Gene-based molecular analysis of COX1 in Echinococcus granulosus cysts isolated from naturally infected livestock in Riyadh, Saudi Arabia

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Abstract

The diversity and importance of Echinococcus species in domesticated animals in Saudi Arabia are poorly understood. In this study, 108 singular (hydatid) cysts were collected from goats (n = 25), sheep (n = 56) and camels (n = 27). DNA was extracted from the protoscoleces of individual fertile cysts and used for polymerase chain reaction (PCR) amplification of mitochondrial subunit 1 of the cytochrome c oxidase 1 (cox1) gene. Amplicon sequencing results revealed the presence of Echinococcus granulosus sensu stricto (s.s.) (genotypes G1–G3) in 16 of the 17 sheep cysts and 2 of the 27 camel cysts of these samples, 18 (2 camel and 16 sheep) were divided into genotypes G1, G2, and G3.

Introduction

Cystic echinococcosis (CE) is a prevalent zoonotic disease caused by parasitic infection [1]. CE is most widespread in rural areas with few to no hygiene facilities and poor living conditions where humans, dogs, and animals reside in close proximity [2]. The parasitic lifecycle includes eggs that are passed by definitive hosts (canids) harboring adult worms. The eggs subsequently develop to reach the cystic stage after ingestion by an intermediate host [3]. CE affects millions of people worldwide and is highly endemic in Mediterranean coastal regions, South America, Eastern Europe, the Middle East, East Africa, China, Central Asia, and Russia [4]. The disease is also estimated to cause yearly financial losses of several billion dollars in domesticated animals. This is partly the result of low eradication rates, mortality in infected animals, and the need to discard the contaminated organs of slaughtered animals [5]. Genetic examinations of hydatid cysts in numerous geographical areas have led to the discovery of ten genotypes: G1–G10 [6–9]. Echinococcus granulosus sensu stricto (s.s.) (G1–G3), Echinococcus ortleppi (G5), Echinococcus equinus (G4) and Echinococcus canadensis (G6–G10) [10, 11]. Although the status of genotype G9
remains uncertain [12], it may be similar to the genotype found in pigs (G7) [13]. The *E. granulosus* s.s. G1 genotype (found in sheep) has been implicated in numerous human CE cases. In addition, human infections have been described for every genotype except G4 [13,14]. A phylogenetic examination of the *cytochrome c oxidase 1* (*cox1*) gene indicated that the primary strains observed in sheep (G1) and buffalo (G3) cycle among domesticated animals and have adapted to goats, camels and cattle. Human infections have been linked to the G1 basic sheep genotype of *E. granulosus*, suggesting that these strains are highly capable of engaging in zoonotic exchange [15]. Better characterization of *Echinococcus* species may improve the development and advancement of control measures, indicative tests, and treatment options [11,16]. Studies performed in Saudi Arabia have investigated the general prevalence of *Echinococcus* [17–20], although little information is available regarding the zoonotic potential of this parasite. In the present study, we analyzed hydatid cysts using a polymerase chain reaction (PCR) sequencing strategy to evaluate the mitochondrial *cox1* gene in domesticated animals (sheep and camels) in Saudi Arabia with the aim of expanding what is known about *E. granulosus*.

**Materials and methods**

**Ethics statement**

The Institutional Committee of Post-graduate Studies and Research at King Saud University (Saudi Arabia) approved this study. Hydatid cysts were collected by veterinarians during post-mortem inspections of slaughtered animals performed at the Al-Sada Abattoir in Riyadh, Saudi Arabia, in April and October 2016. Official approval of the use of hydatid cysts (for research purposes only) was obtained from the university as well as the abattoir veterinarians.

**Sample collection**

A total of 108 hydatid cysts were collected from sheep (n = 56), goats (n = 25) and camels (n = 27). Each cyst was considered an isolate, and all cysts were isolated from the liver. To determine whether the cysts were fertile, the contents of each cyst were aseptically aspirated and dispensed into sterile Petri dishes, and the presence of protoscoleces (fertile cysts) was visually determined. Protoscoleces were specifically collected from single fertile cysts under nuanced aseptic conditions and subsequently washed as many as three times using a sterile saline solution before being fixed in 95% ethanol.

