Genotyping of cystic echinococcosis isolates from clinical samples of human and domestic animals

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Abstract

Cystic hydatid disease is a cosmopolitan important disease in both human and animals. Many strains were investigated in this parasite. The aim of study was to characterize genotype variations of *Echinococcus granulosus* isolates collected from human and domestic animals in Al-Qadisiyah province/ Iraq based on sequencing of *nad1* mitochondrial gene. Eighty hydatid cysts of human (12), sheep (15), cattle (36), and camels (17) were collected from hospital and slaughter house of the province, during October 2014 to June 2015; microscopic examination was made for cysts fluid to determine the fertility. DNAs extraction was done for each sample in addition to purify and concentrate of extracted DNA samples was performed to determine *nad1* (400bp) gene used conventional PCR method. Phylogenetic analysis was performed using NCBI-Blast Alignment identification and Unweighted Pair Group Method with Arithmetic Mean. Twenty five (10 from human and 5 from each studied animals) samples were chosen due to their fertility and high DNA purity, in which three strains (genotypes) were investigated including sheep strain (G1) 40%, buffalo strain (G3) 48% and camel strain (G6) 12%, where human samples related to G1(20%) and G3(80%); sheep samples related to G1(80%) and G3(20%); cattle samples related to G1(60%), G3 (20%) and G6 (20%); camels samples related to G1(20%), G3(40%) and G6(40%). The dominant strain is a buffalo strain (G3); both of buffalo strain (G3) and sheep strain (G1) represented the actual source of human infection. There is no host specificity of detected genotypes.

Keywords: Echinococcus, Hydatid cyst, Genotype, Iraq
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جينوتايب أبواغ الايكانوكوس والمعزولة من عينات سريرية في الانسان والحيوانات المستانسة

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الخلاصة

داء الأكياس العدرية هو مرض مهم واسع الأنتشار في كلا من الأنسان والحيوان؛ هنالك عدة عتر درست في هذا الطفيلي؛ الدراسة الحالية هدفت الى تمييز الاختلاف الجيني لعزلات طفيلي المشوكات الحبيبية التي جمعت من الأنسان والحيوانات المستأنسة في محافظة القادسية/ العراق؛ بالأعتماد على تسلسل جين *nad1* الموجود في المايتوكوندريا. تم جمع ثمانون كيس عدري من الأنسان (١٢) والأغنام (١٥) و الأبقار (٣٦) والجمال (١٧)؛ حيث جمعت من مستشفى ومجزرة المدينة خلال الفترة من تشرين الأول ٢٠١٤ لغاية حزيران ٢٠١٥؛ أجري الفحص المجهري على سائل الأكياس لتحديد خصوبتها وأستخلص الحمض النووي من العينات وتم قياس نقاوة وتركيز الحمض النووي المستخلص. أستخدمت طريقة تفاعل السلسلة المتبلمرة العادية لتحديد الأصابة حسب الجين *nad1* (٤٠٠ زوج قاعدي) ثم أجري التحليل الجيني الوراثي بأستخدام موقع المركز العالمي للمعلومات التقنية الأحيائية وتحديد المحاذاة وطريقة زوج المجموعة الغير مرجح مع المتوسط الحسابي. أختيرت خمسة وعشرون عينة حسب خصوبتها ونقاوة الحمض النووي فيها (١٠ من الأنسان و٥ من كل من الحيوانات المدروسة الأخرى) حيث وجدت ثلاثة عتر (سلالات) شملت عترة الأغنام (G1) بنسبة ٤٠% و عترة الجاموس (G3) بنسبة

٤٨% وعترة الجمال (G6) بنسبة ١٢%؛ وسجلت عترتي الجاموس والأغنام نسبة ٨٠% و ٢٠% حسب التوالي من عينات الأنسان في حين سجلت عكس النسبة بالنسبة للعترتين في الأغنام, و ظهرت في الأبقار ثلاثة عتر وهي عتر الأغنام والجاموس والجمال بنسب ٦٠% و ٢٠% و ٢٠% على التوالي, أما عينات الجمال فكانت نسبة ٢٠% منها تعود الى عترة الأغنام و ٤٠% الى كل من عترتي الجاموس والجمال. إن عترة الجاموس G3 تمثل العترة السائدة, وإن المصدر الحقيقي لأصابة الأنسان هو كل من عترتي الجاموس والأغنام حيث لاتوجد خصوصية الأصابة للمضيف بالنسبة للعتر الموجودة.

Introduction

Echinococcus granulosus is one of the most important zoonotic parasites that cause hydatid cysts in human and domestic animals, which called "dog small tapeworm"; it lives in the small intestine of dogs mainly (1). The adult worm required two hosts to complete its life cycle which are intermediate host like human and domestic animals and definitive host like canids (2).

