

## **Molecular characterization of the human and sheep hydatid cyst strains in Chaharmahal va Bakhtiari province of Iran using restriction fragment length polymorphism (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 *Rsa*<sup>2</sup>, *Hpa*II, *Alu*<sup>2</sup> and *taq*<sup>2</sup>, 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 *Alu*<sup>2</sup> enzyme; three fragments of 100, 180 and 720bp in human isolates and two fragments of 800bp and 200bp in sheep isolates were created. *Rsa*<sup>2</sup> also revealed three segments of 150, 180 and 670bp in human samples and two fragments of 655bp and 345bp in sheep samples. After using *Hpa*II 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 *Taq*<sup>2</sup> 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.

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### **KEYWORDS**

Hydatid cyst;  
RFLP;  
Strain;  
Human;  
Sheep.

## 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<sup>[1,2]</sup>. Several genetically distinct strains differing in several characterizations have been identified<sup>[3]</sup>. In endemic area for this disease there is considerable genetic variation among different isolates of the parasite<sup>[4,5]</sup>. These variations may affect the epidemiology, pathology and control of the disease<sup>[6-8]</sup>. So the identification of the strains is considered a major requirement in the control and prevention of hydatid disease<sup>[2,7]</sup>. Moreover there are evidences indicating that some strains are more pathogenic<sup>[9]</sup>. So far 10 distinct strains of the parasite (G1-G10) have been recognized<sup>[10,11]</sup> from different parts of the world<sup>[3,10,12,13]</sup>.

Hydatidosis is one of the most important zoonotic diseases prevalent in different parts of Iran<sup>[14,15]</sup>. Various surveys have indicated that hydatid cysts are commonly found in livestock throughout the country<sup>[14,16-18]</sup>. Human cases are also regularly reported from different parts of the country<sup>[4,14,15]</sup>. Molecular techniques have been applied successfully for distinguishing the different strains of *Echinococcus granulosus*<sup>[8]</sup>. 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.

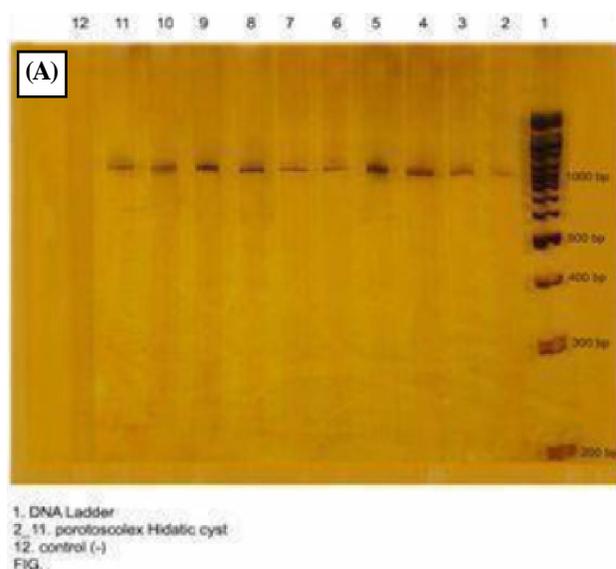
## MATERIALS AND METHODS

Hydatid cysts from 30 infected sheep were collected from several abattoirs in Chaharmahal va Bakhtiari province of Iran. Fifteen 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.

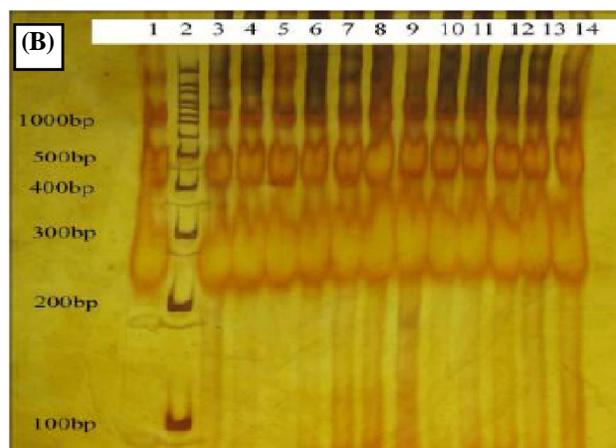
**DNA extraction:** Preserved protoscolices washed three times with distilled water and then digested with SDS and proteinase K. DNA was extracted using Phenol Chloroform method.

