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Short communication

Detection of polymorphism in AgB1 gene from sheep, cattle and human isolates of echinococcus granulosus by SSCP

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ABSTRACT

Antigen B (AgB) is a major protein produced by the metacestode cyst of *Echinococcus granulosus*, the causative agent of cystic hydatid disease. *E. granulosus* AgB is a gene family of at least five gene loci (B1–B5), each one consisting of several minor variants. We used PCR-SSCP followed by DNA sequencing to evaluate sequence variation and polymorphism of AgB1 in 99 isolates which the 43 were from cattle, 25 of sheep and 31 of human. All samples were analyzed with 12S rRNA-PCR for the strain detection and all of were identified as G1–G3 cluster (*E. granulosus* sensu stricto). The 16 human, 35 cattle and 25 sheep isolates were yielded the 102 bp band by PCR and these samples were tested by SSCP. As results of the SSCP, different band profiles were detected one each of cattle and human isolates while the other 74 isolates showed same band patterns. The DNA sequence analysis was performed for these two isolates and the other selected 4 isolates and polymorphism was confirmed.

1. Introduction

Cystic echinococcosis (CE) is a zoonotic infection caused by the larval (metacestode) stages of cestodes belonging to the genus *Echinococcus* and the family *Taeniidae* (Thompson, 1995). Although effective preventive and therapeutic measures have been developed for most parasitic helminths, cystic echinococcosis (CE) infection is still very common in the developing world today (McManus et al., 2003). It affects humans and a wide range of livestock species and is characterized by the presence of steadily growing unilocular cysts filled with a hydatid cyst fluid (HCF) in host internal organs, mostly the liver and lungs. *E. granulosus* larvae produce a lipoprotein known as antigen B (AgB) in the tegumental cells of the protoscolices and, to a lesser extent, in the laminated and germinal layer of the brood capsules before being secreted in the HCF (Arend et al., 2004).

Antigen B (AgB), initially identified in hydatid cyst fluid of *E. granulosus* causing cystic echinococcosis, is a major excretory–secretory antigenic component of the metacestode stage of the parasite (Oriol et al., 1971), and may play an important role in host–parasite interaction during the echinococcal infections, since it is the major component of hydatid cyst fluid (Musiani et al., 1978). The *E. granulosus* AgB is a thermostable polymeric lipoprotein of 160 kDa (Oriol et al., 1971). AgB elicits both cellular and humoral immune responses in intermediate hosts, and has been reported as an immunoregulatory molecule that is involved in some of the host–parasite interactions that are responsible for parasite establishment and survival in chronic CE. Thus, AgB directly or indirectly interferes with the host immune response at several levels, including the early inflammatory response, and innate and adaptive immunity. It suppresses functions of certain immune cell subsets and stimulates others associated to CE immunopathology, modulating the host immune response in a way that benefits the parasite (Monteiro et al., 2008).

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Results of the last molecular studies indicated that, *E. granulosus* AgB is encoded by a multigene family having at least 5 gene loci (B1–B5) (Arend et al., 2004; Haag et al., 2004). Due to polymorphic nature of AgB1 this antigen is related with evasion of immune system (Pan et al., 2010).

Molecular analyses showed that *E. granulosus* can be divided into mainly 10 genotypes (G1–G10), corresponding to the strain definition (Thompson and McManus, 2002). Reviewing the names of synonyms and subspecies, the species has been split into *E. granulosus* sensu stricto (genotypes G1–G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6–G10). However, the species status of *E. canadensis* is still controversial (Nakao et al., 2010). The disease is of worldwide importance and it is widespread in Turkey, especially in the eastern regions (Simsek et al., 2005). Despite the fact that CE continues to be an important problem in Turkey, there exists limited published information on the presence or public health impact of the different genotypes (strains and species) of *E. granulosus* (Utuk et al., 2008; Simsek and Eroksuz, 2009; Saarma et al., 2009; Simsek et al., 2010, 2011a).

The molecular variation of AgB1 gene of the Turkish cattle, sheep and human isolates has not been previously studied. Thus the aim of the current study is to determine the extent of variation and relation with the strains in isolates collected from three different host species which common intermediate hosts by using of SSCP and DNA sequence analysis.

The cattle and sheep were inspected by visual inspection and palpation to detect and collect hydatid cysts at local slaughterhouses in Elazig and Erzurum provinces in east of Turkey. After slaughtering germinal layers and/or protoscoleces were collected from the infected liver and lungs and stored in 70% ethanol untill used.