Cystic echinococcosis disease is caused by the larval stage (hydatid cyst) of *E. granulosus*, the hydatid cyst develops after ingestion of eggs contain oncosphere embryo that shown by King and Fairley (3); the cyst characterized as unilocular filled with fluid surrounded by a two layers of hydatid cyst wall, nucleated inner germinal layer, where protoscolices grow, and a cellular outer laminated layer; these layers are surrounded by fibrous capsules of host (4,5).

Oku (6) and Al-Mutaywiti (7) referred to that the adult worm settles down in the mucosal layer of small intestine of definitive host; general life cycle of *Echinococcus* spp. occur through passing of gravid segment or free eggs by adult parasite with feces of definitive host, where the intermediate host ingested the eggs with contaminated food lead to develop of hydatid cyst containing protoscolices (fertile cysts). The cycle is completing if the definitive host eats the infected part of intermediate host then protoscolices grow to adult cestode in definitive host small intestine (8). Clinical signs of hydatid disease may occur after a highly variable incubation period of several months to years; hepatic cyst may cause abdominal pain and hepatomegaly while pulmonary cyst may cause chronic cough, dyspnea and expectoration (5,9,10). Romig (11) reported that hydatid cysts was cosmopolitan distribution. It remains public health threatened in endemic areas such as Mediterranean countries, North and East Africa, Western and Central Asia, China, South America and Australia. Diagnosis of the hydatid cyst in the infected animals do not explained, but most dependent detections during carcass inspection and at post mortem examination (12,13).

To date more sensitive molecular techniques are used for determination species and strains of *E. granulosus* (14). There are ten distinct genotypes (G1-G10) have been recorded in the world based on nucleotide sequence analysis of the mitochondrial cytochrome C oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 1 *(nad1*) genes. These genes have been related to intermediate hosts (15-17).

McManus and Thampson (18) recorded the most common geographic distribution around the world is sheep strain (G1 genotype); it is also dominant in the Mediterranean area.

Due to there is inadequate study in Iraq related to genotyping diversity and sequence variations of *E. granulosus* isolates from human and animals hydatid cysts, this study was designed and it`s aims were: characterize genotype variations of *E. granulosus* isolates from human and animal's hydatid cysts based on *nad1* gene and determine the relationship between strains in relative countries.

Materials and methods

Eighty hydatid cysts of human (12), sheep (15), cattle (36) and camels (17) were collected from hospital and slaughter house of Al-Qadisiyah province during October 2014 to June 2015. Microscopic examination was made for cysts fluid to determine the cyst fertility through investigation of protoscolices which were rinsed three times with phosphate buffer saline (PBS).

DNA extraction was done for each sample by Genomic DNA extraction kit (Geneaid, USA), according to the company instruction; the purity and concentration of extracted DNA samples were analyzed by Nanodrop. The extracted DNAs were stored at -20°C until used for PCR.

The mitochondrial *nad1* gene was used for PCR amplification the primers were designed by (19) which provided by Bioneer Company, Korea.

PCR amplification was prepared by added of 20 µl including: 5 µl of DNA template, 1.5 µl (10 pmol) of each (forward) 5'-TAAAGAAAGAACATAATGAAAATG-3' and reverse 5'-CCATAATCAAATGGCGTACGAT-3' primers, 12 µl PCR water to the PCR tube of AccuPower PCR PreMix Kit(Bioneer, Korea) which contain other PCR reaction requirements (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl2, stabilizer, and tracking dye), were used to amplify a 400bp fragment of *nad1* gene under the following conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 second; annealing at 50°C for 45 second; elongation at 72°C for 30 second, then the final extension at 72°C for 5 minute and holding at 4°C. The PCR product of

mitochondrial *nad1* gene (400bp) was analyzed by agarose gel electrophoresis.

The PCR products were purified from agarose gel by using commercial purification kit (EZ EZ-10 Spin Column DNA Gel Extraction Kit, Biobasic, Canada) and sent to Bioneer Company in Korea for DNA sequencing by AB DNA sequencing system.

Phylogenetic analyses were performed based on NCBI-Blast Alignment identification and unweight pair Group Method with Arithmetic Mean Tree (UPGMA tree).

Results

Twenty five (10 from human and 5 from each studied animals) samples has been chosen due to their fertility and high purity DNA.

The result of phylogenetic analysis based on *nad1* gene showed that there are three strains (genotypes) of the local *E. granulosus* parasite including sheep strain (G1) 40%, buffalo strain (G3) 48% and camel strain (G6) 12% (Table 1); where human samples related to G1(20%) and G3(80%) (Fig. 1); sheep samples related to G1 (80%) and G3 (20%) (Fig. 2); cattle samples related to G1 (60%), G3 (20%) and G6 (20%) (Fig. 3) and camel samples related to G1 (20%), G3 (40%) and G6 (40%) (Fig. 4).

Among all analyzed samples the G3 strain recorded the highest percentage (48%) followed by the G1 strain (40%) with the lowest rate was appeared in G6 (12%).

The local *E. granulosus* of human, sheep, cattle and camels isolates were showed close related together depending on NCBI-Blast *E.granulosus* human (KJ556994.1), sheep (KP245839.1), cattle (KT005319.1), and camels (AB921091.1). (Fig. 5).

