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Molecular characterization of the human and sheep hydatid cyst strains in Chaharmahal va Bakhtiari province of Iran using restriction fragment length plolymorphism (PCR RFLP)

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ABSTRACT

Background: Hydatidosis caused by larval stage of *Echinococcus granulosus* is a cosmopolitan zoonotic infection. In endemic area for this disease there is considerable genetic variation among different isolates of the parasite. These variations may affect the epidemiology, pathology and control of the disease. In this work strain identification of hydatid cysts isolated from human or sheep in Chaharmahal va Bakhtiari province of Iran has been investigated.

Method: Fertile sheep hydatid cysts were collected from several abattoirs in Chaharmahal va Bakhtiari province of Iran. Human isolates were obtained at surgery from Kashani hospital in the same area. DNA was extracted from preserved protoscoleces and Nested PCR was performed on the extracted DNA samples, the rDNA-ITS fragment was amplified subsequently. Using 4 restriction enzymes include Rsa², HpaII, Alu² and taq², PCR-RFLP procedure was performed on the PCR products.

Results: The size of PCR product in this research was 1000bp in both human and sheep isolates. Using Alu² enzyme; three fragments of 100, 180 and 720bp in human isolates and two fragments of 800bp and 200bp in sheep isolates were created. Rsa² also revealed three segments of 150, 180 and 670bp in human samples and two fragments of 655bp and 345bp in sheep samples. After using HpaII enzyme three segments with 120, 200 and 680bp length in human isolates and two fragments with 700bp and 300bp in sheep isolates were detected. Finally using Taq² enzyme no digestion was occurred on human or sheep samples.

Conclusion: The result of this investigation showed that human hydatid cyst strain in Chaharmahal & Bakhtiati province of Iran is different from sheep ones, so it is recommended to recognize DNA sequence in this human samples in future studies.

KEYWORDS

Hydatid cyst;
RFLP;
Strain;
Human;
Sheep.

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INTRODUCTION

Hydatidosis caused by larval stage of *Echinococcus granulosus*, affects man and livestock and considered as one of the most important cosmopolitan zoonotic infection with different mammalian hosts being involved in the life cycle[1,2]. Several genetically distinct strains differing in several characterizations have been identified[3]. In endemic area for this disease there is considerable genetic variation among different isolates of the parasite[4,5]. These variations may affect the epidemiology, pathology and control of the disease[6-8]. So the identification of the strains is considered a major requirement in the control and prevention of hydatid disease[2,7]. Moreover there are evidences indicating that some strains are more pathogenic[9]. So far 10 distinitic strains of the parasite (G1-G10) have been recognized[10,11] from different parts of the world[3,10,12,13].

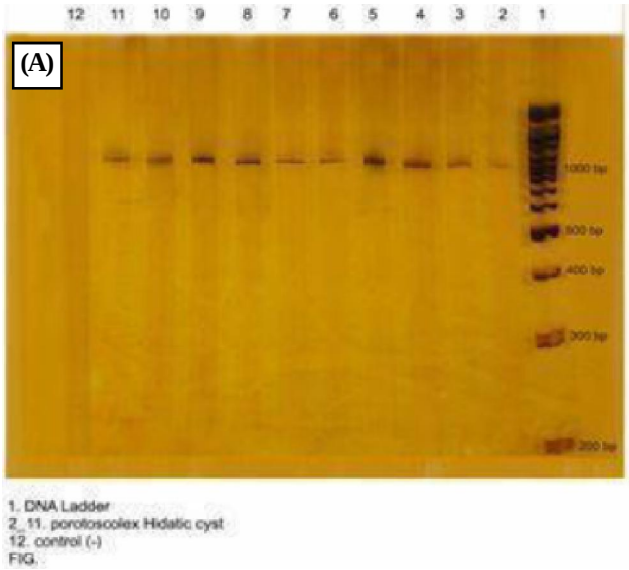
Hydatidosis is one of the most important zoonotic diseases prevalent in different parts of Iran[14,15]. Various surveys have indicated that hydatid cysts are commonly found in livestock throughout the country[14,16-18]. Human cases are also regularly reported from different parts of the country[4,14,15]. Molecular techniques have been applied successfully for distinguishing the different strains of *Echinococcus granulosus*[8]. In this study strain identification of hydatid cysts isolated from human or sheep in Chaharmahal va Bakhtiari province of Iran has been investigated using PCR-RLFP molecular method.