The PCR reaction was performed in a final 25µl

volume containing Taq DNA polymerase 1 unit, 3µl MgCl<sub>2</sub>, 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.

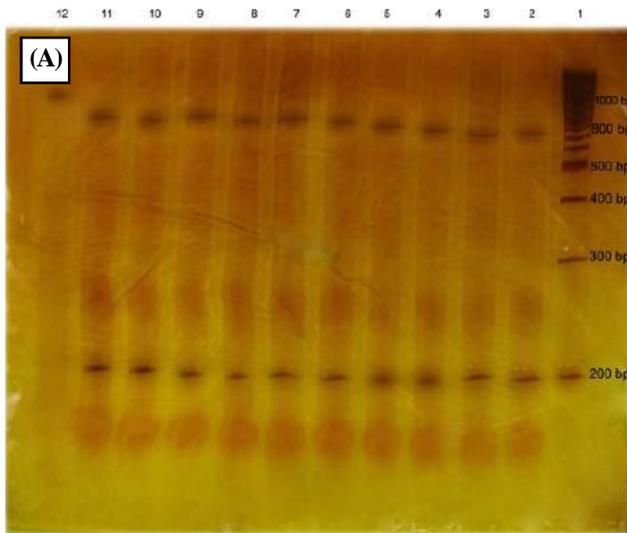
**Figure 1:** PCR amplified ITS1 region from sheep (A) or human (B) hydatid cyst protoscoleces isolates in achrylamide gel.

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**RESULTS**

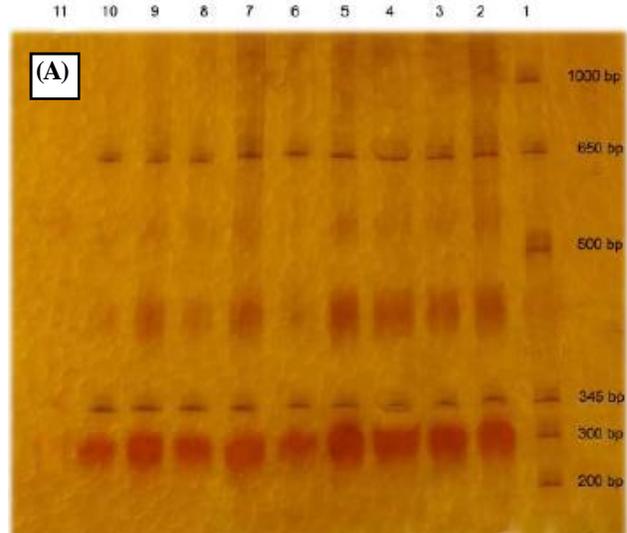
In all human and sheep specimens, a fragment 1000bp was achieved after PCR amplification (figure 1 A&B). PCR products of hydatid cyst protoscoleces

of sheep samples were digested with AluI 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).