Some local *E. granulosus* isolates of human, sheep, cattle and camels were show close related to Egyptian (AB921124.1), Iranian (JF836798.1) and Moroccan (EF367337.1; EF 367330.1; EF367315.1) isolates; whereas other local *E. granulosus* isolates out of tree as unique isolates. (Fig. 6).

Table 1: The genotypes of E. granulosus in human and animals (sheep, cattle and camels) using partial sequence of nad1 gene according to phylogenetic tree analysis and NCBI- BLAST alignment tool

Isolate No.	NCBI –BLAST Genotypes Identity (%)						Diagnostic genotype
	Genotype1 (AJ237632)		Genotype3 (AJ237634)		Genotype6 (HM749616)		
	Max Score	Identity (%)	Max Score	Identity (%)	Max Score	Identity (%)	
EG.H1	724	99%	722	99%	477	87%	Genotype1
EG.H2	722	99%	720	99%	473	86%	Genotype1
EG.H3	722	99%	713	100%	482	87%	Genotype3
EG.H4	717	99%	720	100%	473	86%	Genotype3
EG.H5	695	99%	704	100%	466	87%	Genotype3
EG.H6	717	99%	720	99%	475	86%	Genotype3
EG.H7	717	99%	720	99%	475	86%	Genotype3
EG.H8	713	99%	722	100%	479	87%	Genotype3
EG.H9	717	99%	720	99%	475	86%	Genotype3
EG.H10	713	99%	722	100%	479	87%	Genotype3
EG.S1	711	99%	700	99%	482	87%	Genotype1
EG.S2	600	99%	620	99%	491	87%	Genotype3
EG.S3	711	99%	700	99%	482	87%	Genotype1
EG.S4	620	99%	601	99%	497	88%	Genotype1
EG.S5	720	99%	715	99%	480	87%	Genotype1
EG.C1	583	99%	592	99%	495	88%	Genotype3
EG.C2	725	99%	722	99%	488	87%	Genotype1
EG.C3	720	99%	717	99%	482	86%	Genotype1
EG.C4	226	88%	230	88%	720	100%	Genotype6
EG.C5	489	100%	484	99%	486	87%	Genotype1
EG.CM1	700	99%	669	99%	484	87%	Genotype1
EG.CM2	827	99%	841	100%	562	87%	Genotype3
EG.CM3	122	85%	127	85%	710	100%	Genotype6
EG.CM4	717	99%	720	99%	479	87%	Genotype3
EG.CM5	241	88%	244	89%	729	100%	Genotype6

EG.H: *E. granulosus* Human, EG.S: *E. granulosus* Sheep, EG.C: *E. granulosus* Cattle, EG.CM: *E. granulosus* Camel

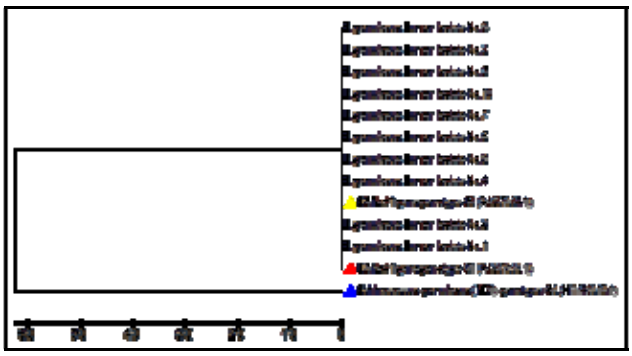

Figure 1: Phylogenetic tree analysis based on *nad1* gene partial sequence that used for *E. granulosus* genotyping detection of human isolates. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version).

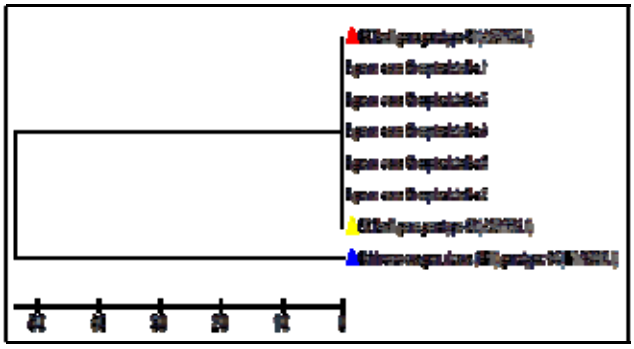

Figure 2: Phylogenetic tree analysis based on *nad1* gene partial sequence that used for *E. granulosus* genotyping detection of sheep isolates. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version).

