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Original Article

Epidemiological and Molecular Characterization of *Echinococcus granulosus* Isolated from Small Ruminants in Kashmir Valley, India

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Abstract

Background: Cystic Echinococcosis (CE) is an emergent or re-emergent zoonosis and remains a public health and economic problem all over the world.

Methods: The present study was carried on the prevalence and genotypes of *Echinococcus* present in small ruminants in Kashmir valley. A total of 2100, sheep (2052) and goats (48), slaughtered or spontaneously dead, from various areas of Kashmir valley were screened for the presence of hydatidosis. In case of goat none of the cases were found positive for hydatidosis, whereas, all the positive cases (85) were recorded in sheep only. The overall prevalence of hydatidosis was 4.04%. The prevalence was higher in female sheep (5.46%) compared to males (2.83%). Season-wise highest prevalence was in summer (4.55%), followed by autumn (4.1%), spring (3.89%) and winter (2.5%).The liver was observed to be the most frequently infected organ with relative prevalence of 61.17% followed by lungs (38.82%).The rDNA-ITS1 fragment of positive samples was amplified with BD1 / 4S primers.

Results: The length of amplified fragment for all isolated samples was 1000bps. The products obtained on PCR were digested with four restriction enzymes (*Rsa 1, Alu 1, Msp 1* and *Taq*1). *Rsa 1, Alu 1, Msp 1* yielded identical fragments, 300 and 700 bp in sheep. TaqI restriction enzyme had no effect on PCR product and after digestion; intact 1000bps fragment was seen.

C*onclusion:* Phylogenetic analysis of ITS1 gene revealed that the common sheep strain (G1) is the predominant genotype in sheep in Kashmir valley.

Introduction

Cystic Echinococcosis (CE), a zoonotic infection by larval forms of the tapeworm (metacestodes) of the genus *Echinococcus* is found in the small intestines of carnivores (1, 2) has definitive hosts as carnivores (like dogs) and the intermediate hosts omnivores and herbivores (3). Humans are not a part of the natural life cycle of the parasite, are infected accidently. The adult worm, *Echinococcus granulosus,* develops within the small intestine of carnivores and intermediate stage develops within the internal organs (mainly liver and lungs) of herbivores (such as sheep, horses etc.) and humans in the form of fluid-filled cysts.

The hydatid cyst fertility is an important factor in the epidemiology of *E. granulosus* and in humans; it is an essential element for the progress of formation of secondary echinococcosis (4). Fertility varies depending on the different geographical conditions and intermediate hosts (5, 6). *E. granulosus* has different strains throughout the world (7, 8) affecting the epidemiology, pathology and hence, control and prevention of the cystic hydatid disease (9).

Echinococcosis is diagnosed by different ways using X-ray, CT scan, immunological and serological tests including modern diagnostic technique i.e polymerase chain reaction (PCR), which is very sensitive and specific in detecting echinococcosis infection. Further, PCR has also been used in genotyping of *E. granulosus* to facilitate treatment and vaccination. PCR-based technique, have been used widely for strain characterization within *E. granulosus*. In order to delineate the link among strains of the genus *Echinococcus* mitochondrial (COI and ND1) and nuclear (ribosomal ITS1) nucleotide data sets have been analyzed (10). To date, ten distinct genetic types (G1-G10) of *E. granulosus* sensulato (s.l.) have been identified (11-14). *E. granulosus* and *E. multilocularis* exist as different ITS1 sequence variants, which represent as many as four evolutionary lineages: (i) a sheep strain of *E. granulosus*, (ii) cervid and camel *E. granulosus* ITS1 variants (iii) ITS1 variants of horse, bovine and camel strains of *E. granulosus* (iv) ITS1 variants including *E. granulosus* strains and *E. multilocularis* (15).

Molecular approaches are the best option for *Echinococcus* spp. identification and differentiation. RFLP analysis is an accurate technique to confirm the distinctiveness among *Echinococcus* spp. RFLP, is a technique by which *Echinococcus* isolates are easily identified based on sequence and size of the nuclear genomic rDNA ITS 1 region (16). In addition, PCR-RFLP analysis is important tool for *Echinococcus* spp. genotyping (8, 17).

