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Genotyping of *Echinococcus granulosus* from domestic animals and humans from Ardabil Province, northwest Iran

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Abstract

Cystic echinococcosis is endemic in Iran, particularly in Ardabil Province, where it causes health and economic problems. The genetic pattern of *Echinococcus granulosus* has been determined in most parts of Iran, except in this area. In the present investigation, 55 larval isolates were collected from humans (11), sheep (19), goats (4) and cattle (21). For analysis of the genetic characteristics of *E. granulosus* isolates, DNA sequencing of mitochondrial cytochrome c oxidase subunit 1 (cox1) and NADH dehydrogenase subunit 1 (nad1) genes was applied. Fifty isolates were successfully analysed, with 92% (46) and 8% (4) identified as G1 and G3 genotypes, respectively. The sequence analyses of the isolates displayed nine characteristic profiles in cox1 sequences and eight characteristic profiles in nad1 sequences. Based on these results, the sheep strain (G1 genotype) was the most prevalent in humans, sheep, goats and cattle. The buffalo strain (G3 genotype) was not only demonstrated in sheep (1 isolate) and cattle (1 isolate), but also for the first time in two human isolates. These findings will provide information for local control of echinococcosis.

Introduction

The *Echinococcus granulosus* metacestode, the aetiological agent of cystic echinococcosis, is an important medical, veterinary and economic problem worldwide (Thompson, 2008). During the past 40 years, several variants have been described within the species (Thompson & McManus, 2002). Recognition of these strains and genotypes is important for the formulation and development of vaccines, diagnosis, epidemiology, therapeutics and prevention and control of hydatid disease (McManus & Thompson, 2003).

Investigations using mitochondrial DNA (mtDNA) sequences have characterized ten genotypes (G1–G10) within *E. granulosus sensu lato* (Scott et al., 1997; Lavikainen et al., 2003). These comprise two sheep strains (G1 and G2), two bovid strains (G3 and G5), a horse strain (G4), a camel strain (G6), two pig strains (G7 and G9) and cervid strains (G8 and G10) (McManus & Thompson, 2003; Lavikainen et al., 2006). Since the taxon *E. granulosus sensu lato* is paraphyletic (Moro & Schantz, 2009), a
taxonomic revision established that there are four valid species in the *E. granulosus* complex: *E. granulosus* *sensu stricto* (G1–G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6–G10) (Nakao et al., 2010).

Echinococcosis is endemic in Iran (Ahmadi & Dalimi, 2006) with hydatid cysts prevalent in sheep, cattle, camels and goats throughout the country (Mobedi et al., 1970; Rokni, 2009). Adult worms have been reported in dogs, wolves and jackals (Esami & Hosseini, 1998; Maleky & Moradkhani, 2000; Dalimi et al., 2002; Rokni, 2009). Human cases are regularly reported in medical centres in Iran (Ahmadi & Dalimi, 2006; Rokni, 2009). Ardabil Province is among the most important areas of echinococcosis infection, due to the nomadic lifestyle of its inhabitants which brings humans and dogs into close contact.

The genetic pattern of *E. granulosus* has been determined in most parts of Iran ((Sharbatkhor et al., 2010; Hajialilo et al., 2011; Pour et al., 2011), but there are no data from Ardabil Province. The aim of this study was to identify the population genetic structure and genetic variability of *E. granulosus* isolated from livestock and humans by sequencing cytochrome *c* oxidase subunit 1 (*cox*1) and NADH dehydrogenase subunit 1 (*nad*1) mitochondrial genes.

Materials and methods

Collection and examination of hydatid cysts

Ardabil Province is located in north-western Iran bordering the Republic of Azerbaijan and the provinces of East Azerbaijan and Zanjan. The Talesh mountain range to the east separates Ardabil from Gilan Province. Ardabil East Azerbaijan and Zanjan. The Talesh mountain range bordering the Republic of Azerbaijan and the provinces of

<table>
<thead>
<tr>
<th>Host</th>
<th>Organ</th>
<th>G1 genotype</th>
<th>G3 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep (19)</td>
<td>Liver (10)</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Lung (9)</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Cattle (19)</td>
<td>Liver (4)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Lung (15)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Goats (3)</td>
<td>Liver (1)</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Lung (2)</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Humans (9)</td>
<td>Liver (5)</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Lung (2)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Spleen (2)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total (50)</td>
<td></td>
<td>46</td>
<td>4</td>
</tr>
</tbody>
</table>

*(n) = number examined

DNA extraction and PCR amplification

The ethanol was removed by washing all samples twice with PBS. DNA was extracted using a High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer’s protocol. The genomic DNA was stored at −20°C until polymerase chain reaction (PCR) amplification.