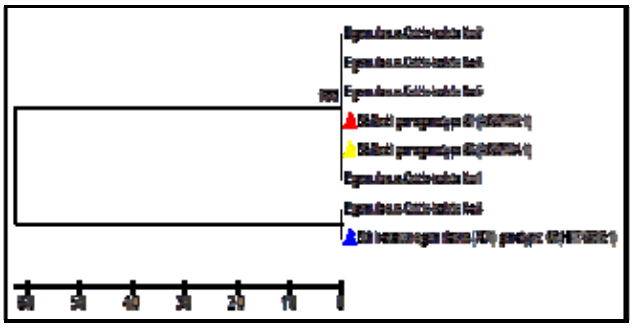

Figure 3: Phylogenetic tree analysis based on *nad1* genes partial sequence that used for *E. granulosus* genotyping detection of cattle isolates. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version).

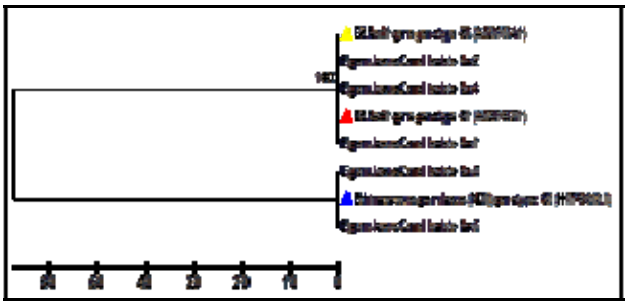

Figure 4: Phylogenetic tree analysis based on *nad1* genes partial sequence that used for *E. granulosus* genotyping detection of camel isolates. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version).

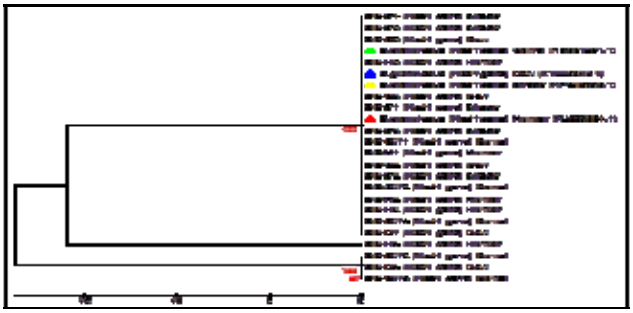

Figure (5): Phylogenetic tree analysis based on *nad1* gene partial sequence that used for *E. granulosus* host relationship study. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). E.g = *E. granulosus*, H = human, S = sheep, C = cattle, CM = camel, 1-5 samples numbers.

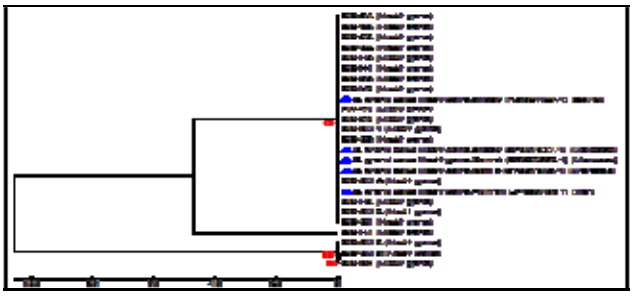

Figure (6): Phylogenetic tree analysis based on *nad1* genes partial sequence that used for *E. granulosus* genotyping detection relationship to other relative countries. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). E.g = *E. granulosus*, H = human, S = sheep, C = cattle, CM = camel, 1-5 samples numbers.

Discussion

Genotyping of *E. granulosus* is the initial step in detection of parasite and controlling its virulence then minimize the infection by genotype determination. The results showed there are three common genotypes existed in Iraq depending on *nad1* gene sequencer analysis, which are: sheep strain (G1); buffalo strain (G3) and camel strain (G6). Two strains were existed in human isolates which are: sheep strain (G1) and buffalo strain (G3); and the most predominant genotype that infects Iraqi people was G3 (80%).

The results mismatched the previous studies in Iraq such as (20) who detected G1 in human and animals where the result showed 100% matching sheep strain (G1), also (21) detected G1 strain in all human isolates in Turkey. Anyway, present result agreed with (16) in Italy; (22) in Romania and (19,23) in Iran. This may be attributed to the study encircled by a determinant such as the reality of high risk of surgery in such infections; many people prefer to visit more developed hospitals and might necessity to involvement a wide provinces in Iraq for accurate genotypes diagnoses. Two strains were existed in sheep isolates which are: sheep strain (G1) and buffalo strain (G3) depending on *nad1* gene; G1 was the predominant (80%) genotype that infects sheep in Iraq. The result matches most of previous studies in Iraq such as (20,24) that sequenced *nad1* gene and (25) who detected G1, G3 and G7 genotypes in Turkey. The reason may attribute to sheep obviously sensitive to sheep strain (G1) of *E. granulosus*; shortest life span of parasite depending on sheep slaughtering age in compare to other animals and the hydatid cysts in this intermediate host being predominantly fertile so the sheep is essential source of echinococcosis in dogs (26).

