

Evaluation of four DNA extraction methods for the detection of *Echinococcus granulosus* genotype 1

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ABSTRACT

Aim: The aim of this survey was to compare four DNA extraction methods from Iranian sheep strain *E. granulosus* isolates.

Background: Cystic echinococcosis (CE) caused by the metacestode of the dog tapeworm *Echinococcus* spp., is a global zoonotic infection which is economically important and constitutes a major threat to public health in many countries. Strains characterization is essential for the establishment of a preventive and control strategy in every endemic area.

Patients and methods: Forty five infected organs from cattle, sheep and goat were collected from different abattoirs of Iran. All cysts were examined by microscopic observation of protoscoleces. For each cyst, protoscoleces were aspirated and DNA of each cyst was extracted with 4 different methods including tissue Kit extraction, modified Cinnagen extraction kit, Phenol-chloroform (Sambrook1999) and modified Phenol chloroform methods. Efficiency of the DNA was determined by degree of success in PCR amplification.

Results: Cinnagen modified extraction and modified Phenol chloroform methods were equally effective and superior to other methods after DNA electrophoresis and PCR reaction. Inhibition was observed in PCR with DNA isolated from protoscoleces, and a 1/100 dilution was able to alleviate this problem with DNA extracted.

Conclusion: The result of this study show that the quality of extracted DNA using modified Cinnagen extraction kit and modified phenol–chloroform are very high and gave identical results after RCR reaction using 12S rRNA gene. Further evaluation is required for its utilization in other clinical specimens.

Keywords: *Echinococcus granulosus*, DNA, Extraction methods.

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Introduction

Echinococcosis is one of the major zoonotic parasitic diseases that occurs throughout the world and causes considerable economic losses and public health problems in many countries (1). Both cystic and alveolar Echinococcosis has been reported from many areas. Cystic Echinococcosis is more prevalent than alveolar Echinococcosis

and has been reported from all countries in the Middle East and Arabic North Africa (2-4). This disorder has been recognized as a major public health problem in sheep raising regions of the world (5-7), such as Iran, especially in rural and nomadic communities. Around half of the Iranian population lives in rural areas as farmers and shepherds. Given that sheep act as intermediate and dogs as definitive hosts of *Echinococcus granulosus*, hydatidosis poses both a human health risk and an economic loss to the country (8).

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Human's contamination takes place through close contact with infected dogs. Humans are usually a "dead-end" host (9). The high specificity, sensitivity, and rapid turn-around time of polymerase chain reaction (PCR), combined with morphological, biological, biochemical characterization, has led to ten main genotypes (G1–G10) for *E. granulosus* has being identified. However, enzyme inhibitory factors may be co-extracted with the target nucleic acid that will hinder the performance of PCR. The aim of this study was to compare the quality of extracted DNA using four different extraction methods.

Methods

Forty five infected organs of cattle, sheep and goat were collected from different abattoirs of Iran. The viability of protoscoleces was determined by microscopic test. Protoscoleces were aspirated from cysts, rinsed in saline, fixed in 70% (v/v) ethanol, stored at 70 °C. The protoscoleces were rinsed several times with PBS to remove the ethanol prior to DNA extraction.

Different DNA extraction methods

The quality of *E. granulosus* DNA were evaluated with four different methods, including the phenol–chloroform (10), a modified Cinnagen extraction kit (Cinnagen, Iran), Tissue Extraction Kit (Qiagen, Germany), and modified phenol chloroform methods. Efficiency of the DNA extraction method was evaluated by the degree of success in PCR. The presence of DNA generic to all species and strains of *E. granulosus* used in this study was ascertained to amplify a fragment of the mitochondrial 12S rRNA gene.