The strain distribution in different regions G1 (sheep strain), G2, G3 (buffalo strain) and G4 (equine strain) have been observed from Lebanon, Italy, Spain and Syria. G6 strain (in camels) is dominantly found in North Africa and the Middle East (18). A pig strain (G7), and a cervid strain (G8) and (G9) have been reported in swine in Poland and tenth strain (G10) in reindeer in Eurasia (19, 20).With the exception of G4 genotype remaining other strains infect the humans. Studies have suggested that *E. granulosus* sensu stricto (G1-3) has the widest global distribution among all the genotypes (21, 22, 23, 24).Worldwide, most of hydatidosis cases in human beings have been found to be infected with sheep strain (G1) (25,26).

Enough studies have not been carried out on the molecular and genetic variations of *E. granulosus* that could open new clues identification and determination of strains infective to the humans and can help in determination of their pathogenic behavior in domestic ruminants in Kashmir valley. The aim of present study was to find out the genotypes of *E. granulosus* currently infecting Sheep and humans in Kashmir valley, using polymerase

chain reaction-restriction fragment length polymorphism (PCR-RFLP) and to estimate the genetic variability within the strains by sequencing rDNA-ITS1 gene.

Materials and Methods

Study Material

The present study was conducted on small ruminants, including both slaughtered and spontaneously dead, from local farms, post-mortem houses, local abattoirs and local butcher shops of different regions of Kashmir valley. A total of 2100 sheep were screened. Total 85 isolates were collected from sheep and none of the isolates was collected from goats.

Collection of parasite

Fertile cysts of *E. granulosus* were recognized based on Protoscoleces presence. Protoscoleces were isolated from the fertile cysts. Prior to DNA extraction, Protoscoleces were washed almost three times using distilled water and preserved in 70 % alcohol and then stored in refrigerator until used.

DNA extraction

DNA was isolated from protoscoleces as described earlier (27).

PCR amplification

The PCR amplification was performed as described by Bowles and McManus (12) in the rDNA-ITS1 region of the parasite using the following primer pairs (BD1 and 4S):

(BD1: 5´ GTC GTA ACA AGG TTT CCG TA 3´), (4S: 5´ '- TCT AGA TGC GTT CGA TGT CGA TG 3´).The PCR was carried out in a 25 µl reaction mixture containing:10x Buffer (fermentas) 2.5µl, dNTPS (10 mM)(fermentas) 0.75µl, MgCl2 (25mM) 1.5µl, BD1-F (12.5 pmol) 0.5µl, 4S -R (12.5 pmol) 0.5µl, Taq polymerase (fermentas) 0.3µl, Distilled water 16.95µl, Template DNA 2.0µl.The PCR conditions were: Primary denaturing step at 95°C for 5 minutes, Denaturing step at 95°C for 30 sec, Annealing step at 50°C for 30 sec, Extension step at 72°C for 5 minutes x 30 times, Final extension at 72°C for 5 minutes, Hold at 4°C.

After completion of PCR, amplified products were confirmed and analyzed on Agarose gels (1%) and stained with ethidium bromide. Any nonspecific reaction or difference in size of band was observed by running the 100bp DNA ladder (Fermentas) along with PCR product.

Restriction fragment length polymorphism-PCR (RFLP-PCR)

Then the PCR products were digested by 4-base cutting restriction endonucleases *Rsa 1, Alu, Msp 1* and *Taq*1 (10 U) using buffers recommended by the manufacturer (Thermo Fischer), which were effective on different regions of ITS1; in defined heat and time.

Alu 1 = (5' AG ↓CT 3'), 37°, 6h
Rsa 1 = (5' GT ↓AC 3'), 37°, 6h
Taq 1 = (5' T ↓CGA 3'), 65°, 6h
Msp 1 = (5' C ↓ CGG 3'), 37°, 6h

7.5 µl PCR product was used, the total volume was increased to 25 µl (NFW=14 µl, Buffer =2.5 µl and Enzyme= 1 µl) for digestion. The sizes of the restricted products were assessed by electrophoresis in 2% (w/v) TBE agarose gel, stained with 0.5µg/ml ethidium bromide.