Three strains were existed in cattle and camels isolates which are: sheep strain (G1); buffalo strain (G3) and camel strain (G6) depending on *nad1* gene. The G1 was the predominant (60%) genotype in cattle; the result matched (20,24) who detected G1 in all cattle isolates in Iraq whereas (27) detected G3 in all Indian livestock. G3 and G6 are the predominant genotypes in camel's isolates that which mismatched to the study of (28) in Tunisia who detected most of camels infected with sheep strain (G1) whereas (29) detected G1, G3, and G6 in camels isolates in Iran.

Phylogenetic analysis of Iraqi isolates showed that hydatid cysts were produced by G3, G1 and G6 respectively. This study indicated that commonly circulating genotype which cause hydatid cystic disease in Iraq was buffalo strain (G3) in general, but the result mismatched to (24) who referred to that G1 is the common genotype in Iraqi sheep, cattle and camels.

The commonest of G3 strain infectivity may be due to its highly fertility that aid in infects not typical host rather than other strains as proved by (27); additionally Al-Qadisiyah is agricultural province and existence of buffalo's breeders and settle down in such province then enhancement of buffalo strain to transmission easily to human and animals; likewise G6 could not be predominant strain mostly attributed to the fact of little use of camel's meat, thus providing little or no access for dog to camel carcass.

The result of phylogenetic analysis that based on *nad1* gene revealed there is close relationship between strains that infects human, sheep, cattle and camels; so each strain do not specific to infects one host without another.

Results of this study indicated that G1 genotype detected in ten (40%) isolates and could be infective for human, sheep, cattle and camels that agreed with (25,30-32). Bowles (33) explained when cattle were infected with sheep strain (G1) it be considered as accidental host and the cyst mostly infertile so they do not agreed with the present study. G3 strain represented of most of isolates (twelve isolates 48%). Studies on G3 strain revealed this genotype is most predominant strain in human, sheep, cattle and camels as reported by (34-36); but Grosso (37) disagreed with the current study and explained that G3 has no susceptibility among human. Pednekar (27); Sharbatkhori (29); Rostami (38) investigated that G3 genotype was detected in sheep, cattle and camels and it is the predominant strain in Iranian cattle and camels, while Capuano (39) detected G1 in Italy in most of buffalo's isolates, so G3 rarely infected its typical host.

G6 strain composed of few isolates (only three isolates 12%); the study agreed with the previous investigation in some points; such study of Fasihi-Harandi (40) that used PCR-RFLP method on the internal transcribed spacer (*Its1*) region and reviewed that camels' strains have a cross-transmitted between human, sheep, cattle and camels. Sadjjadi (1); McManus and Thompson (18) and McManus (41) also detected that G6 related to infection of studied hosts with hydatidosis; while (34) identified G6 in human isolates; furthermore (42,43) recorded G6 in sheep and cattle samples while recorded in camels also in addition to sheep and cattle by (44). In Egypt, all human, sheep, buffalo and camels isolates indicated to presence of G6 strain (45,46). The mitochondrial genes have more power than nuclear genes in reconstruction of the phylogenetic relationship among closely related species due to their rapid sequence evolution (44).

Demonstrated of G3 was not exclusively infect its typical host (buffalo), but it can be considerably ingested by human and other animals. Higher frequency of strains with G3 genotype compared with other reports is of great concern that suggested human, cattle and camels as a new appropriate host for G3 genotype. G1, G3 and G6 have a possibility to transmission between livestock and human (47).

Relationship of detected genotypes in Iraq with others of relative countries indicated that the phylogenetic analysis of *nad1* gene revealed that some of sheep, cattle and camels isolates related to Egyptian, Iranian and Moroccan isolates whereas other local isolates considered as unique isolates due to it is out of tree. Sheep strain is the worldwide predominant genotype among extent of intermediate hosts (48). In Ilam province, Iran, G1 have been detected by isolation of DNA from protoscolices of human, sheep and cattle hydatid cysts (49). G1 is the predominant genotype in Turkey; it is essential agent in human and animal hydatidosis as reported by (50); majority of sheep and cattle were infected with G1 strains in different Turkish regions (30). In Greece, G1 have been detected in sheep isolates (51).

Sadjjadi (1); Bardonnet (52) and Azab (53) detected in Africa and Middle East the sheep strain in human and animals, additionally common circulating genotype in Tunisia and Libya was G1 which be identified in camels also as investigated by (28, 54). Zhong (55) collected of 45 hydatid cysts belongs to human and sheep in China and detected G1 in all samples when he used *cytb* gene; in contrast to Grosso (37) who could be find G1 and G6 as the predominant strains that infects human.

Buffalo strain (G3) detected in human and domestic animals in Iran whereas G1 was the common infective strain in them as reported by (23); while Sharbatkhouri (29) identified buffalo strains as a predominant in dromedaries that matched the present investigation.

G6 have been detected in human and animal hydatidosis in Iran (1,29,40) and in Sub-Saharan Africa where as it is proven as predominant strain in Egypt (45,46).