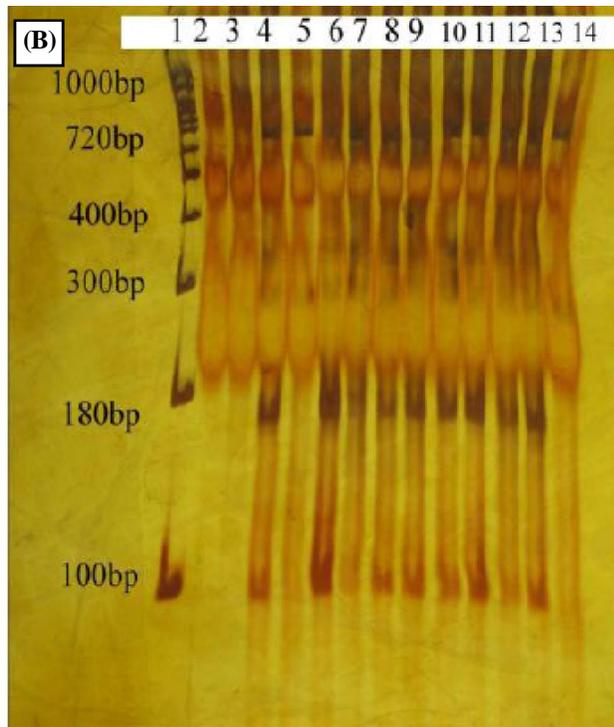
1. DNA Ladder  
2\_11. samples  
12. Control (+)  
FIG. ALU1

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



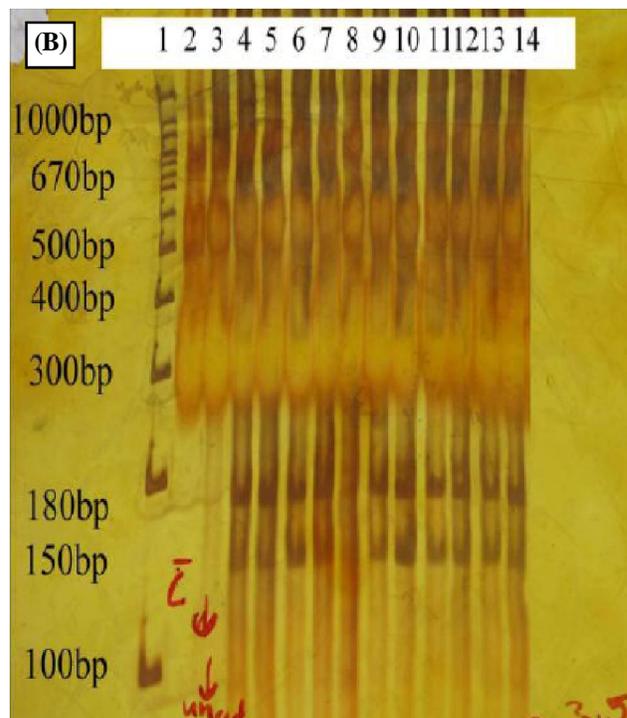
1. DNA Ladder  
2\_10. Samples  
11. Control (+)  
FIG. Rsa1

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



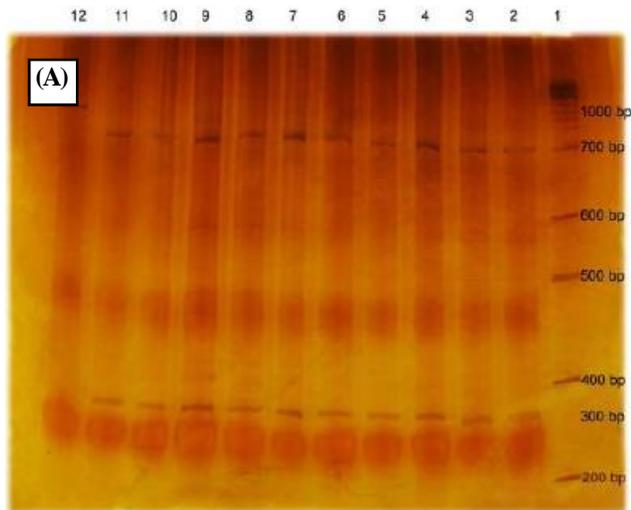
Lane 1 DNA ladder, lane 2 negative control, lane 3 positive control, lanes 4-14 human isolates.

