detection of specific coproantigens of *E. granulosus* (CHEKIT® ECHINOTEST, Bommeli Diagnostics, Switzerland). The test procedure was performed according to the instructions of the manufacturer. Briefly, fecal samples were mixed in a 1:4 ratio with CHEKIT-Echinotest-sample-diluent. These suspensions were mixed within shaking for 15 minutes, and then centrifuged at 3.000x g for 10 minutes. 90 µl CHEKIT-Echinotest-sample-diluent was dispensed into each well of the microtiter plate. 10 µl of the sample supernatants and controls were added in to appropriate wells of the microtiter plate. Microtiter plates were incubated for 90 minutes at room temperature in a humid chamber. After the incubation, each microtiter plate was washed with at least 300 µl CHEKIT-washing solution for five times. At the end of the washing, 100 µl CHEKIT-TMB-Substrate, pre-warmed to 25 °C, were dispensed in to each well. The substrates were incubated at room temperature for 15 mi-

nutes. 100 µl CHEKIT-Stopping TMB-Solution, pre-warmed to 25 °C, was added in to each well for stopping color reaction. The results were read using a photometer at a wavelength of 450 nm.

Statistical analysis

Chi-square test was used to determine whether there was a significant difference in dog prevalence between sexes, ages, and possible risk factors associated with canine echinococcosis. A significance level of 5 % or less was used throughout.

Results

Out of 79 faecal samples in 13 ascarid or taenia eggs were detected (16.4 %). Mean age of positive dogs with *E. granulosus* was 2.5 years with a range of five months to ten years. Taeniid eggs were found in 7 samples (8.86 %) The prevalence of *E. granulosus* infection using the coproantigen ELISA was 8.86 % (7/79). Only one sample was positive in both microscopy and coproantigen ELISA; however, other six coproantigen ELISA positive samples were microscopically negative (Table 2).

Table 2. Comparison of coproantigen ELISA and coprological examination in dogs from Antakya region

Tests	Positive (%)			Negative
	Male	Female	Total	
Coproantigen ELISA	4 (57.1)	3 (42.9)	7 (8.86)	72
Coprological examination (<i>Taenia spp.</i>)	5 (71.4)	2 (28.6)	7 (8.86)	72
Both	1	-	1 (14.3)	-
Total	8 (61.5)	5 (38.5)	13 (16.4)	66

The analysis of questionnaire data was carried out to measure the association between coproantigen ELISA results and several possible factors including sex, age (<5 years versus >5 years), restraint of the dogs, and deworming practice. Mean age of the dogs with *E. granulosus* was found to be 2.3 years old with a range of one to five years. There was no statistically significant difference regarding the prevalence between male and female dogs. The prevalence of *E. granulosus* in male and female dogs was found to be 8.5 % (4/47) and 9.4 % (3/32), respectively. A significant difference was detected between unrestrained (or free roaming) and indoor or chained dogs ($p < 0.05$). Twenty-seven unrestrained dogs have also never received anthelmintic drugs. Six of 27 (22.2 %) untreated and unrestrained dogs were positive with coproantigen ELISA test.

Discussion

Detection of the infection rate in dogs is probably the best

index of the extent of transmission potential for this zoonotic parasite in a local area (Jenkins *et al.*, 2000; Lopera *et al.*, 2003). It is more difficult to obtain using traditional diagnostic approaches such as faecal examination, arecoline purgation, and autopsy. Coprological examinations have low sensitivity since excretion of the egg occurs sporadically. Additionally, eggs of *Echinococcus* and *Taenia* species cannot be differentiated by light microscopy (Allan *et al.*, 1992). Coproantigen ELISA test have been developed for diagnosis of canine echinococcosis incorporating polyclonal antibodies against somatic or excretory/secretory antigens of adult *E. granulosus* (Deplazes *et al.*, 1992; Allan & Craig, 2006). Use of the coproantigen ELISA test would be of great value in determination of the risk of canine echinococcosis in endemic areas (Lopera *et al.*, 2003). They have successfully been used in Libya (Buishi *et al.*, 2005), Peru (Lopera *et al.*, 2003), Cyprus (Christofi *et al.*, 2002) and Uruguay (Malgor *et al.*, 1997) demonstrating their usefulness for epidemiological studies. In this study, prevalence of canine echinococcosis was detected as 8.86 % by coproantigen ELISA; however, six of 27 (22.2 %) untreated and unrestrained dogs were detected as positive. The coprological survey, which is considerably underestimating the true prevalence of the infection (Jenkins *et al.*, 2000), revealed the presence of taeniid eggs in 8.86 % of the stool samples. Six coproantigen ELISA positive samples were microscopically negative. Despite the fact that the coproantigen has high sensitivity; this low ratio maybe attributable to the erratic excretion of the *Echinococcus* egg (Benito *et al.*, 2006). In the present study we did not evaluate adult worms directly; therefore, it should be unwise to comment on worm burden, besides it is not easy to compare two methods that were used. Although, the prevalence of *E. granulosus* in this area was lower than that in most of the other regions of Turkey (Table 1), the incidence was quite higher in unrestrained and untreated dogs. In a recent study carried out in northern Spain, Benito *et al.*, (2006) reported a prevalence of *E. granulosus* of 10.3 % performed by coprological examination and 14.0 % by coproantigen ELISA in sheep dogs, similarly to the results obtained in our study. In a prior research by Reiterová *et al.* (2005) evaluated copro-ELISA kit for the presence of *E. multilocularis* in red foxes. According to the results of these studies copro-ELISA test is advantageous in terms of allowing the intravital diagnostics for the epidemiological survey of *Echinococcus* occurrence.

Previous studies have showed a pattern of age-related infection, with young dogs bearing the highest abundance and prevalence rates, whereas older animals have lower parasite burdens and prevalence (Lahmar *et al.*, 2001; Torgerson *et al.*, 2003; Buishi *et al.*, 2005; Moro *et al.*, 2005). It also supports the suggestion that there is an increase in specific immunity against *E. granulosus* with dog age (Torgerson *et al.*, 2003). Similar results have also been revealed in this study. The mean age was 2.3 years with a range of one to five years in the dogs. Dogs older than five years old were associated with a decreasing risk of being

coproantigen positive in comparison with dogs younger than five years old. There was no significant difference in prevalence of *E. granulosus* between the male and female dogs, which is a contrary to the findings of Moro *et al.* (2005). They reported that female dogs were infected more significantly compared with male ones.

In conclusion, although the prevalence of the *E. granulosus* infection in dogs in Antakya region is generally lower than that in other parts of Turkey, the incidence was quite high in the unrestrained and undewormed dog. Therefore, a preventive control program based on regular treatment of dogs should be a considered thereby.

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RECEIVED DECEMBER 11, 2007

ACCEPTED AUGUST 26, 2008