Nucleotide sequencing

DNA derived from individual hydatid cysts was subjected to sequencing by the primers employed in the PCR. The purified PCR product was sequenced in Macrogen Inc. Lab. (Geumcheon-gu, Seoul,Korea). Multiple sequence alignment was done using the MUSCLE (v3.8.31) configured for highest accuracy (MUSCLE with default settings).Data obtained were compared with the NCBI nucleotide gene bank (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov/BLAST/).

Results

Prevalence

Table 1 summarizes the prevalence of hydatidosis in sheep screened in three different regions of Kashmir valley. In general, 85 sheep revealed one or more hydatid cysts in various organs, giving an overall prevalence of 4.04%.

Table 1: Prevalence of hydatidosis in local and non-local sheep in Central, Northern and Southern regions of the Kashmir valley

Sheep	*Northern region*		*Central region*		*Southern region*		*Total*	
	No. screened	No. +ve	No. screened	No. +ve	No. screened	No. +ve	No. screened	No. +ve
Total	412	18(4.36%)	942	36(3.82%)	746	31(4.15%)	2100	85(4.04%)

Sex-wise distribution of hydatidosis

Sex-wise distribution is given in Table 2. The prevalence was higher in female sheep (5.46%) compared to males (2.83%), respectively.

Table 2: Sex-wise distribution of sheep infected with hydatidosis in Kashmir valley

Sex	*Male*	*Female*
No. screened	1130	970
No. positive	32	53
%age	2.83	5.46

Season-wise distribution of hydatidosis

Species-wise distribution of hydatidosis

In case of goat, none of the cases was found positive for hydatidosis, whereas, all the positive cases were recorded in sheep only (Tables 3 and 4).

Table 3: Season-wise distribution of hydatidosis in Kashmir valley

Sheep	*Spring*		*Summer*		*Autumn*		*Winter*		*Total*	
	No. screened	No. +ve	No. screened	No. +ve	No. screened	No. +ve	No. screened	No. +ve	No. screened	No. +ve
Total	565	22 (3.89%)	944	43 (4.55%)	414	17 (4.1%)	70	3 (2.5%)	2100	85 (4.04%)

Table 4: Specie-wise distribution of hydatidosis in Kashmir valley

Specie	*Northern region*		*Central region*		*Southern region*		*Total*	
	No. screened	No. +ve	No. screened	No. +ve	No. screened	No. +ve	No. screened	No. +ve
Sheep	405	18	919	36	728	31	2052	85
Goat	7	0	23	0	18	0	48	0
Total	412	18(4.36%)	942	36(3.82%)	746	31(4.15%)	2100	85(4.04%)

Organ-wise distribution pattern

The sheep infected with hydatidosis revealed cysts in one or more organs. The frequently infected organs were lungs and liver. The liver was observed to be the most frequently infected organ with relative prevalence of 61.17 % followed by lungs (38.82%).

Molecular techniques

PCR - RFLP analysis

The ITS1 region was used to characterize genotypes of *E. granulosus*. The rDNA-ITS1 fragment of samples including 85 from sheep, were amplified with BD1 / 4S primers (Bowles and McManus, 1993b). The length of amplified fragment for all isolated was 1000bps. However, no amplification was observed in the negative controls (Fig. 1).

The PCR product obtained was subsequently digested with four restriction enzyme (*Rsa 1, Alu, Msp 1* and *Taq*1).*Rsa 1, Alu 1, Msp 1* yielded identical fragments, 300 and 700 bp. TaqI restriction enzyme had no effect on PCR product and after digestion intact 1000bps fragment was seen (Fig. 2).The patterns obtained in sheep were identical to common sheep strain of *E. granulosus*.

