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Molecular characterization and prevalence of cystic echinococcosis in slaughtered water buffaloes in Turkey

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ABSTRACT

The present study was carried out between March 2006 and June 2010. During the study nine abattoirs were visited and 166 water buffalo internal organs were examined in Black Sea Region of Turkey. It was found that 10.24% buffaloes were infected with cystic echinococcosis (CE). The rate of CE found as 3.77% in males and 21.66% in females, 37.93% in older and 4.38% in young animals. The degree of prevalence according to age and sex was statistically significant ($p < 0.05$). CE was observed 29.41% only in liver, 47.06% only in lungs and 23.53% in both liver and lungs. Therefore, the lungs were the predominant sites of the CE in buffaloes. Molecular identification on nine isolates, based on mitochondrial *cox1* sequencing analyses, revealed that six cysts belonged to G1 genotype (domestic sheep strain) while 3 samples showed variant genotypes of *Echinococcus granulosus* complex G1–G2–G3. Two of them showed a thymine in position 52, like G2 strain, but the rest of sequences were completely identical to strain G1; also one specimen showed a single nucleotide change compared to strain G1 (C122T).

1. Introduction

Cystic echinococcosis (CE) is caused by the larval stage of *Echinococcus granulosus*; it is one of the major zoonotic diseases in the world and it induces economic loses and public health problems. Parasite definitive hosts are canids and intermediate hosts are mammalians, especially wild and domestic ruminants or humans. Intraspecific variants of strains have been associated to a wide spectrum of intermediate host species and geographical region (Thompson and McManus, 2002). In total, 10 different strains have been formerly genetically characterized: G1 (common sheep strain), G2 (Tasmania sheep strain), G3 (buffalo strain), G4 (horse strain), G5 (cattle strain), G6 (camel strain), G7 (pig strain), G8 (cervid strain), G9 (human strain) and G10 (Fennoscandian cervid strain), albeit the strict validity of G9 genotype has been questioned (McManus, 2002; Lavikainen et al., 2003; Snabel et al., 2009). Recent taxonomic revisions suggested the existence of at least 4 valid species in *E. granulosus* species complex, namely *E. granulosus* s.s. (G1–G3 genotypes), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), *Echinococcus canadensis* (G6–G10), with leaving species status of *Echinococcus intermedius* in the G6–G10 group for further considerations (Thompson and McManus, 2002; Nakao et al., 2010). *E. granulosus* has a worldwide distribution, and used to be particularly common in developing and undeveloped countries; CE is also endemic in Turkey and neighboring countries (Urquhart et al., 1996; Eckert et al., 2001; Umur, 2003; Kebede et al., 2009).

In Turkey, many studies have been performed regarding the prevalence of the disease in sheep and cattle and few studies about genetic characterization of *Echinococcus* variants (Bowles et al., 1992; Bowles and McManus, 1993; Ütük et al., 2008; Vural et al., 2008; Snabel et al., 2009). Previous studies showed various prevalences of CE

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Table 1
Examined buffaloes ages.

	Male ♂	Female ♀	Total
Age < 3	93	44	137
Age > 3	13	16	29
Total	106	60	166

in livestock among the regions: 8.96–46.41%, 3.50–70.91%, 1.6–29.28% in cattle, sheep and goats respectively (Dik et al., 1992; Arslan and Umur, 1997; Öge et al., 1998; Ulutaş and Tüzer, 2007). The most important animal for CE is the sheep, due to the most prevalent intermediate host and usually carries more fertile cysts. For these reasons, the domestic sheep variant (genotype G1) appears to be the most relevant for zoonotic potential (Arslan and Umur, 1997; Şenlik, 2000), as it is found to be the most prevalent genotype in Turkey (Ütük et al., 2008; Vural et al., 2008; Snabel et al., 2009). In addition, buffalo hydatidosis have been investigated in a few studies in Turkey with 16.66–41.1% prevalence rates (Zeybek and Tokay, 1990; Türkmen, 1992; Umur and Aslantaş, 1993).