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



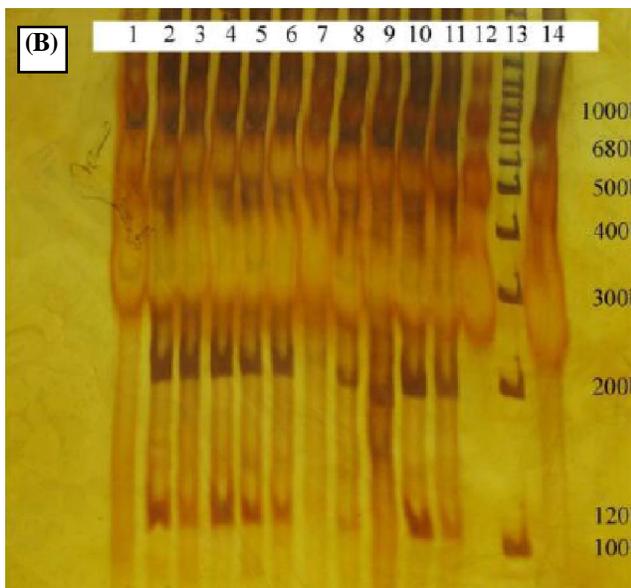
Lane 1 DNA ladder, lane 2 negative control, lane 3 positive control, lanes 4-14 human isolates.

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



1. DNA Ladder  
2\_11. Samples  
12. Control (+)  
FIG. PCR\_RLFP\_Hpa11

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

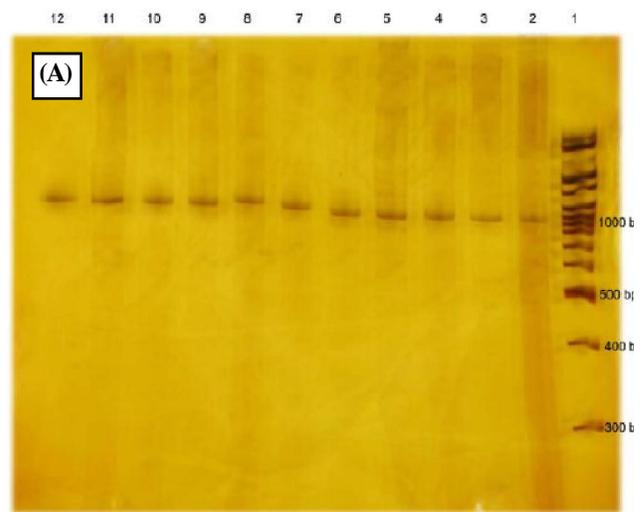


Lanes 1-11 human isolates, lane 12 negative control, lane 13 DNA ladder.

Figure 4 : PCR-RFLP patterns of hydatid cyst protoscoleces DNA following digestion with HpaII enzyme.

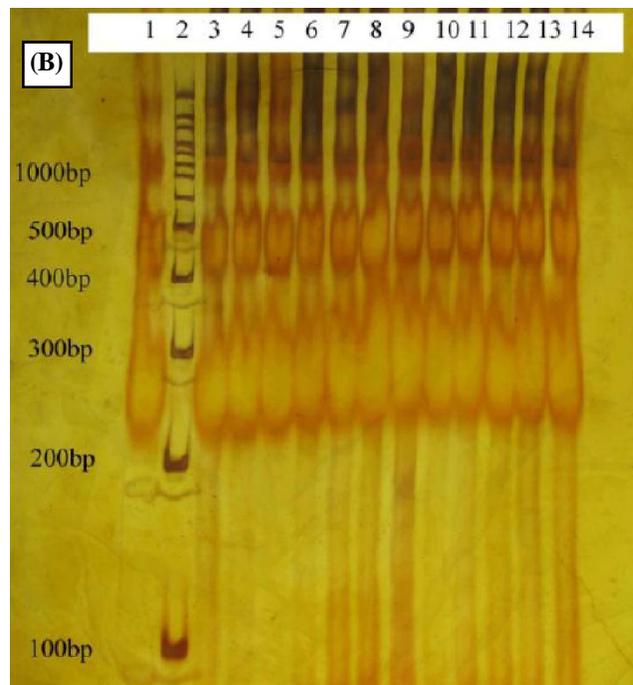
Using RsaI enzyme, two bands with size of 655bp and 345bp were detected in sheep isolates of hydatid cyst protoscoleces (Figure 3A), and three bands with the size of 670bp, 180bp and 150bp were detected in human isolates (figure 3B). HpaII enzyme digested 1000bp DNA of hydatid cyst protoscoleces to 700bp and 300bp in sheep isolates (Figure 4A) and to 680bp, 200bp and 120bp in human isolates (figure 4B). No digestion was happened in PCR products of hydatid cyst protoscoleces in sheep (figure 5A) or human (figure