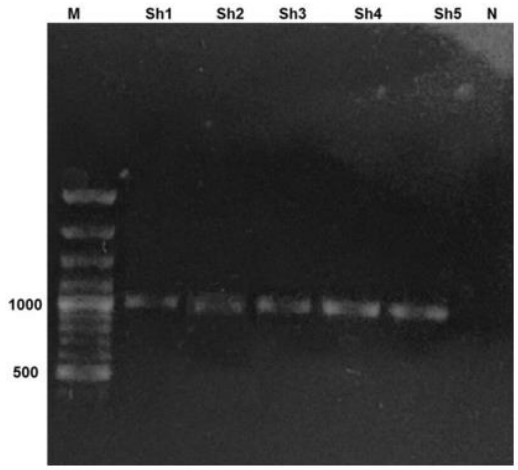

Fig. 1: PCR amplified ITS1 fragments from *E. granulosus* isolates from sheep. Lanes: M, DNA marker (100 bp); N, negative control (without DNA template); isolates from sheep (Sh1 –Sh5)

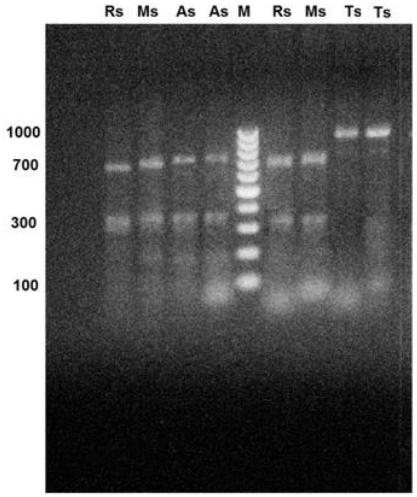

Fig. 2: RFLP fragments of sheep isolates of E. granulosus, digested with: Alu I (As), MspI (Ms), Rsa I (Rs), Taq I (Ts) enzymes.Lanes:M, DNA size marker (100 bp)

Sequencing and phylogenetic analysis

The ITS1 gene fragments of hydatid cyst were sequenced. With the BLAST program GenBank (http://www.ncbi.nlm.nih.gov/) was searched for identical sequences (11, 16, 28, 29, 30) and a significant homology was detected with *E. granulosus* sequences. All of the isolates examined (GenBank accession nos. KY129666, KY129667, KY129668, KY129669 and KY129670) were identified as corresponding to the sheep strain (G1) of *E. granulosus* and no other genotypes were detected.

Discussion

Hydatidosis in small ruminants has been reported throughout the world. The occurrence has been recorded as low, medium and high in different sheep rearing areas (31).The prevalence of 4.04% for hydatidosis in sheep, observed in present study, is comparable with reports from other parts of globe (32-35). However, it has been observed that prevalence varies greatly from one geographical area to other and different workers have reported

prevalence varying from as low as <1% to >70% (36-40).

The prevalence of hydatidosis in urban centers has shown a consistent decline over the past few decades. This could be attributed to the increase in the number of government-controlled abattoirs, where veterinary inspection of carcasses and proper disposal of offal is routinely practiced (26).The higher rate of infection in rural areas has been attributed to the outdoor rearing of cattle and sheep besides having a high number of stray dogs (34). The absence of hydatidosis in goats could be attributed to the feeding habits of the animal. Goats are browsers while as the sheep are grazers. Browsing affords a least chance to the goats to pick up the infection.

In the current study, females were observed to have higher prevalence than males. Reports of higher prevalence in females have been documented earlier which might be attributed to longer lives of females than males (41, 42). Further, they have high ages at the time of slaughtering and are more prone to the stresses of pregnancy, parturition and lactation (43).

Bowles et al (10) sequenced three nucleotide data sets (CO1, ND1 and ITS1) in order to delineate relationships among strains and species of the genus *Echinococcus*. A highly specific identification of *E. granulosus* strains requires approaches for its DNA characterization (23). Extensive literature on the molecular biological methods have been reported to discriminate *Echinococcus* strains (44, 45).They provided evidence that *E. granulosus* was not a monophyletic taxon and strains within this species fall into groups which might merit recognition as separate species.

In this study, rDNA-ITS1 fragment was amplified with BD1 / 4S primers. The length of amplified fragment for all isolated samples with sheep origin was 1000bp and with human origin was between 1000bps and 1100bps characteristics of the sheep strain. Similar results were reported by other workers (17, 20, 27,29, 40, 46-51). In contrast, PCR amplification of ITS1 gene of hydatid cysts from sheep and cattle showed similar pattern of PCR product of all isolates with amplified DNA band of the same molecular size at 1115bp (35). Vahedi et al., (52) reported that size of amplicon for ITS1-PCR in case of humans in Azerbaijan province was 900 bp.