The concourse of these results with Iran and other lands may due to reality of these countries are neighboring to Iraq and shares borders overlapping naturally in addition to these countries specialized in breeding different species of animals to each other; therefore the pathogenic strains in human and animals are the same in these countries and this is analogous to that proven by in addition to there are no studies in other surrounding countries involving genotyping and phylogenetic analysis (21,56).

References

1. Sadjjadi SM. Present situation of echinococcosis in the Middle East and Arabic North Africa. Parasitol Int. 2006;55:197-202.
2. Roberts LS, Janovy JJ, Schmidt GD. Cestoidea: Form, Function, and Classification of Tapeworms. In Roberts LS, Janovy JJ. (eds.) Foundations of Parasitology, 8th ed.) New York: McGraw-Hill. 2009:pp. 313-340.
3. King, CH, Fairley JK. Cestodes (Tapeworms). In: Mandell GL, Douglas RG, Bennett A. eds. Principles and Practice of Infectious Diseases. 7th ed. Philadelphia, PA: Elsevier Inc, Ch. 2010:pp.3607-3617.
4. Rigano R, Profumo E, Bruschi F, Carulli G, Azzara A, Ioppolo S, Buttari E, Ortona P, Teggi A, Siracusano A. Modulation of human immune response by Echinococcus granulosus antigen B and its possible role in evading host defense. Infect Immun. 2001;69(1):288-296.
5. Eckert J, Deplazes P. Biological and clinical aspects of Echinococosis, A zoonosis of increasing concern. Clin Microbiol. 2004;17(1):107-135.
6. Oku Y, Malgor R, Benavidez U, Carmona C, Kamiya H. Control program against hydatidosis and the decreased prevalence in Uruguay. Interna Cong Series. 2004;18(13):1425-1437.
7. Al-Mutaywiti YSS. Effect of lipopolysaccharide extracted from Escherichia coli in immune response of BALB/c mice against fection with secondary hydatid disease. M.Sc. Thesis, College of Education/Baghdad University. 2005.
8. Eckert J, Deplazes P, Craig PS, Gemmell MA, Gottstein B, Heath D, Jenkins DJ, Kamiya M, Lightowlers M. Echinococcosis in animals: Clinical aspects, diagnosis and treatment. In: WHOI/OIE Manual on Echinococcosis in humans and animals: A public health problem of global concern. (Eds) Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, Paris, France. OIE and WHO. 2001:pp.72- 95.
9. Budke CM, Deplazes P, Torgerson PR. Global socioeconomic impact of cystic echinococcosis. Emerg Infec Dis. 2006;12: 296-303.
10. Ahmadi NA, Bodi F. Clinical presentation, localization and morphology of hepato-pulmonary hydatid cysts in patients operated in Tehran. World Appl Sci J. 2011;12(9):1544-1548.
11. Romig T. Epidemiology of echinococcosis. Langenbecks Arch Surg. 2003;388:209-217.
12. Torgerson PR, Dowling PM, Abo-Shehada MN. Estimating the economic effects of cystic echinococcosis. Part 3: Jordan, a developing country with lower-middle income. Ann Trop Med Hyg. 2001;95(6):595-603.
13. Kittelberger R, Reichel MP, Jenner J, Heath D, Lightowlers M W, Moro P, Ibrahem MM, Craig PS. O'Keefe JS. Evaluation of three enzyme linked immunosorbent assays (ELISAs) for the detection of serum antibodies in sheep infected with Echinococcus granulosus. Vet Parasitol. 2002;110(1-2):57-76.
14. Salih NE and AL-Jamain, A. DNA analysis of Echinococcus of human and sheep origin in Ninevah province, Iraq by PCR-RAPD technique. J Rivista di Parassitol. 2001;17(3):221-232.
15. Lavikainen A, Lehtinen MJ, Meri T, Hirvelä-Koski V, Meri S. Molecular genetic characterization of the Fennoscandian cervid strain, A new genotypic group (G10) of Echinococcus granulosus. Parasitol. 2003;127:207-215.
16. Busi, M, Snábel V, Varcasia A, Garippa G, Perrone V, De Liberato C, D'Amelio S. Genetic variation within and between G1 and G3 genotypes of Echinococcus granulosus in Italy revealed by multilocus DNA sequencing. Vet Parasitol. 2007;150:75-83.
17. Moks E, Jõgisalu I, Valdmann H, Saarma U. First report of Echinococcus granulosus G8 in Eurasia and a reappraisal of the phylogenetic relationships of 'genotypes' G5-G10. Parasitol. 2008;135:647-654.
18. McManus DP, Thompson RC. Molecular epidemiology of cystic echinococcosis. Parasitol. 2003;127:37-51.
19. Nikmanesh B, Mirhendi H, Ghalavand Z, Alebouyeh M, Sharbatkhori M, Kia EB, Mohebali M, Eghbali M, Rokni MB. Genotyping of Echinococcus granulosus isolates from human clinical samples based on sequencing of mitochondrial genes in Iran, Tehran. Iranian J Parasitol. 2014;9(1):20-27.
20. Hama AA, Mero WM, Jubrael JM. Molecular identification of Echinococcus granulosus (G1) strain in human and animals. J of university of Zakho (JOUZ). 2013;1(1):1-6.
21. Ergin S, Saribas S, Yuksel P, Zengin K. Midilli K, Adas G, Arikan S, Aslan M, Uysal H, Caliskan R, Oner A, Kucukbasmaci O, Kaygusuz A, Torun MM, Kocazeybek B. Genotypic characterization of Echinococcus granulosus isolated from human in Turkey. Afri J Microbiol Res. 2010;4(7):551-555.
22. Piccoli L, Bazzocchi C, Brunetti E, Mihailescu P, Bandi C, Mastalier B, Cordos I, Beuran M, Popa LG, Meroni V, Genco F, Cretu C.

