Molecular Characterization of *Echinococcus* Species in Khyber Pakhtunkhwa, Pakistan

Ijaz Ali, Maria Khan Panni, Aqib Iqbal, Iqbal Munir, Sohail Ahmad & Abid Ali

**ABSTRACT**

**Background:** Species belonging to genus *Echinococcus* are cestode parasites well known for helminthic infections globally. Diseases caused by these parasites are serious health threats for public and veterinary sectors. DNA-based characterization confirmed genetic variability among *Echinococcus* species and resulted in the identification of 10 genotypes (G1-10). Among identified *Echinococcus* species, *E. granulosus* and *E. multilocularis* are clinically most important responsible for cystic echinococcosis and alveolar echinococcosis, respectively. Identification and genetic characterization of these cestode parasites at species level is essential for disease diagnosis and control measures. This study aimed at narrowing gap of missing knowledge on *Echinococcus* spp. and their genotypes in Khyber Pakhtunkhwa (KP), Pakistan.

**Materials, Methods & Results:** Hydatid cysts of human source were obtained under aseptic conditions from thoracic surgery unit of the Lady Reading Hospital (LRH) at Peshawar, KP, Pakistan. Hydatid cysts from animal source (cattle) were collected at Peshawar visiting numerous abattoirs. Theses cyst samples (n = 40) were collected from animals (cattle) (n = 30) and human sources (n = 10). Nucleic acid was extracted from aspirates obtained from cysts, and investigated using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of a mitochondrial coding gene (*rrnL*, large subunit of ribosomal RNA). A pair of primers (ECH-LSU/F and ECH-LSU/R) were used to amplify a 570-bp DNA fragment of a mitochondrial gene (*rrnL*, large subunit of ribosomal RNA) containing a species-specific *SspI* restriction site for the differentiation among *E. granulosus* and *E. multilocularis*. Overall results indicated that among the cysts collected from animal and human sources, majority were positive for *E. granulosus* (n = 24, 60%) and *E. multilocularis* (n = 16, 40%). Genotyping of the positive *E. granulosus* samples revealed that *E. granulosus sensu stricto* (G1–3 genotype) (n = 22, 91%) was highly prevalent as compared to G6 genotype (n = 2, 9%). Out of 30 animal (cattle) source samples, 17 (56.6%) and 13 (43.3%) were found positive for *E. granulosus sensu stricto* (G1-3) and *E. multilocularis*, respectively. On the other hand, among 10 human source samples, 7 (70%) and 3 (30%) were found positive for *E. granulosus sensu stricto* (G1-3) and *E. multilocularis* respectively. Among 17 animals (cattle) source *E. granulosus* positive samples, 16 (94%) were identified as *E. granulosus sensus stricto* (G1-3) whereas only 1 sample (6%) was identified as G6 genotype. In case of human source *E. granulosus* positive samples, 6 (85%) were confirmed as *E. granulosus sensu stricto* (G1-3) and only 1 (15%) sample was confirmed as G6 genotype.

**Discussion:** *E. granulosus* and *E. multilocularis* are prevalent in KP, Pakistan. The presence of *E. granulosus sensu stricto* (G1-3) and G6 genotypes in KP, Pakistan is responsible for both human and animal infections. Since genotype determination is the first step in preventing *Echinococcus* spp. and minimizing subsequent infections, therefore, information provided in this report may have important consequences and direct impact on public health. According to our information, this is the first report of *Echinococcus* species and their genotypes prevalent in KP, Pakistan. This pioneering effort will provide tool for future research agenda, devising proper prevention strategies and control programs against *Echinococcus* species prevalent in KP, Pakistan.

**Keywords:** *Echinococcus*, echinococcosis, hydatid cysts, KP Pakistan.
INTRODUCTION

Echinococcosis is a helminthic infection caused by *Echinococcus* spp. (family Taeniidae) affecting public sector and cause huge economic losses in livestock industry [11,15,23]. Among identified *Echinococcus* spp., *E. granulosus* and *E. multilocularis* are clinically most important responsible for cystic echinococcosis and alveolar echinococcosis respectively [9,27,29,39,46,47]. Based on genetic studies using mitochondrial DNA sequences resulted into differentiation of ten *E. granulosus* genotypes (G1 to G10) [27,29]. Among these genotypes, numerous studies suggest the widest global distribution of *E. granulosus* sensu stricto (G1-3), (sheep strain) [12,27,38,42,44].

Molecular approaches have been commonly employed for identification and differentiation of *Echinococcus* spp. Among these approaches, PCR-RFLP analysis [4-6] is accurate to confirm the distinctiveness among *Echinococcus* spp. Also, PCR-RFLP analysis is also of paramount importance for *Echinococcus* spp. genotyping.