MATERIALS AND METHODS

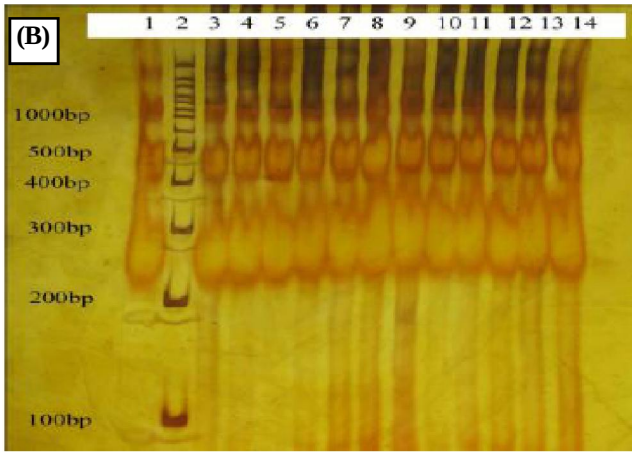
Hydatid cysts from 30 infected sheep were collected from several abattoirs in Chaharmahal va Bakhtiari province of Iran. Fiftheen human isolates were obtained at surgery from Kashani hospital in Shahrekord, Iran. In the laboratory, cysts were tested for protoscoleces and any protoscolices present were aspirated and collected. Protoscoleces from each cyst were washed in isotonic saline and then preserved in 75% ethanol.

DNA extraction: Preserved protoscolices washed three times with distilled water and then digested with SDS and proteinase K. DNA was extracted using Phenol Chloroform method.

The PCR reaction was performed in a final 25µl volume containing Taq DNA polymerase 1unit, 3µl MgCl2, 1µl primers, 1µl DNA, 2.5µl PCR buffer and 1µl mix dTNP. The PCR conditions were as follows: an initial denaturing step (95° C for 3 min) followed by 33 cycles 95 ° C for 1min (denaturation), 55 ° C for 1min (annealing), and 72 ° C for 1min (extension), and a final extension of 72 ° C for 3min. PCR products were electrophoresed through polyacrylamide gel and stained with silver nitrate. The PCR products of each amplification were then digested with the 4-base cutter restriction endonucleases, AluI, HpaII, RsaI and TaqI, as per instruction of the manufacturer. The restriction fragments were separated by running through 6% polyacrylamid gels, and visualized after staining with nitrate silver.



Lane 1 DNA ladder, lanes 2-11 sheep isolates, lane 12 negative control



Lane 1 Negative control, lane 2 DNA ladder, lane 3 positive control, lanes 4-14 human isolates.

Figure 1: PCR amplified ITS1 region from sheep (A) or human (B) hydatid cyst protoscoleces isolates in achrylamide gel.

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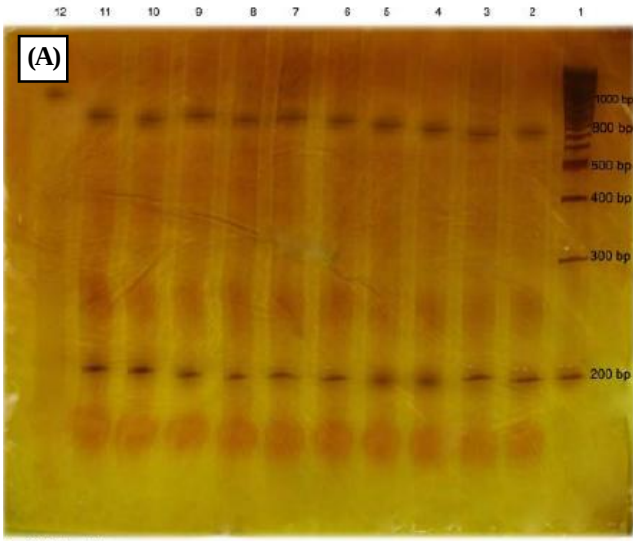
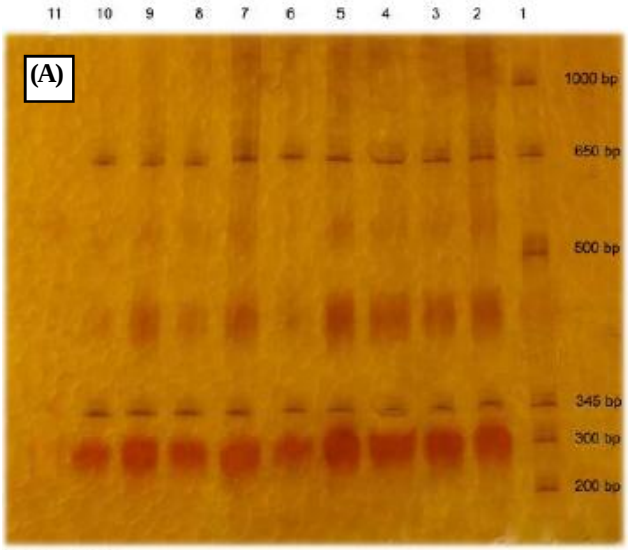
RESULTS

In all human and sheep specimens, a fragment 1000bp was achieved after PCR amplification (figure 1 A&B). PCR products of hydatid cyst protoscoleces of sheep samples were digested with Alu1 enzyme, two bands with 800bp and 200bp were created (figure 2A). However digestion of PCR products of human samples with the same enzyme revealed 3 bands with size of 720bp, 180bp and 100bp (figure 2B).