Two mitochondrial loci were amplified from distinct genomic DNA by PCR. The primers and PCR conditions were as described previously by Bowles et al. (1992) for the *cox*1 gene and Sharbatkhor et al. (2009) for the *nad*1 gene. The PCR products were separated by electrophoresis in 1.5% (w/v) agarose gel and visualized by ethidium bromide staining.

DNA sequencing

Each band was cut from the gel and purified using the QIA quick Gel Extraction Kit (QIagen, Germany), according to the manufacturer’s instructions and was sequenced in two directions by Bioneer Company (Korea). The sequences were compared to one another and with reference sequences using the BLAST system (http://www.ncbi.nlm.nih.gov/) and BioEdit software (Hall, 1999) and adjusted manually. Phylegenetic analyses were carried out applying concatenated *cox*1 and *nad*1 sequence data from this study as well as reference sequences of all identified *E. granulosus* genotypes (G1–G10) and *Echinococcus* species, with *Taenia saginata* as the outgroup (see fig. 1) (Bowles et al., 1992; Bowles & McManus, 1993, 1994; Gasser et al., 1999). The sequence-based Bayesian inference (BI) method was used for the analyses. BI was executed using the program MrBayes v.3.1.2 (http://mrbayes.csit.fsu.edu/index.php). Posterior probabilities (pp) were planned for 2,000,000 generations (ngen: 2,000,000). The TreeviewX v.0.5.0 Program (Page, 1996) was used to create the consequence tree.

Results

The *cox*1 gene and *nad*1 gene amplicons produced by PCR were 450 and 400 bp long, respectively. The consensus lengths of *cox*1 gene and *nad*1 gene sequences were 366 bp and 378 bp, respectively. A total of 55 isolates were sequenced, of which five failed (table 1).

The sequence alignments of the isolates displayed nine characteristic profiles in *cox*1 sequences and eight characteristic profiles in *nad*1 sequences. Sequence profiles for *cox*1 and *nad*1 were submitted to GenBank with accession numbers AB677806–AB677814 and AB677815–AB677822, respectively. A dendrogram based on the phylogenetic analyses of *cox*1 and *nad*1 sequences, and a consensus tree of the concatenated data, revealed two distinct clusters. One cluster contained a G1–G3 complex (pp = 1.00), and the other clustered with G6–G10 complex (pp = 1.00). Thirteen haplotypes (H1–H13) were found in the G1–G3 cluster (fig. 1). Forty-six isolates (92%) were recognized as G1 or G1 variations (haplotype 1–12) and 4 isolates (8%) identified as G3 genotypes (haplotype 13).
Echinococcus granulosus genotyping in Iran

Discussion

Ardabil Province has a mountainous climate and suitable pastures for traditional rearing of domestic animals, including those of migrating tribes. The presence of stray dogs infected with *E. granulosus* creates the potential for transfer of parasites. The reported prevalence of hydatid cyst in the province is 74.4% in sheep, 38.3% in cattle, 20% in goats and 11.9% in buffalo (Daryani et al., 2007). This is the first extensive genotyping investigation of *E. granulosus* infected intermediate hosts in Ardabil Province using mtDNA sequencing.

Studies using various techniques have documented the existence of G1, G3 and G6 genotypes in Iran (Zhang et al., 1998; Harandi et al., 2002; Ahmadi & Dalimi, 2006; Karimi & Dianatpour, 2008; Rostami Nejad et al., 2008; Sharbatkhori et al., 2009, 2010, 2011; Kia et al., 2010; Pour et al., 2011). The results of this investigation demonstrated that the G1 genotype (sheep strain), in addition to being in sheep (95%), was also in cattle (95%), goats (100%) and humans (80%). The G3 genotype (buffalo strain) was also detected in sheep, cattle and human isolates. This is the first report of the G3 genotype of *E. granulosus* in humans in Ardabil Province of Iran.

Moreover, some researchers have demonstrated the presence of the G3 genotype in intermediate hosts such as camel, buffalo, cattle and sheep in parts of Iran (Sharbatkhori et al., 2010; Hajialilo et al., 2011; Pour et al., 2011). Our results are in agreement with previous studies in Iran showing G1 to be the predominant *E. granulosus* genotype in livestock and humans. As all of the cysts were infertile in cattle, it seems that sheep play a significant role in *E. granulosus* transmission to dogs, providing an important source for human infection in this area.

Although there are many reports about the intermediate hosts of *E. granulosus*, limited information is available about infections in dogs and other canids. Further study should focus on identifying *E. granulosus* strains in canids in order to develop appropriate methods for prevention and control of the disease.

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