5B) isolates when TaqI enzyme was used.



1. DNA Ladder  
2\_11. Samples  
12. Control (+)  
FIG. TAQ1

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



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

Figure 5 : PCR-RFLP patterns of hydatid cyst protoscoleces DNA following digestion with TaqI enzyme.

## DISCUSSION

In this investigation nuclear rDNA-ITS1 region of sheep and human hydatid cysts protoscoleces was amplified. In stained polyacrylamide gel a band with

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size of about 1000bp was detected. This is in agreement with what Ahmadi, Scott and Bowles detected in human or sheep isolates of hydatid cyst<sup>[13,19,20]</sup>.

PCR-RFLP technique provides a rapid method for discriminating *Echinococcus* strains<sup>[21]</sup>. In this work *Alu*I, *Rsa*I, *Hpa*II and *Taq*I were used for DNA digestion. *Alu*I enzyme digested 1000bp DNA band to 800bp and 200bp bands in sheep isolates and to 720bp and 180bp in human isolates. These results are in agreement with Scott et al.'s findings. They showed that DNA of human isolates digested differently from sheep isolates<sup>[13]</sup>. With *Rsa*I enzyme also different digestion patterns were detected in human and sheep isolates of hydatid cyst. Scott et al. also achieved different digestion patterns in animal and human isolates of hydatid cyst with this enzyme<sup>[13]</sup>. Gonzalez et al. used *Rsa*I enzyme on human and animal isolates of hydatid cyst. They showed that the pattern of digestion was not similar between animal and human isolates. Furthermore the pattern was not also similar among human isolates<sup>[22]</sup>. When *Hpa*II enzyme was used the pattern of digestion again was different in human and sheep hydatid cyst isolates. Santivañez et al. showed that 95% of human isolates were G1 genotype<sup>[23]</sup>.

In this research work we found that human hydatid cyst isolates in Chahar Mahal va Bakhtiari province of Iran was very different from that of sheep strain. Further work is recommended to clarify genotype of human hydatid cyst isolates.