In the present study two clearly distinguishable patterns were obtained with *Rsa 1, Alu 1, Msp 1*, which yielded identical fragments, 300 and 700 bp in sheep and 325 and 700 bp in humans which are identical to sheep strain (G1) of *E. granulosus.* Whereas, *TaqI* had no effect on PCR product, which is in accordance with other studies (17, 47, 48) who reported similar results. Molecular analysis by PCR-RFLP of *ITS1* of cattle, buffalo and sheep showed similar patterns with *Msp1* and *Rsa.1* (29). Molecular analysis of ITS1 gene of cattle and sheep isolates by RFLP showed no variations and showed similar patterns in all the isolates with Msp1 and Rsa1. Digestion of amplification product of ITSI with MSP1 yielded 661 bp and 406 bp, while as with RSA1 yielded 745 bp and 360 bp fragments (45). Similarly, (49) reported that *Rsa1* showed two bands approximately 655bp and 345bp (49). *Alu1* yielded 800bp and 200bp and *Taq1* had no effect on PCR product. *Rsa*I restriction endonucleases showed two different bands, 300 and 600 bps in cattle and sheep.

Conclusion

The ITS1 sequence data obtained in this study confirmed RFLP patterns and were identified as corresponding to the G1 strain of *E. granulosus.* The sheep strain, confirmed as G1 at the DNA sequence level, showed remarkable uniformity with isolates from sheep from geographically diverse regions. The study inferred that G1 strain in sheep in Kashmir valley is a potential zoonotic parasite and its control both in definitive and intermediate host would in a long way help to curb the disease.

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Conflict of interest

There is no conflict.

References

1. Endalew D, Nurradis I. Prevalence and Economic Importance of Echinococcosis in Cattle Slaughtered at North Gonder Elfora Abattoir. Eur J Appl Sci.2013; 5: 29-35.
2. Ahmad ME, Rahim MA, Fatima MA. Hydatid disease, a morbid drop needs awareness. Sudan Med J.2011;47: 1.
3. Ould CB, Schneegans F, Chollet JY, Jemli MH. Prevalence and aspects of lesions of echinococcosis in camel in Northern Mauritania. Revue ElevMédVét Pays Trop. 2010;63: 23-28.
4. Oudni-M rad MS, M'rad M, Gorcii M, et al. Cystic echinococcosis in children in Tunisia: Fertility and cysts location. Bull Soc Pathol Exot. 2007; 100(1): 10-13.
5. Kose M, Sevilmi FK. Prevalence of cystic echinococcosis in slaughtered cattle in Afyonkarahisar. Turkiye Parazitol Derg.2008;32(1): 27-30.
6. Scala A, Mazette R. Cystic echinococcosis in the sheep: causes of its persistence in Sardinia. Vet Res Commun. 2009;33 Suppl 1: 41-45.
7. McManus DP, Smyth JD. Hydatid disease (Echinococcosis): changing concepts in epidemiology and speciation. Parasitol Today.1986; 2(6): 163-168.
8. Bowles J, McManus DP. Molecular variation in *Echinococcus*. Acta Trop. 1993; 53(3-4): 291-305.
9. Thompson RCA,Lymbery AJ. The nature, extent and significance of variation within the genus *Echinococcus*. Adv Parasitol.1988; 27: 209-258.
10. Bowles J, Blair D, Mc-Manus DP. A molecular phylogeny of the genus *E. granulosus*. Parasitology.1995;110(Pt 3): 317-328
11. Bowles J, Mc-Manus DP. Rapid discrimination of *Echinococcus* species and strains using a PCR-based method. Mol Biochem Parasitol. 1993; 57(2): 231-239.
12. Bowles J, Blai, D, Mc-Manus DP. Molecular genetic characterization of the cervid strain" (northern form)"of *E. granulosus*. Parasitology.1994; 109(Pt 2): 215-221.
13. Snabel V, D'Amelio S, Mathiopoulos K, Turcekova L, Dubinsky P. Molecular evidence for the presence of a G7 genotype of *Echinococcus granulosus* in Slovakia. J Helminthol.2000; 74(2): 177-181.
14. Nakao M, Mcmanus DP, Schantz PM, Craig PS, Ito A. A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. Parasitology.2007;134(Pt 5): 713-722.
15. Van Herwerden L, Gasser RB, Blair D. ITS-1 ribosomal DNA sequence variants are maintained in different species and strains of *Echinococcus*. Int J Parasitol.2000; 30(2):157-169.
16. Bowles J, Mc-Manus DP. NADH dehydrogenase 1 gene sequence compared for species and strains of the genus *Echinococcus*. Int J Parasitol.1993; 23(7): 969-972.
17. Bowles J, Blair D, Mc-Manus DP. Genetic variants within the genus *Echinococcus* identified by mitochondrial sequencing. Mol Biochem Parasitol.1992; 54(2): 165-173.
18. Hosseinzadeh S, Mehdi F, Arsalan H, Shahram S. Molecular Characterization of *Echinococcus granulosus* in South of Iran. Open J Vet Med.2011; 2: 201-206.
19. Scott JC, Stefaniak J, Pawlowski ZS, McManu DP. Molecular genetic analysis of human cystic hydatid cases from Poland: identification of a new genotype group (G9) of *Echinococcus granulosus*. Parasitology. 1997; 114(Pt 1): 37-43.
20. McManus DP, Thompson RC Molecular epidemiology of cystic echinococcosis. Parasitology. 2003; 127 Suppl:S37-51.
21. McManus DP, Gray DJ, Zhang W, Yang Y. Diagnosis, treatment, and management of echinococcosis. BMJ. 2012; 344: e3866.
22. Snabel V, Altintas N, D'amelio S, Nakao M, Romig T, Yolasigmaz A, Gunes K, Turk M,