Despite its endemic nature due to common herd keeping and frequent contacts between animal and human, studies on the prevalence of *Echinococcus* spp. and their genotypes diversity in KP, Pakistan are scarce. Poor local hygienic conditions and unawareness about *Echinococcus* parasites life cycle have made situations favorable for disease perpetuation [21,22,24,36]. A better characterization of *Echinococcus* spp. and their genotypes prevalent in KP, Pakistan is important to understand related pathological conditions and implementation of control strategies. This study was designed to detect *Echinococcus* spp. and their genotypes prevalent in KP, Pakistan.

MATERIALS AND METHODS

Sample site and isolated source

Echinococcosis hydatid cysts of human source were obtained under aseptic conditions from thoracic surgery unit of the Lady Reading Hospital (LRH) at Peshawar, KP, Pakistan. LRH is the main health care facility at Peshawar visited by patients coming from all parts of KP. The history and locality of human patients with echinococcosis was also obtained. In addition, hydatid cysts from animal source (cattle) were collected at Peshawar visiting numerous abattoirs. Cyst contents were aspirated and washed with phosphate buffer saline (PBS, pH 7.4) by centrifugation at 2,000 g for 15 min. The obtained pellets were kept in 95% ethanol at -20ºC until DNA extraction.

DNA extraction and PCR

DNA was extracted using a GF-1 Nucleic Acid extraction Kit1. The extracted DNA was used as a template for PCR reaction. A pair of primers, ECH-LSU/F and ECH-LSU/R (5'-GGTTATTTTGCCTTTTTGATCGTGC-3') and Ech-LSU/R (5'-ATCACGTCAAAACCATTCAAAAGC-3') were used to amplify a 570-bp DNA fragment of a mitochondrial gene (*rrnL*), large subunit of ribosomal RNA) containing a species-specific *Ssp*I restriction site for the differentiation among *E. granulosus* and *E. multilocularis*. The primers were designed on the basis of conserved regions of *rrnL* sequences among *E. granulosus* sensu stricto (accession no, AF297617) and *E. multilocularis* (AF297617). PCR was conducted in a final volume of 50 μL containing extracted genomic DNA templates using the protocol mentioned earlier [46,47] with slight changes. Negative PCR (no-DNA) as control were included in each set of reaction. Thermal conditions were optimized at initial denaturation of 94°C for 5 min followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 57ºC for 45 s and extension at 72ºC for 45 s with a final extension at 72°C for 10 min. Six μL of PCR products were electrophoresed on 2% agarose gel and stained with ethidium bromide. A 100 bp ladder2 was used as a DNA size marker. The gels were visualized by UV trans-illuminator3.

Species and genotype determination

The partial *rrnL* gene of the genotype G6 could be amplified using the primer set Ech-LSU/F and Ech-LSU/R; however, it cannot differentiate the genotype G6 from *E. granulosus* sensu stricto (G1-3 genotypes) because both *Echinococcus* lacked *Ssp*I restriction site inside the target gene fragment [45-47]. The target gene sequence analysis as shown previously [46, 47] suggests that *BglII* restriction site is specific to the G6 genotype. Therefore, this additional cleavage site (*BglII*) was used in PCR-RFLP method to differentiate the genotype G6 from *E. granulosus* sensu stricto (= G1-3).

PCR based amplified *Echinococcus* spp. products were purified using the GENECLEAN II kit (MP Biomedicals) according to the manufacturer instructions. After DNA purification, the ampiclons were...
subjected to digestion using SspI restriction enzyme according to manufacturer instructions for species differentiation. Restriction products were electrophoresed and visualized on 2% agarose gel pre-stained with ethidium bromide (Figure 1). After confirmation of *E. granulosus*, samples were subjected to digestion using BglII restriction enzyme for genotype determination. The pattern of digestion visualized on 2% agarose gel was compared with previous report as standard for confirmation of the genotypes [46,47].

RESULTS

Observed lesions of human and animal caused by *Echinococcus* spp. after surgical examination were confirmed by PCR. Analyses of all 40 cyst samples showed that majority of them were positive for *Echinococcus* spp. Out of 30 animal (cattle) source samples, 17 (56.6%) and 13 (43.3%) were found positive for *E. granulosus sensu stricto* (G1-3) and *E. multilocularis* respectively. On the other hand, among 10 human source samples, 7 (70%) and 3 (30%) were found positive for *E. granulosus sensu stricto* (G1-3) and *E. multilocularis* respectively (Table 1). Among 17 animals (cattle) source *E. granulosus* positive samples, 16 (94%) were identified as *E. granulosus sensu stricto* (G1-3) and only 1 sample (6%) was identified as G6 genotype. In case of human source *E. granulosus* positive samples, 6 (85%) were confirmed as *E. granulosus sensu stricto* (G1-3) and only 1 (15%) sample was found G6 genotype (Table 1).