### ACKNOWLEDGEMENTS

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### REFERENCES

- [1] D.P.McManus, W.Zhang, J.Li, P.B.Bartley; Echinococcosis, *The Lancet*, **362**, 1295-1304 (2003).
- [2] R.Thompson; Echinococcosis. Principles and Practice of Clinical Parasitology, 585-612 (2001).
- [3] A.Lavikainen, M.Lehtinen, T.Meri, V.Hirvelä-Koski, S.Meri; Molecular genetic characterization of the Fennoscandian cervid strain, a new genotypic group (G10) of *Echinococcus granulosus*. *Parasitology*, **127**, 207-215 (2003).
- [4] B.Bastani, F.Dehdashti; Hepatic hydatid disease in Iran, with review of the literature. *The Mount Sinai journal of medicine, New York*, **62**, 62 (1995).
- [5] D.McManus, J.Smyth; Hydatidosis: changing concepts in epidemiology and speciation. *Parasitology Today*, **2**, 163-168 (1986).
- [6] J.Eckert, R.Thompson; Intraspecific variation of *Echinococcus granulosus* and related species with emphasis on their infectivity to humans. *Acta Tropica*, **64**, 19-34 (1997).
- [7] R.Thompson, A.Lymbery, C.Constantine; Variation in *Echinococcus*: towards a taxonomic revision of the genus. *Advances in Parasitology*, **35**, 145-175 (1995).
- [8] R.Thompson, D.P.McManus; Towards a taxonomic revision of the genus *Echinococcus*. *Trends in Parasitology*, **18**, 452-457 (2002).
- [9] J.Bowles, D.P.McManus; Molecular variation in *Echinococcus*. *Acta Tropica*, **53**, 291-305 (1993).
- [10] J.Bowles, D.Blair, D.McManus; Molecular genetic characterization of the cervid strain ('northern form') of *Echinococcus granulosus*. *Parasitology*, **109**, 215-221 (1994).
- [11] J.Bowles, D.Blair, D.P.McManus; Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Molecular and Biochemical Parasitology*, **54**, 165-173 (1992).
- [12] J.Bowles, D.McManus; NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. *International Journal for Parasitology*, **23**, 969-972 (1993).
- [13] J.Scott, J.Stefaniak, Z.Pawlowski, D.McManus; Molecular genetic analysis of human cystic hydatid cases from Poland: identification of a new genotypic group (G9) of *Echinococcus granulosus*. *Parasitology*, **114**, 37-43 (1997).
- [14] H.Y.Darani, M.Avijgan, K.Karimi, K.Manouchehri, J.Masood; Seroepidemiology of Hydatid Cyst in Chaharmahal va Bakhtiari Province, Iran. *Iranian Journal of Public Health*, **32**, 31-33 (2003).
- [15] I.Mobedi, A.Dalimi; Epidemiology of hydatid cyst in Iran and World. Tehran: Moghaddam Publication, 132-147 (1994).
- [16] A.Dalimi, G.Motamedi, M.Hosseini, B.Mohammadian, H.Malaki, Z.Ghamari, F.G.Far; Echinococcosis/hydatidosis in western Iran. *Veterinary parasitology*, **105**, 161-171 (2002).
- [17] D.Mehrabani, A.Oryan, S.Sadjjadi; Prevalence of *Echinococcus granulosus* infection in stray dogs and herbivores in Shiraz, Iran. *Veterinary parasitology*, **86**, 217-220 (1999).
- [18] A.Oryan, N.Moghaddar, S.Gaur; Metacestodes of sheep with special reference to their epidemiologi-

- cal status, pathogenesis and economic implications in Fars Province, Iran. *Veterinary parasitology*, **51**, 231-240 (1994).
- [19] N.Ahmadi, A.Dalimi; Characterization of *Echinococcus granulosus* isolates from human, sheep and camel in Iran. *Infection, Genetics and Evolution*, **6**, 85-90 (2006).
- [20] J.Bowles, D.Blair, D.McManus; A molecular phylogeny of the genus *Echinococcus*. *Parasitology-Cambridge*, **110**, 317-328 (1995).
- [21] J.Bowles, D.P.McManus; Rapid discrimination of *Echinococcus* species and strains using a polymerase chain reaction-based RFLP method. *Molecular and Biochemical Parasitology*, **57**, 231-239 (1993).
- [22] L.González, K.Daniel-Mwambete, E.Montero, M.Rosenzvit, D.McManus, T.Gárate, C.Cuesta-Bandera; Further molecular discrimination of Spanish strains of *Echinococcus granulosus*. *Experimental parasitology*, **102**, 46-56 (2002).
- [23] S.J.Santivañez, A.M.Gutierrez, M.C.Rosenzvit, P.M.Muzulin, M.L.Rodriguez, J.C.Vasquez, S.Rodriguez, A.E.Gonzalez, R.H.Gilman, H.H.Garcia; Human hydatid disease in Peru is basically restricted to *Echinococcus granulosus* genotype G1. *The American Journal of Tropical Medicine and Hygiene*, **79**, 89 (2008).