Busi M,Hüttner M. Cystic echinococcosis in Turkey: genetic variability and first record of the pig strain (G7) in the country. Parasitol Res. 2009;105(1): 145-154.

23. Utuka AE, Simsek S, Koroglub E, McManus DP. Molecular genetic characterization of different isolates of *Echinococcus granulosus* in east and southeast regions of Turkey. Acta Trop. 2008; 107(2): 192-194.
24. Vural G, Baca AU, Gauci CG, Bagci OY, Lightowlers MW. Variability in the *Echinococcus granulosus* Cytochrome C oxidase1 mitochondrial gene sequence from livestock in Turkey and a re-appraisal of the G1-3 genotype cluster. Vet Parasitol. 2008; 154(3-4):347-350.
25. Moro P, Schantz PM. Echinococcosis: a review. Int J Infect Dis.2009; 13(2): 125-133.
26. BhattacharyaD, Bera AK, Bera BC, Pan D, Das SK. Molecular appraisal of Indian animal isolates of *Echinococcus granulosus*. Indian J Med Res.2008; 127: 383-387.
27. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual (2nd edn.).1989; Cold Spring Harbor Laboratory Press, New York.
28. Bhattacharya D, Bera AK, Bera BC, Maity A, Das SK. Genotypic characterization of Indian cattle, buffalo and sheep isolates of *Echinococcus granulosus*. Vet Parasitol.2007; 143(3-4): 371-374.
29. Huttner M, Siefert L, Mackenstedt U, Romig T. A survey of *Echinococcus* species in wild carnivores and livestock in East Africa. Int J Parasitol. 2009; 39(11):1269-1276.
30. Khademvatana S, Yousefia E, Rafieia A, Rahdara M, Sakia J. Molecular characterization of livestock and human isolates of *Echinococcus granulosus* from south-west Iran. J Helminthol.2013; 87(2): 240-244.
31. Jenkins DJ, Romigb T, Thompsonc RCA. Emergence/re-emergence of Echinococcus spp.-a global update. Int J Parasitol.2005;35(11-12): 1205-1219.
32. Fakhar M, Sadjjadi SM. Prevalence of Hydatidosis in Slaughtered Herbivores in Qom Province, Central Part of Iran. Vet Res Commun. 2007; 31(8): 993-997.
33. El-Ibrahim, JH. Prevalence of Sheep Hydatidosis in North West Bank- Palestine. 2009. Master's thesis, Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.
34. Kebede W, Hagos A,Girna Z, Lobago F. Echinococcosis/ hydatidosis: Its prevalence, economic and public health significance in Tigray region. Trop Anim Health Prod. 2009; 41(6): 865-871.
35. Madawy RSE, Nashwa OK, Jehan SAA. Epidemiological and molecular studies of hydatid cyst in slaughtered cattle and sheep in Toukh, Egypt. Benha Vet Med J. 2011;95-101.
36. SenlikB. Prevalence of hydatidosis and its relationship to the age, breed and sex of sheep in the province of Bursa. Turkiye Parazitol Derg. 2000; 24(3): 304-308.
