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Original Article

A Serological Survey of Cystic Echinococcosis in Equids in East of Turkey

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ABSTRACT

Background: Cystic echinococcosis (CE), caused by hydatid cysts, is a widespread and hazardous disease in humans and animals worldwide. The disease is very common in Turkey, causing serious economic losses and public health problem. In this study, the seroprevalence of equine CE was determined by enzyme-linked immunosorbent assay (ELISA).

Methods: Partially purified cyst fluid antigen from sheep hydatid cyst fluid was used as antigen in ELISA. A total of 250 equids consisting of 206 donkeys and 44 horses from various regions of Erzurum province of Eastern Turkey.

Results: Anti- *Echinococcus granulosus* antibodies were detected in 78 (31.2%) of 250 equids. The prevalence rate was 20.4% for horses and 33.5% for donkeys. There was no statistically difference between sex and ages groups for both horses and donkeys ($P>0.05$).

Conclusion: Equine CE is quite endemic in Eastern Turkey. The high prevalence of CE suggests that equids in the transmission cycles is possible as a source of infection for definitive hosts.

Keywords: Cystic echinococcosis, Donkey, Horse, ELISA, Seroprevalence, Turkey

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Introduction

Cystic echinococcosis (CE) caused by hydatid cyst is a zoonotic disease that occurs all around the world and causes economic losses and public health problems. Domestic intermediate hosts are major reservoirs for the disease. Infection of humans occurs during the natural transmission of the parasite between the canid definitive hosts and domestic livestock intermediate hosts. Together with other *Echinococcus* spp., it is recognized as one of the most important parasitic zoonoses (1). In addition, large hydatid cysts in the liver and lungs of sheep and cattle can result in significant economic loss to the meat industry through condemnation of the infected organs (2). Results of studies on CE in eastern regions of Turkey, with large pastures showed considerable variations of infection rates among farm animals. The high figures reported were 33.9% (necropsy) and 63.3% (ELISA) in cattle, 62% (ELISA) and 66.4% (Western blotting) in sheep and 25.1% (necropsy) in goats (3-6). However, there are a few reports about equine CE in Turkey and all of them based on postmortem examination. Alibasoglu and Yalciner (7) reported the equine CE was 0.78% and Gonenc et al. (8) were reported 0.15%. Oge et al. (9) examined 80 equines, between 1 and 35 years of age that were slaughtered to feed carnivores in Ankara Zoo. Among the equines cyst hydatid infection was detected only in donkeys, 2 out of 35 donkeys (5.7%) were found to be infected, one of which had six cysts in the liver and the other had six cysts in the spleen. The cysts were found to be fertile. The prevalence data of CE in livestock are most commonly obtained after slaughter. Abattoirs which are strictly controlled can often provide admissible record. However, in some areas where the disease is common, home slaughter without

veterinary supervision is performed. Thus, the development of a sensitive and specific serological assay for intermediate hosts would provide useful epidemiological data for the antemortem study and control of CE (10).

The objective of the present study was to determine the seroprevalence of CE in equids in east of Turkey by indirect ELISA. This study also served to correlate sex and age with ELISA results in equine.

Materials and Methods

The samples were collected from towns of Erzurum province and their surrounding villages, in Eastern Anatolia in the year of 2009. Serum samples were obtained from the jugular vein in sterile air-vacutained tubes in these locations. All the equine were older than 1 year old and usually grazed in the pasture for at least one season. All animals especially for donkeys were using for the transportation of freight and grazing in the pastures together with the other livestock such as cattle, sheep and goats.

An *Echinococcus granulosus* antigen B (AgB) enriched antigen was prepared from hydatid cyst fluid (HCF) obtained from sheep infected with hydatid cysts. The procedure was performed as previously described by Oriol et al (11). Briefly, HCF was aspirated under sterile conditions and examined microscopically for the presence of protoscoleces. HCF was centrifuged at 2000 g for 15 min to remove the protoscoleces and any other solid materials and 100 ml of the clarified supernatant fluid was first dialyzed overnight at 4°C against 0.005 M acetate buffer, pH 5.0. The cyst fluid was then centrifuged at 15,000 g for 30 min at 4 °C. The pellet was collected and dissolved in 10 ml of 0.2 M phosphate buffer, pH 8.0, boiled

in a water bath for 15 min and re-centrifuged at 20,000 g for 1 h at 4 °C. The precipitate pellet was discarded, and the supernatant containing AgB was assayed for protein concentration and reactivity, and stored in aliquots at -20 °C until used. Protein concentration was determined by the method described by Lowry et al. (12).