**DNA extraction and PCR amplification**

Protoscoleces obtained from cysts were washed with distilled water and ethanol before they were centrifuged. Genomic DNA (gDNA) was then extracted using a High Pure PCR Template Preparation Kit (Qiagen GmbH, Hilden, Germany, Cat. No.51304). The mitochondrial *cox1* gene was amplified with the reverse primer 5’ - TAAAGAAAGAACATAATGAAAATG-3’ [6] and the forward primer 5’ - TTTTTTGGGCATCCTGAGGTTAT-3’ in a 40-μl reaction mixture containing 8 μl of master mix, 25.6 μl of deoxynucleotides (dNTPs), 2.4 μl of primers and 4 μl of DNA template. The PCR program consisted of an initial denaturation step at 94˚C for 5 minutes, followed by 40 cycles of denaturation at 94˚C for 45 seconds, annealing at 50˚C for 45 seconds, and extension at 72˚C for 10 minutes, and a final extension step at 72˚C for 10 minutes. The PCR products were analyzed by 1% agarose gel electrophoresis.

**DNA sequencing and phylogenetic analysis**

The sequences of the forward strands were aligned using ClustalW [11] implemented in Genious software version 10.0.7 [21]. Multiple sequence alignment was performed once for each
group of samples (camel, sheep, and a combination of all samples). Multiple sequence align-
ment included the genotypes G1, G2, and G3, and G1 was set as the reference sequence. Low-
quality sequence ends were trimmed to obtain better results. A phylogenetic tree was generated
from the trimmed sequences obtained in this study in addition to standard sequences for *E.
granulosus* genotypes (G1–G10) and other *Echinococcus* species. *Taenia saginata* was used as
the out-group (Table 1). The neighbor-joining method [22] was used with the Tamura Nei
model to generate the phylogenetic tree. The bootstrap method was used for resampling with
the number of replicates set to 1000.

| Haplotype, genotype or species | Host | Accession number (cox1) | Reference |
|--------------------------------|------|-------------------------|-----------|
| Echs1                          | Sheep| -                       | The present study |
| Echs2                          | Sheep| -                       | The present study |
| Echs3                          | Sheep| -                       | The present study |
| Echs4                          | Sheep| -                       | The present study |
| Echs5                          | Sheep| -                       | The present study |
| Echs6                          | Sheep| -                       | The present study |
| Echs7                          | Sheep| -                       | The present study |
| Echs8                          | Sheep| -                       | The present study |
| Echs9                          | Sheep| -                       | The present study |
| Echs10                         | Sheep| -                       | The present study |
| Echs11                         | Sheep| -                       | The present study |
| Echs12                         | Sheep| -                       | The present study |
| Echs13                         | Sheep| -                       | The present study |
| Echs14                         | Sheep| -                       | The present study |
| Echs15                         | Sheep| -                       | The present study |
| Echs16                         | Sheep| -                       | The present study |
| Echs17                         | Sheep| -                       | The present study |
| Echs18                         | Sheep| -                       | The present study |
| Echc1                          | Camel| -                       | The present study |
| Echc2                          | Camel| -                       | The present study |
| G1                             | Sheep| U50464                  | [23]       |
| G2                             | Sheep| M84662                   | [6]        |
| G3                             | Buffalo| M84663                  |           |
| G4                             | Horse | M84664                   |           |
| G5                             | Cattle| M84665                   |           |
| G6                             | Camel | M84666                   |           |
| G7                             | Pig   | M84667                   |           |
| G8                             | Moose | AB235848                 | [10]       |
| G10                            | Reindeer| AF525457                | [9]        |
| *E. multilocularis*            | Human | M84668                   | [6]        |
| *E. multilocularis*            | Rodent| M84669                   | [6]        |
| *E. vogeli*                    | Rodent| AB208064                 | [10]       |
| *E. shiquicus*                 | Pika  | M84670                   | [6]        |
| *E. oligarthrus*               | Rodent| M84671                   | [6]        |
| *E. felidis*                   | Lion  | EF58356                  | [24]       |
| Outgroup: *Taenia saginata*    | Cattle| AB465239                 | [25]       |

https://doi.org/10.1371/journal.pone.0195016.t001
Results
Hydatid cyst collection
After the collected cysts were assessed, 17 of the 56 cysts extracted from sheep and 2 of the 27 cysts collected from camels were found to be fertile. All 25 cysts derived from goats were infertile. PCR amplification and DNA sequencing were performed for all fertile cysts. Unfertile cysts were not processed for molecular analysis.