1- Phenol–chloroform

According to Sambrook et al. study, genomic DNA was extracted with phenol chloroform method. At the first step the sediment resulting from protoscoleces were suspended in 500µl of lysis buffer, containing 1% SDS and 2 µl proteins

k. The mixture was incubated at 60 °C for 2h, and then boiled for 15 min. DNA was extracted once with phenol-chloroform and then precipitated with absolute ethanol. The DNA was resuscitated in 50 µl distilled water, and stored at -20 °C until PCR amplification. The DNA concentration was determined by measuring optical absorbance at 260 and 280 nm.

2- Modified Cinnagen extraction kit

At first, DNGe- Plus solution (Cinnagen, Iran) was warmed at 37°C for 20 min with gentle shaking. Then 600 ml of DNGe-Plus was mixed with 300 ml of sample and vortexed for 20–25 s. In the next step, 450 ml of isopropanol was inserted and mixed by vortexing. For low concentrations of DNA, samples were kept at minus 20 °C for 20 min. Then samples were centrifuged for 10 min at 12,000 rpm and the supernatant gently poured off. Two ml of 75% ethanol were added to the pellet, mixed by vortexing for 3–5 seconds and centrifuged at 12,000 rpm for 5 min. Once again the supernatant was smoothly poured off and the pellet dried at 65 °C. The DNA pellet was dissolved in 50 ml of sterile distilled water by gentle shaking at 65 °C for 5 min. A final centrifugation for 30 s at 12,000 rpm pelleted undissolved material, leaving pure DNA in the supernatant.

3- Tissue Extraction Kit

In case of the Tissue Extraction Kit (Qiagen, Germany), DNA was extracted according to the manufacturer's guidelines.

4- Modified phenol–chloroform

Protoscoleces were washed three times with PBS. Then 500 ml lysis buffer containing 1% SDS was added to 300 ml protoscoleces. At the next step 2 ml of proteinase K was added, vortexed and then incubated for 2 h at 55 °C and 15 min at 80 °C. Next, 650 ml phenol–chloroform–isoamyl alcohol (25:24:1) was added and spun at 12,000 rpm for 2

min. The supernatant was removed and, after chloroform addition (same volume), mixing and spinning (12,000 rpm for 2 min), 1/10 the volume of sodium acetate (3M, pH¼ 5.2) and 500 ml ethanol were added and the tube kept at 20 °C for 30 min. Thereafter, it was spun at 12,000 rpm for 10 min and the pellet was washed using 70% ethanol. Later, the pellet was dissolved in 30 ml sterile super double-distilled water, placed at 37 °C for 15 min and subsequently stored at 20 °C until required for PCR.

Results

In all investigated cysts from different hosts in this study, there were no significant differences of quality observed between the modified Cinnagen extraction kit and modified phenol–chloroform methods and these methods gave identical results and yielded superior-quality DNA compared to the other methods.

The g1 PCR selectively amplified the G1 genotype of *E. granulosus* with specific bands of 259 and both methods (modified Cinnagen extraction kit and modified phenol–chloroform) produced identical bands with the G1 genotype. As result, G1 genotype was identified in all cyst samples by specific primer pair.

Discussion

Hydatidosis disease is is one of the most important zoonotic diseases, prevalent in many countries, including Iran (11, 12). In Iran, studies have confirmed that hydatid cysts are commonly found in sheep, cattle and goats throughout the country (13-17).

Mitochondrial and nuclear DNA analyses have previously been used successfully in molecular studies on *E. granulosus* in Iran and confirmed that the G1 genotype is the most common genotype in Iranian domestic animals. The result of our study showed that G1 genotype was found

to be present in bovine and ovine isolates. This result is in keeping with previous studies.

DNA-based methods are useful for taxonomy at the level of genus, species and subspecies, use of such methods often requires careful attention to preparation of pure DNA in adequate quantities (18, 19).

In the present study, we improved DNA extraction procedures and the result of this study show that modified Cinnagen extraction kit and modified phenol–chloroform methods were equal and yielded superior-quality DNA compared to the other methods. These methods were also more efficient at removing inhibitory factors and required the least labor and completion time.

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