Molecular characterization of Echinococcous granulosus in South-Eastern Romania: Evidence of G1-G3 and G6-G10 complexes in humans. Clin Microbiol Infect. 2013;19(6):578-582.
23. Pezeshki A, Akhlaghi L, Sharbatkhori, M, Razmjou E, Oormazdi H, Mohebali M, Meamar AR. Genotyping of Echinococcus granulosus from domestic animals and humans from Ardabil province, northwest Iran. J Helminthol. 2012;10:1-5.
24. Al-Fatlawi MAA. The diagnosis of Echinococcus granulosus strain in sheep, cattle and camel in Al-Qadisyiah governorate. Al-Qadisyiah J Vet Med Sci. 2006;2(5):53-60.
25. Snabel V, Altıntas N, D'Amelio S, Nakao M, Romig T, Yolasıgmaz A, Günese K, Türk M, Busi M, Hüttner M, Sevcova D, Ito A, Dubinsky P. Cystic Echinococcosis in Turkey: Genetic Variability and First Record of the Pig Strain (G7) in the Country. Parasitol Res. 2009;105:145-154.
26. Rahimi H, Sadjjadi SM, Sarkari B. Performance of antigen B isolated from different hosts and cyst locations in diagnosis of cystic Echinococcosis. Iranian J Parasitol. 2011;6:12-19.
27. Pednekar RP, Gatne ML, Thompson RC, Traub RJ. Molecular and morphological characterization of Echinococcus from food producing animals in India. Vet Parasitol. 2011;165:58-65.
28. Lahmar S, Debbek H, Zhang LH, McManus DP, Souissi A, Chelly S, Torgerson PR. Transmission dynamics of the Echinococcus granulosus sheep-dog strain (G1 genotype) in camels in Tunisia. Vet Parasitol. 2004;121:151–156.
29. Sharbatkhori M, Fasihi-Harandi M, Mirhendi H, Hajialilo E, Kia EB. Sequence analysis of cox1 and nad1 genes in Echinococcus granulosus G3 genotype in camels (Camelus dromedarius) from central Iran. Parasitol Res. 2011;108(3):521-527.
30. Vural G, Baca AU, Gauci, C G, Bagci O, Gicik Y, 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;154:347-350.
31. Simsek S, Balkaya I, Koroglu E. Epidemiological survey and molecular characterization of Echinococcus granulosus in cattle in an endemic area of eastern Turkey. Vet Parasitol. 2010;172:347-349.
32. Gholami Sh, Sosari M, Fakhar M, Sharif M, Daryani A, Hashemi M, Vahadi M. Molecular characterization of Echinococcus granulosus from Hydatid cysts isolated from human and animals in Golestan province, north of Iran. Iranian J Parasitol. 2012;7(4):8-16.
33. Bowles J, Blair D, McManus DP. A Molecular phylogeny of the genus Echinococcus. Parasitol. 1995;110:317-328.
34. Simsek S, Balkaya I, Ciftci AT, Utuk, AE. Molecular discrimination of sheep and cattle isolates of Echinococcus granulosus by SSCP and conventional PCR in Turkey. Vet Parasitol. 2011;178:367-369.
35. Singh BB, Sharma JK, Ghatak S, Sharma R, Bal MS, Tuli A, Gill JP. Molecular epidemiology of Echinococcosis from food producing animals in north India. Vet Parasitol. 2012;186(3-4):503-506.
36. Sharma M, Sehgal R, Fomda BA, Malhotra A, Malla N. Molecular Characterization of Echinococcus granulosus Cysts in North Indian Patients: Identification of G1, G3, G5 and G6 Genotypes. PLOS Neglec Trop Dis. 2013;7(6):1-6.
37. Grosso G, Gruttadauria S, Biondi A, Marventano S, Mistretta A. Worldwide epidemiology of liver hydatidosis including the Mediterranean area. World J Gastroenterol. 2012;18(13):1425-1437.
38. Rostami S, Talebi S, Babaei Z, Sharbatkhori M, Ziaali N, Rostami H, Harandi MF. High resolution melting technique for molecular epidemiological studies of cystic Echinococcosis: differentiating G1, G3, and G6 genotypes of Echinococcus granulosus ensu lato. Parasitol Res. 2013;112(10):3441-3447.
39. Capuano F, Rinaldi L, Maurelli MP, Perugini AG, Veneziano V, Garippa G, Genchi C, Musella V, Cringoli G. Cystic Echinococcosis in water buffaloes: Epidemiological survey and molecular evidence of ovine (G1) and buffalo (G3) strains. Vet Parasitol. 2006;137:262-268.