Total genomic DNA (gDNA) was isolated from each individual cyst samples of sheep and cattle isolates using a commercial tissue DNA isolation kit (Genomic DNA Purification Kit-K0512, Fermentas) with addition of 40 μl Proteinase-K (20 mg/ml) (Sigma, USA) at 56 °C within 400 μl lysis buffer. After digestion, kit procedure was performed and at last step the pellet was resuspended in 80 μl sterile distilled water, and the gDNA samples were stored at −20 °C until use. Mitochondrial 12S rRNA gene was amplified for the aim of strain detection by PCR which was described by Dinkel et al. (2004). Fragments of the CO1 mitochondrial gene were amplified as reported by Bowles et al. (1992) using the JB3/JB4.5 primers and sequenced for the detection of strains. The all procedures were performed as previously reported (Simsek et al., 2011a).

Tissues samples from human patients with histologically confirmed CE, operated on between 2000 and 2009 at the University Hospital in the Elazig province were investigated in a previous study (Simsek et al., 2011b) were used for the AgB polimorphisim analysis in this study. All patients were identified as being infected with CE by histo-pathological examination (detection of PAS-positive laminated layers and/or of protoscoleces and/or hooklets) of the resected tissues. For the extraction of genomic DNA (gDNA) from human isolates, 10 μm thick sections were prepared from tissue blocks and excess paraffin was trimmed. Sections were deparaffinized with 1 ml xylene for 10 min at 37 °C. After deparaffinization, rehydration in 100%, 90%, 80% and 70% ethanol followed. Thereafter the 70% ethanol was removed and a commercial kit of lysis buffer was added. Total gDNA was extracted from each individual sample using a commercial tissue DNA isolation kit (Genomic DNA Purification Kit-K0512, Fermentas) with little modifications. The tissues were digested overnight at 56 °C within 400 μl of the lysis buffer with the addition of 40 μl Proteinase-K (20 mg/ml) (Sigma, USA). After digestion, the gDNA extraction kit procedure was performed. At the last step the pellets were resuspended in 80 μl sterile distilled water and the gDNA samples were stored at −20 °C until use (Simsek et al., 2011b).

Coding nuclear EgAgB1 gene of *E. granulosus* was amplified for all isolates using specific primers previously described by Frosch et al. (1994): *Eg*AgB1.fw. 5′-CGTGATCCGTTGGGTCAG-3′ and *Eg*AgB1.rev. 5′-GGCACCTCTATTCACCTTCA-3′. PCR products were carried out in a final volume of 50 μl, containing 5 μl 10× PCR buffer, 5 μl 25 mM $MgCl_2$, 250 μM each of dNTP, 20 pmol of each primer, 200 ng of template DNA, and 1.25 IU of TaqDNA polymerase (MBI Fermentas). The PCR procedure consisted of a pre-denaturating step at 95 °C for 5 min and 35 cycles of denaturating (45 s at 95 °C), annealing (45 s at 55 °C), extension (90 s at 72 °C) and a final extension (10 min at 72 °C). The amplified products were separated by gel electrophoresis in 2% agarose gel with a Tris–boric acid–EDTA (TBE, pH 8.3) buffer at 90 V for 45 min. Following electrophoresis, the amplified products were visualised with ethidium bromide (0.5 μg/ml) staining for 45 min at room temperature. A visible band of 102 bp in the PCR was considered to be a positive result.

Single Stranded Conformation Polymorphism (SSCP) method was performed by Simsek et al. (2011a). Eight microliters of each PCR product were mixed with an 12 μl of denaturing buffer (10 mM NaOH, 95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanole). After denaturation at 95 °C for 10 min and subsequent snap-cooling on a freeze block (−20 °C) and 5 μl of individual samples were loaded into the gel (18 cm × 18 cm × 0.1 cm). The electrophoresis was carried out in 12% acrylamide–bisacrylamide (49:1) non-denaturing gels containing 10% of glycerol, at 200 V for 3 h at room temperature. The pattern of bands was visualized by silver staining.

The EgAgB1 sequences were automatically obtained using a 377 ABI PRISM system (Applied Biosystems). Nucleotide sequence analysis was undertaken by BLAST algorithms and databases from the National Center for Biotechnology (http://www.ncbi.nlm.nih.gov).

A total of 99 isolates collected from Erzurum and Elazig provinces of east-Turkey were examined. The 43 of these were obtained from cattle (8 of liver and 35 of lungs), 25 of sheep (9 of liver and 16 of lungs) and 31 of human isolates. The 12S rRNA-PCR with the *E.g.ss*1fw and *E.g.ss*1rev primers yielded 254 bp of amplification product with all samples analyzed. Thus, these samples were identified as G1–G3 cluster (*E. granulosus* sensu stricto). The 99 samples were analyzed with EgAgB1 primers and 15 human and 8 cattle isolates did not yield any band although repeated examination. The 102 bp band was amplified in the other