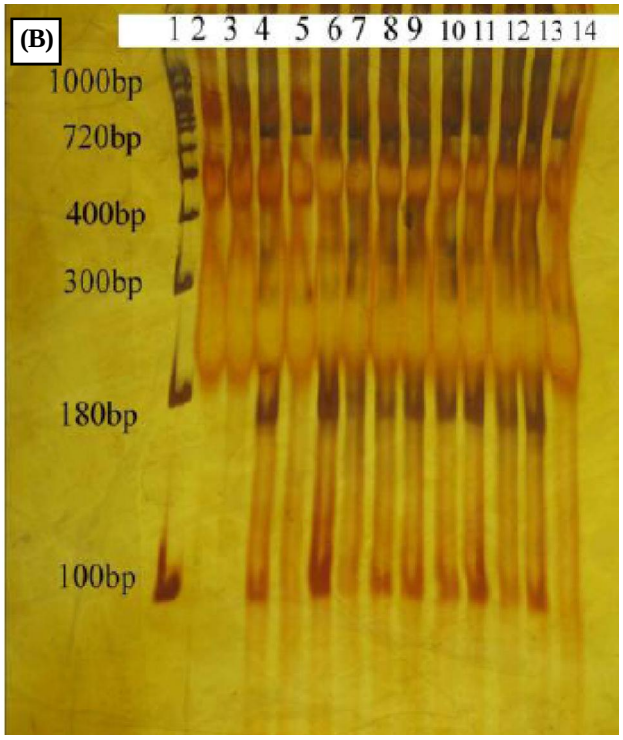
1. DNA Ladder
2_11. samples
12. Control (+)
FGI. ALU1

Lane1 DNA ladder, lanes 2-11 sheep isolates, lane 12 PCR product of sheep isolate without digestion.



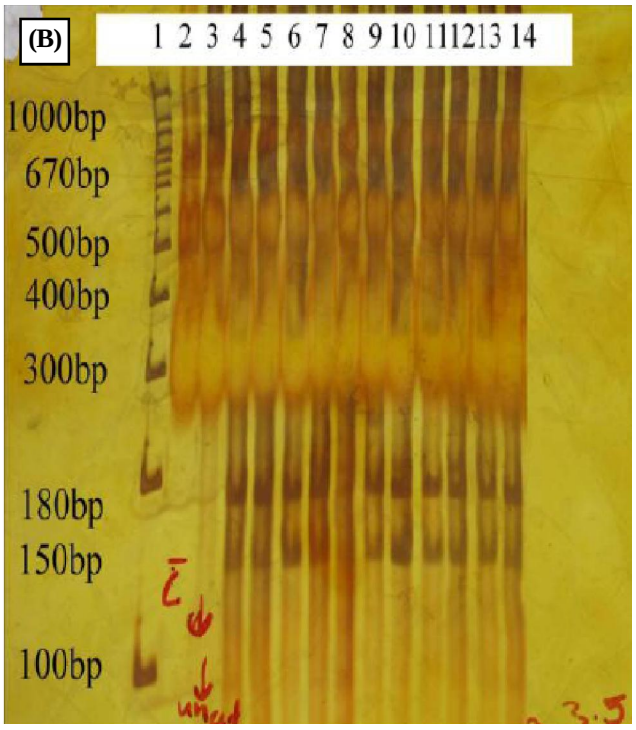
1. DNA Ladder
2_10. Samples
11. Control (+)
FIG. RSa1

Lane 1 DNA ladder, lanes 2-11 sheep isolates, lane 12 PCR product of sheep isolate without digestion.



Lane 1 DNA ladder, lane 2 negative control, lane 3 positive control, lanes 4-14 human isolates.

Figure 2 : PCR-RFLP patterns of hydatid cyst protoscoleces DNA following digestion with Alu1 enzyme.



Lane 1 DNA ladder, lane 2 negative control, lane 3 positive control, lanes 4-14 human isolates.

Figure 3 : PCR-RFLP patterns of hydatid cyst protoscoleces DNA following digestion with Rsa1 enzyme.