40. Fasihi-Harandi M, Hobbs RP. Adams PJ, Mobedi I, Morgan-Ryan UM, Thompson RC. Molecular and morphological characterization of Echinococcus granulosus of human and animal origin in Iran. Parasitol. 2002;125:367-373.
41. McManus DP. The Molecular epidemiology of Echinococcus granulosus and cystic hydatid disease. Trans R Soc Trop Med Hyg. 2002;96(Suppl.1):151e7.
42. Sharbatkhori M, Mirhendi H, Jex AR, Pangasa A, Campbell BE, Kia EB, Eshraghian MR, Harandi MF, Gasser RB. Genetic categorization of Echinococcus granulosus from humans and herbivorous hosts in Iran using an integrated mutation scanning phylogenetic approach. Electrophoresis. 2009;30:2648–2655.
43. Kia EB, Rahimi H, Sharbatkhori M, Talebi A, Fasihi-Harandi M, Mirhendi H. Genotype identification of human cystic Echinococcosis in Isfahan, central Iran. Parasitol Res. 2010;107:757-760.
44. Rostami-Nejad M, Taghipour N, Nochi Z, Mojarad EN, Mohebbi SR, Fasihi-Harandi M, Zali MR. Molecular identification of animal isolates of Echinococcus granulosus from Iran using four mitochondrial genes. J Helminthol. 2012;86:485-492.
45. Khalifa NO, Khater HF, Fahmy HA, Radwan MEI. A fifty JSA genotyping and phylogenetic analysis of cystic Echinococcosis isolated from camels and humans in Egypt. Amer J Epidemiol Infect Dis. 2014;2(3):74-82.
46. Amer SM, Helal IB, Kamau E, Feng Y, Xiao L. Molecular characterization of Echinococcus granulosus Sensu lato from farm animals in Egypt. PLoS One. 2015;10(3):1-12.
47. Romig T, Dinkel A, Mackenstedt U. The present situation of echinococcosis in Europe. Parasitol Int. 2006;55:187-191.
48. Craig PS, Rogan MT, Campos-Ponce M. Echinococcosis: disease, detection and transmission. Parasitol. 2003;127(Suppl.): S5-S20.
49. Dousti M, Abdi J, Bakhtiyari S, Mohebali M, Mirhendi Sh, Rokni MB. Genotyping of hydatid cyst isolated from human and domestic animals in Ilam province, Western Iran Using PCR-RFLP. Iranian J Parasitol. 2013;8(1):47-52.
50. Eryıldız C, Şakru N. Molecular characterization of human and animal isolates of Echinococcus granulosus in the Thrace region, Turkey. Balkan Med J. 2012;29:261-267.
51. Varcasia A, Tosciri G, Pedes T, Pipia AP, Marrosu R, Scala A, Garippa G. Cystic Echinococcosis in Pigs and wild boars of Sardinia (Italy). Proc 6th International symposium on the mediterranean, ISSN 2035-4088. 2007;pp.11-13.
52. Bardonnet K, Benchikh-Elfegoun MC, Bart JM, Harraga S, Hannache N, Haddad S, Dumon H, Vuitton DA, Piarroux R. Cystic Echinococcosis in Algeria: cattle act as reservoirs of a sheep strain and May contribute to human contamination. Vet Parasitol. 2003;116(1):35-44.
53. Azab ME, Bishara SA, Helmy H, Oteifa NM, El- Hoseiny LM, Ramzy RM, Ahmed MA. Molecular characterization of Egyptian human and animal Echinococcus granulosus isolates b RAPD-PCR technique. J Egypt Soc Parasitol. 2004;34:83-96.
54. M'rad S, Filisetti D, Oudni M, Mekki M, Belguith M, Nouri A, Sayadi T, Lahmar S, Candolfi E, Azaiez R, Mezhoud H, Babba H. Molecular evidence of ovine (G1) and Camel (G6) strains of Echinococcus granulosus in Tunisia and putative role of cattle in human contamination. Vet Parasitol. 2005;129:267-272.
55. Zhong X, Wang N, Hu D, Wang J, Liu T, Gu X, Wang Shm, Peng X, Yang, G. Sequence Analysis of cytb Gene in Echinococcus granulosus from Western China. Korean J Parasitol. 2014;52(2):205-209.
56. Rostami-Nejad M, Roshani M, Lahmi F, Mojarad EN. Evaluation of four DNA extraction methods for the detection of Echinococcus granulosus Genotype 1. Gastroenterol Hepatol Bed Bench. 2011;4(2):91-94.