![Figure 1.](image_url)

**Table 1.** *Echinococcus* spp. and their genotypes detected in animals and humans isolates of KP, Pakistan.

<table>
<thead>
<tr>
<th>Species</th>
<th>Animals</th>
<th>Humans</th>
</tr>
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<tbody>
<tr>
<td><em>E. granulosus sensu stricto</em></td>
<td>17 (16&lt;sup&gt;a&lt;/sup&gt;, 1&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>7 (6&lt;sup&gt;a&lt;/sup&gt;, 1&lt;sup&gt;b&lt;/sup&gt;)</td>
</tr>
<tr>
<td><em>E. multilocularis</em></td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>30</td>
<td>10</td>
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<sup>a</sup>G1-3 genotype; <sup>b</sup>G6 genotype.
Infections caused by species belong to the genus *Echinococcus* have potential implications for public and veterinary sector. Metacystic stages of *Echinococcus* spp. cause chronic cystic echinococcosis and alveolar echinococcosis in human. Out of 2-3 million of echinococcosis global cases, most are cystic [9]. Reports published by Office International des Epizooties databases showed that the global burden for human cystic echinococcosis resulted in a loss of $760 million a year [9]. As compared to cystic echinococcosis, alveolar echinococcosis is less common however difficult to treat. There are 0.4 million human alveolar echinococcosis cases with 18,000 new cases annually [40,41]. Although considerable impacts by echinococcosis on human and animal health; less has been taken into account for its preventive measures. Since, studies on prevalence of *Echinococcus* spp. and their genotypes are limited in Pakistan; therefore, it stresses need for further investigation concerning local *Echinococcus* spp. and their genotypes detection. Present study aimed at PCR-RFLP based detection of *Echinococcus* spp. and their genotypes prevalent in KP, Pakistan. PCR-RFLP assay based on mitochondrial gene (*rml*, large subunit of ribosomal RNA) was developed which allowed rapid discrimination between *Echinococcus* spp. and their genotypes.

Despite of high global prevalence, studies employed on characterization of *Echinococcus* spp. in present study area are scares. Hydatid surgeries and the prevalence of cystic echinococcosis have not been reported previously in KP, Pakistan. Indeed, this is the first report of molecular characterization and genotyping of *Echinococcus* species prevalent in KP, Pakistan. The prevalence of *E. granulosus* and *E. multilocularis* provide evidences for the infection caused by these parasites both in human and livestock.

*E. granulosus sensu stricto* (G1-3) has been reported as the most prevalent and a source of infection for both public and animals health in numerous countries [3,7,10,17,18,25,26,28,31,32,35,43]. Accordingly, our results are in agreement with reports showing *E. granulosus sensu stricto* (G1-3) responsible agent for human and animal infections [2,10,16,19,24,30,34,37]. Two hypotheses are under discussion to answer the question of predominance of G1 genotype (i) humans are refractory or poorly susceptible to G6 infection (48,49) and/or (ii) the level of contamination of the environment with G6 genotype eggs from dog feces is not very high [1,38,42,44]. On epidemiological ground, camels appear to be an important reservoir for human infections; however, studies suggest that the G6 genotype has a low or no infectivity to human [14,48,49]. We report the first cases of *E. granulosus* G6 genotype both in human and animal (cattle) samples from KP, Pakistan. The record history of a human patient with G6 genotype during current study shows he was labor worker in Middle East (Saudi Arab), where G6 genotype has been reported [33].

Supposedly, *E. granulosus* may introduce into Pakistan from China and Iran, where *Echinococcus* spp. have been previously reported [20,30,46,47]. Further demographic expansions of the parasite may occurred by anthropogenic movements of host carrier hosts. Unavailable abattoirs, house slaughtering practices for daily meat requirements and lack of care during proper disposal of offal carcasses also facilitate parasite spread, disease transmission and subsequent pathological conditions [36,50]. KP, Pakistan has suitable pathological conditions for traditional rearing of domestic animals where exposure to echinococcosis due to animal husbandry is common. The presence of stray dogs with local migratory tribes infected with *Echinococcus* spp. creates further potential for parasites migration and transmission [13].

**CONCLUSION**

Summarizing our conclusion, *E. granulosus* and *E. multilocularis* are prevalent in KP, Pakistan. Identification of *Echinococcus* spp. in human and animal isolates supports the argument for further research concerning the pathological role and public health risks associated with these species. Moreover, the prevalence of *E. granulosus sensu stricto* (G1-3) and G6 genotypes in KP, Pakistan is responsible for both human and animal (cattle) infections. Since genotype determination is the first step in preventing *Echinococcus* spp. and minimizing subsequent infections, therefore, information provided in this report may have important consequences and direct impact on public health of KP, Pakistan.

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**Declaration of interest.** The authors declare no commercial or financial relationships that could be construed as a potential conflict of interest.

**MANUFACTURERS**

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3. UVitec Limited. Cambridge, UK.
REFERENCES