Positive control sera kindly requested from Dr. Georgios Theodoropoulos from Greece. Negative control sera was obtained from an uninfected young equid which was negative in the IFA (1/4) and IgG-ELISA (1/20) tests. This technique was performed according to Simsek et al. (13) with slight modifications. ELISA plates (Dynatech Laboratories, IA, USA) were coated with 100 µl of 5 µg/ml of partially purified HCF antigens in 0.1 M carbonate/bicarbonate buffer (pH 9.6) per well. Following overnight incubation the plates were washed twice with PBS containing 0.01% Tween-20 (PBS/Tween) and blocked with 130 µl per well of a solution containing 5% skimmed-milk powder in 0.01 M PBS (pH 7.4) for 1.5 h at 37°C. After blocking, the plates were washing three times with PBS/Tween, 100 µl sera diluted 1:200 in PBS containing 0.05% Tween-20 were added to the wells and incubated for 1.5 h at 37 °C. The plate was again washed five times and 100 µl of a 1:5000 goat anti-horse IgG HRP (Santa Cruz Biotechnology, sc2906, Lot: B212) for horses and rabbit anti-donkey IgG (Santa Cruz Biotechnology, sc3989, Lot: C082) for donkeys were added to the wells and the plate incubated at 37°C for 2h. Finally, after five washes, 100 µl of substrate at a containing of O-phenylene diamine and hydrogen peroxide in citrate/phosphate buffer were added to each well conveniently readable results were obtained after 15 minutes incubation of room temperature. Enzymatic reaction was stopped with 50 µl per well of 1 N sulphuric acid and the plate was read out 450 nm on an ELISA reader (Bio-Tek instruments,

USA). The results expressed as the mean of the optical density. All samples were worked duplicate and repeated which sera were different up to 10% between. Cut-off value was calculated as the mean of the negative control sera absorbance values plus 3 standard deviations.

Data management and statistical analysis, such as chi square, were performed using SPSS 10.1 software for Windows.

Results

Blood samples were collected from 250 equids consisting of 206 donkeys and 44 horses from various regions of Erzurum province and the vicinity (Table 1 and 2). The donkey samples were collected from Horasan, Oltu, Cat and Olur districts of Erzurum province and horse samples from Horasan, Aziziye and Yakutiye districts. The overall prevalences of antibodies against *E. granulosus* for donkeys and horses are presented in Table 1 and 2, respectively. Anti-*E. granulosus* antibodies were detected in 78 (31.2%) of 250 equids. The prevalence rate was 20.4% for horses and 33.5% for donkeys. CE incidence was higher in females for donkeys (40.2%) and horses (30.4%) than males for donkeys (29.4%) and horses (9.5%). However these were not significant for statistically ($p < 0.05$). Majority of infected donkeys (50%) were 0-2 ages group followed 3-5 ages (43.1%), 9 and up to ages (39.1%) and 6-8 ages (30.8%) ($P < 0.05$). Similarly, the highest seroprevalence rate for horses was found in 0-2 ages (50%) and the other rates were 33.3%, 25% and 8.3% for 3-5 ages, 9 and up to ages and 6-8 ages groups, respectively ($P < 0.05$). The highest seroprevalence for donkeys was found in the region of Cat (48.7%) followed by Horasan (41.5%), Olur (25%) and Oltu (18.4%). While the rates for horses were 14.2%, 18.2% and 23.1% for Horasan, Aziziye and Yakutiye, respectively.

There was no statistically difference between sex and ages groups for both horses and donkeys.

Discussion

This study revealed that the seroprevalence of the equine CE investigated is high in Turkey. Our data show that higher seroprevalence of CE in equids occur in the districts with more rural populations of Erzurum province of eastern Turkey. Already, this province where a high number of people live in rural settings and the highest livestock population exists has the highest percentage of CE in both livestock and human. In the last reports, a cattle CE was reported as 33.9% (6). A total of 111 CE cases had been diagnosed in University Hospital of Erzurum between 1999 and 2004 years (14). This suggests that rural lifestyles are associated with a higher risk of CE infection.

The prevalence of equine CE was documented in several European countries. Varcasia et al. (15) examined the livers and the lungs of 2,231 horses from various Italian regions presence at the time of slaughter for CE between March 2003 and February 2007 and founded it in eight horses. Kouam et al. (16) investigated the IgG antibodies against *Echinococcus* by ELISA in 773 equids including 753 horses, 13 mules and seven ponies in four regions of Greece and detected only 0.1% seropositivity. However 553 of horses was racing, 84 of recreation and only 123 of horses was farming and they are not free-ranging horses and only feed on processed food in stables or in houses. Azlaf and Dakkak (17), carried out an epidemiological study on cystic echinococcosis (CE) in Morocco (2001-2004) and inspected 455 equines (325 horses, 60 mules and 70 donkeys) after slaughter. They detected 17.8% positivity in examined equines.

The seropositive rate of equids in Turkey is not surprising since CE is already endemic especially in livestock in eastern Turkey (4-6). Also *E. granulosus s.s.* (G1-G3 complex) is the predominant strain in the area and highly prevalent in sheep, cattle and human of Turkey (6, 18, 19). Both *E. granulosus s.s.* and *E. equinus* (G4 or horse strain) occur in horses and use dogs as definitive hosts (15). Because of the equids and dogs lived very closely in the study areas, the higher seropositive status of equids may be related to their feeding habits. Besides we know that all of them have access to pasture, they are free-ranging animals and feed on free pastures together with the other livestock. In conclusion, the results obtained in the present work confirm that equine CE is quite endemic in eastern Turkey. The high prevalence of CE suggests that equids in the transmission cycles is possible as a source of infection for definitive hosts.

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