Amplification of the mitochondrial cox1 gene
A 446-bp fragment of the cox1 gene was PCR amplified from gDNA extracted from each of the fertile hydatid cysts (Figs 1 and 2).

Camel samples. A portion of the multiple sequence alignment of camel samples Echc1 and Echc2 with genotypes G1, G2, and G3 is shown in Fig 3. We found a one-nucleotide substitution (C to T) in one sample at position 69 of the reference sequence. Fig 4 shows the phylogenetic tree of the camel samples and reference sequences. The results indicate that G1 was the only genotype found in the camel samples. Table 2 shows the genetic distance matrix.

Sheep samples. A portion of the multiple sequence alignment of the 17 sheep samples with genotypes G1, G2, and G3 is shown in Fig 5. We found the following one-nucleotide substitutions: in 7 samples, C to T at position 69 of the reference sequence (SNP analysis indicates that this change is a transition SNP); in 1 sample, C to T at position 79; in 1 sample, A to T at position 98; in 1 sample, G to T at position 100; in 1 sample, T to G at position 101; in 1 sample, C to T at position 105; in 1 sample, C to T and C to A at position 124; and in 2 samples, T to C at position 270. Fig 6 shows the phylogenetic tree of the sheep samples and reference sequences. The two samples, Echs15 and Echs16, formed a clade with bootstrap support of 96.9%. The pair Echs2 and Echs6 also formed a clade with bootstrap support of 94.6%. Similarly, Echs9 and Echs11 formed a clade with bootstrap support of 85.2%. Echs1 and Echs13 formed a
clade with bootstrap support of 73.7%. *Echs4* and *Echs7* formed a clade with bootstrap support of 71%.

Overall, the results grouped 16 out of the 17 samples in a clade with genotypes G1, G2, and G3, with bootstrap support of 99.7%. Sample *Echs18* seemed less related to the other sheep sequences and more related to the outgroup.

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**Fig 2.** Agarose gel (1.0%) electrophoretogram with 100-bp DNA ladder. PCR analysis of the *cox1* gene revealed a 446-bp band derived from different camel hydatid cyst isolates (2, 3).

https://doi.org/10.1371/journal.pone.0195016.g002

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**Fig 3.** Multiple sequence alignment of two camel samples and genotypes G1, G2, and G3.

https://doi.org/10.1371/journal.pone.0195016.g003
Sheep and camel samples. The multiple sequence alignment of the sheep and camel samples with genotypes G1, G2, and G3 is shown in Fig 7. We found the following one-nucleotide substitutions: C to T at position 69 of the reference sequence (SNP analysis indicates that this change is a transition SNP appearing in 8 of the samples, including one camel sample); C to T at position 79; A to T at position 98; G to T at position 100; T to G at position 101; C to T at position 105; and T to C at position 270. Fig 8 shows the phylogenetic tree of the sheep samples and reference sequences. Two sheep samples, Echs1 and Echs13, formed a clade with camel sample Echc1, with bootstrap support of 85.2%. Overall, the results grouped 18 of the samples (2 camel and 16 sheep) in a clade with G1, G2, and G3, with bootstrap support of 98.7%.

Discussion

The results of this study show that as many as three separate genotypes of *E. granulosus*, including G1, G2, and G3, are present in Saudi Arabia and that they are frequently found in...
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Fig 5. Multiple sequence alignment of sheep samples and genotypes G1, G2, and G3.

https://doi.org/10.1371/journal.pone.0195016.g005

Fig 6. Phylogenetic tree of the sequences of 17 sheep samples and reference sequence obtained from previous studies.

https://doi.org/10.1371/journal.pone.0195016.g006
sheep, cattle, and buffalo, respectively. These findings are similar to those of studies performed in Iran suggesting that G1 remains the most prevalent *E. granulosus* genotype in livestock [26].