CE in animals causes yield losses and serious economic problems due to losses of internal organs and decreases of other products. Human-associated economic losses arise through diagnostic procedures, treatment, hospitalization, life impairment, etc. CE is still being a major problem in Turkey for reasons such as low socio-economic status, lack of public education, slaughtering unhealthy and uncontrolled animals (Macpherson and Craig, 2000; Umur, 2003).

Data on the prevalence of CE in water buffaloes could be very important, because they can indicate the importance of each type of animal as a potential source of infection to dog. Genetic identification of *E. granulosus* isolates from water buffalo also appears relevant due to the lack of information about the etiologic agent of CE in this specific intermediate host which is considered a poorly characterized form (Jenkins et al., 2005; Capuano et al., 2006).

The aim of the present study is to determine the frequency and intensity of infection, the rate of fertile cysts, the effect of host age and sex on the prevalence, the distribution of cysts in buffalo's internal organs and the genetic characterization of *E. granulosus* isolates.

2. Materials and methods

2.1. Epidemiological study

Between March 2006 and June 2010, 166 water buffaloes (60 females and 106 males) were examined at nine abattoirs in Samsun, Ordu and Amasya cities (Middle Black Sea Region, Turkey). They were categorized according to the 3-year-age threshold as young and old animals (Table 1).

Age, sex and origins of slaughtered water buffaloes were recorded. Each animal internal organ, especially lungs, livers, hearts and spleens were examined by inspection and palpation to detect the hydatid cysts. The cysts found in infected organs were divided in three groups: fertile, sterile and calcified/caseous. The fertility of the cysts was determined by the presence of protoscolex.

Statistical analyses to detect possible correlation of infection with age and sex were performed using the SPSS 15.

2.2. DNA isolation and PCR amplification

The genomic DNA extraction of each isolate was performed from cysts washed with PBS and preserved at −20 °C, using the Fermentas® purification kit (Fermentas), according to the manufacturer's protocol. Samples were mixed with lysis solution and then Proteinase K (17.5 μl at 20 mg/ml) was added to each tube, which was incubated at 56 °C to digest samples. Subsequently, chloroform and precipitation solution were added. Finally, NaCl was added and the DNA precipitated with ethanol.

Genomic DNA (5.0 μl, 20–40 ng) was used as a template to amplify the mitochondrial cytocrome oxidase 1 (*cox1*) region by PCR, as described by Bowles and McManus (1993), using following primers: JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and JB4.5 (5′-TAAAGAAAGAACATAATGAAAATG-3′).

PCR was performed with a mix containing 5 μl of 10× PCR buffer (pH 8.8) (Fermantas), 5 μl of 25 mM $MgCl_2$ (Fermantas), 4 μl of each nucleotide set (dNTP) (Fermantas), 20 pmol/μl of each primer, 28.8 μl of dH_2O and 0.2 μl of 1.25 U Taq DNA polimerase (Fermantas). Thermal reactions were performed with an initial denaturation of 10 min at 95 °C, followed by 35 cycles of 94 °C for 30 s (complete DNA denaturation), 52 °C for 40 s (primers annealing) and 72 °C for 45 s (extension) and a final elongation step of 7 min at 72 °C. A negative control (without genomic DNA) was included in each set of amplifications.

Aliquots (10 μl) of individual PCR products were separated by electrophoresis using agarose gels (1%), stained with ethidium bromide (10 mg/ml) and detected using ultraviolet transillumination. Gel images were captured electronically and analysed by KODAK Gel Logic 200 Imaging System.

2.3. Sequencing and variability analyses

Positive amplicons were sent to sequencing company Iontek (İstanbul, Turkey) to purification and sequencing. Sequences were first manually analysed and edited to check the electropherograms quality and subsequently were aligned with verified sequences of *Echinococcus* strains available in GenBank for comparison (for specimen codes and GenBank accession numbers see Table 2), using ClustalW implemented in MEGA 4 (Tamura et al., 2007). Sequences analysed in the present study were finally deposited in GenBank.

3. Results

3.1. Epidemiological results

CE was found in 17 of the 166 examined water buffaloes (prevalence = 10.24%; 95% CI = 6.2–15.89%). The prevalence of infection was higher in females (prevalence = 21.66%; 95% CI = 12.73–34.06%) rather than in male buffaloes (prevalence = 3.77%; 95% CI = 1.30–9.29%). The chi-square