37. Parto P, Nazari AR, Razavi, S.M. A study on the rate of prevalence, fertility and viability of the protoscolices of hydatid cyst of *E. granulosus* in sheep slaughtered in Fars industrial slaughterhouse. Iran J Vet Res.2004;5 (1): 113-121.
38. Daryani A, Alaei R, Arab R, Sharif M, Dehghan MH, Ziaei H. Prevalence of hydatid cyst in slaughtered animals in Northwest Iran. J Ani Vet Adv. 2006;5(4): 330-334.
39. Mokhtaria K, Fatima BJ, Abboud B, Ammar SSM. Cystic Echinococcosis in Small Ruminants in Tiaret (Algeria). GloVeteri. 2013; 11 (6): 753-758.
40. Hayajneh MF, Althomali AMH, Abdelrahman TMN. Prevalence and characterization of hydatidosis in animals slaughtered at Al Taif abattoir, Kingdom of Saudi Arabia. Open J Ani Sci.2014;4(1): 38-41.
41. Lahmar S, Trifi SM, Ben-Naceur S, et al. Cystic echinococcosis in slaughtered domestic ruminants from Tunisia. J Helminthol. 2013; 87(3)318-25.
42. Rahmani K, Radfar MH, Adinehbeigi K. Hydatidosis: prevalence and biometrical studies in sheep in Kerman area, southeast of Iran. Comp Clin Pathol. 2012; 1-6.
43. Iqbal HJ, Maqbool A, Lateef M, et al. Studies on hydatidosis in sheep and goats at Lahore. Pak J Ani Sci.2012;22: 894-897.
44. McManus DP. Molecular characterization of taeniid cestodes. Parasitol Int.2006; 55Suppl:S31-7.
45. Craig PS, Mc-Manus DP, Lightowlers MW, et al. Prevention and control of cystic echinococcosis. Lancet Infect Dis.2007; 7(6): 385-394.
46. Shahnazi M, Hejazi H, Salehi M, Andalib AR. Molecular characterization of human and ani-

mal *Echinococcus granulosus* isolates in Isfahan, Iran. Acta Trop.2011;117(1):47-50.

47. Gholami SH, Sosari M, Fakhar M, et al. Molecular characterization of *Echinococcus granulosus* from hydatid cysts isolated from human and animals in Golestan province, north of Iran.Iran J Parasitol. 2012; 7(4): 8-16.
48. Eryıldız C, Sakru N. Molecular Characterization of Human and Animal Isolates of *Echinococcus granulosus* in the Thrace Region, Turkey. Balkan Med J.2012; 29(3): 261-267.
49. Hanifian H, Diba K, Tappeh KH, Mohammadzadeh H, Mahmoudlou R. Identification of *Echinococcus granulosus* Strains in Isolated Hydatid Cyst Specimens from Animals by PCR-RFLP Method in West Azerbaijan - Iran. Iran J Parasitol. 2013; 8(3): 376-381.
50. Hashemi TGR, Razmi GR, Mirshekar F. Morphological and Molecular Studies (ITS1) of Hydatid Cysts in Slaughtered Sheep in Mashhad Area. Biomed and Bio Eng. 2015;2: 10.
51. Villalobos N, Gonza´lez LM, Morales J, et al. Molecular identification of *Echinococcus granulosus* genotypes (G1 and G7) isolated from pigs in Mexico. Vet Parasitol. 2007.147(1-2): 185-189.
52. Vahedi A, Mahdavi M, Ghazanchaei A, Shokouhi B. Genotypic characteristics of hydatid cysts isolated from humans in East Azerbaijan Province (2011-2013). J Res Clin Med 2014; 2(3): 152-7.