**Fig 7.** Multiple sequence alignment of sheep and camel samples and reference sequences obtained from previous studies.

https://doi.org/10.1371/journal.pone.0195016.g007

**Fig 8.** Phylogenetic tree of all samples and reference sequences obtained from GenBank.

https://doi.org/10.1371/journal.pone.0195016.g008
and that G2 is the second most common genotype in both cattle and sheep [27,28]. A study performed in Turkey assessed nearly 208 isolates (19 cattle, 179 sheep, 7 goats, 1 dog and 1 camel) and detected only the G1 genotype. However, that study mainly utilized PCR-based restriction fragment length polymorphism (PCR-RFLP) targeted to the internal transcribed spacer (ITS)-1 region of ribosomal DNA and performed a cox1 sequence analysis for only a handful of isolates [29]. Genotype G3 of *E. granulosus* has been isolated from cattle, buffalo and sheep in Turkey, Italy, India and Chile [30–34], and studies worldwide have demonstrated many similarities between the distributions of genotypes G1 and G3. For example, in southern Italy, an investigation of 48 wild water oxen detected G3 in 31.25% and G1 in 68.75% of CE cases [35]. Other studies have reported comparable patterns with varying proportions of G1 and G3. For example, in a study of 112 cattle and sheep performed in Turkey, 95.5% of CE cases were G1, while 4.5% were G3 [36]; in a study of 30 cows, sheep and humans performed in Tunisia, 93.3% of CE cases were G1, and 6.7% were G3 [37]; in a study of 80 cattle and water buffaloes performed in Italy, 78.75% of CE cases were G1, and 12.5% were G3 [30]; in a study of 38 animals performed in southeastern Iran, 73.7% of CE cases were G1, and 13.2% were G3 [38]; and in a study of 18 humans and dogs performed in southern Brazil, 77.8% of CE cases were G1, and 11.1% were G3 [39].

Another study identified four different *E. granulosus* genotypes, G1, G2, G3, and G5, in 46 household animals in India [40]. Of these genotypes, G3 was the most prevalent, accounting for 63% of the CE cases, whereas G1 was observed in only six (13%) cases. Furthermore, a unique situation was observed in a study of 19 different hydatid cyst samples extracted from camels in central Iran. In that study, the nad1 and pcox1 genes were sequenced, and the G3 genotype was identified in 42.1%, the G6 genotype in 31.6% and the G1 genotype in 26.3% of the cases [41]. The camel genotype clearly differs from the genotypes found in other domestic animals throughout numerous regions in Iran. While only the G6 genotype has been found in some African countries [42–44], no G6 isolates were identified in the current study. Several studies based on cox1 analysis have also demonstrated different haplotypes within genotypes G3 and G1 in various hosts [34, 45–47]. For example, one study of 112 cattle and sheep hydatid cyst isolates (in Turkey) identified 5 G3 isolates, 107 G1 isolates, and 5 unique haplotypes [37]. In the present study, 18 samples (2 camel and 16 out of 17 sheep) were grouped with genotypes G1, G2, and G3, and these isolates were compared with other genotypes. The horizontal branches in a phylogenetic tree indicate genetic distances (i.e., the amount of genetic change), while longer horizontal branches are associated with greater divergence. Our analysis of the combined sheep and camel samples revealed the following one-nucleotide substitutions: C to T at position 69, C to T at position 79, A to T at position 98, G to T at position 100, T to G at position 101, C to T at position 105, and T to C at position 270 of the reference sequence. The substitution at position 69 of the reference sequence was identified in 8 of the samples, including one camel sample. A phylogenetic tree constructed using all the isolates (Fig 8) showed that the G1, G2 and G3 genotypes comprise a deeply related complex that is distinct from other genotypes (G4 to G10), as previously described by other investigators [35,47,48]. Because most of the sheep used by humans in Saudi Arabia are imported from Sudan, these animals are the likely source of the *E. granulosus* s.s. observed in Saudi Arabia. These harmful cysts are economically important because of their impact on animal health, and the findings of the present work are therefore valuable because they establish the exact genotypes present in each species; this will enable appropriate preventative measures and therapeutic strategies to be implemented in the various animal populations affected by CE. Obtaining additional isolates from other hosts, such as humans and stray canines, and from other geographic areas may be necessary to increase our understanding of the distribution of CE in Saudi Arabia.
Supporting information

S1 Dataset. All samples in the present study provided in two formats: FASTA and chromatogram.
(ZIP)

Acknowledgments

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research through the Research Group project no. RG-1439-034.

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References

1. Faraji R, Javadi GR, Barshahi PM, Ahmadian F, Sarebanhassanabadi M, Firoozabadi AD, et al. Prevalence of Hydatid Cyst in Slaughtered Livestock in Kermanshah (West Iran). Advances in Microbiology. 2015 Apr 1; 5(04):252.
2. Elzein FE, Aljaberi A, Asiri S, Alghamdi A. Isolated Hydatid Cyst of the Kidney. World Journal of Nephrology and Urology. 2016 Mar 31; 5(1):16–9.
3. Eckert J, Gemmell MA, Meslin F, Pawlowski ZS. WHO/OIE manual on echinococcosis in Humans and Animals: A Public Health Problem of Global Concern. World Organisation for Animal Health (Office International des Epizooties) and World Health Organisation. 2001; 1–250.
4. Zhang W, Zhang Z, Wu W, Shi B, Li J, Zhou X, et al. Epidemiology and control of echinococcosis in central Asia, with particular reference to the People’s Republic of China. ActaTropica. 2015 Jan 31; 141:235–43.
5. Budke CM, Deplazes P, Torgerson PR. Global socioeconomic impact of cystic echinococcosis. Emerging infectious diseases. 2006 Feb; 12(2):296. https://doi.org/10.3201/eid1202.050499 PMID: 16494758
6. Bowles J, Blair D, McManus DP. Genetic variants within the genus Echinococcus identified by mitochondrial DNA sequencing. Molecular and biochemical parasitology. 1992 Sep 1; 54(2):165–73. PMID: 1435857
7. Bowles J, Blair D, McManus DP. Molecular genetic characterization of the cervid strain (‘northern form’) of Echinococcus granulosus. Parasitology. 1994 Aug; 109(2):215–21.
8. Scott JC, Stefaniak J, Pawlowsk i ZS, McManus DP. Molecular genetic analysis of human cystic hydatid cases from Poland: identification of a new genotypic group (G9) of Echinococcus granulosus. Parasitology. 1997 Jan; 114(1):37–43.

9. Lavikainen A, Lehtinen MJ, Meri T, Hirveiä-Koski V, Meri S. Molecular genetic characterization of the Fennoscandian cervid strain, a new genotypic group (G10) of Echinococcus granulosus. Parasitology. 2003 Sep; 127(3):207–15.

10. Nakao M, McManus DP, Schantz PM, Craig PS, Ito A. A molecular phylology of the genus Echinococcus inferred from complete mitochondrial genomes. Parasitology. 2006 May; 134(5):713–22.

11. Thompson RA, McManus DP. Towards a taxonomic revision of the genus Echinococcus. TRENDS in Parasitology. 2002 Oct 1; 18(10):452–7. PMID: 12377596

12. Kedra AH, Swiderski Z, Tkach V, Dubinsky P, Pawlowski Z, Stefaniak J, et al. Genetic analysis of Echinococcus granulosus from humans and pigs in Poland, Slovakia and Ukraine. A multicenter study. Acta-Parasitologica. 1999; 44(4):248–54.

13. Rojas CA, Romig T, Lightowlers MW. Echinococcus granulosus sensu lato genotypes infecting humans–review of current knowledge. International Journal for Parasitology. 2014 Jan 31; 44(1):9–18. https://doi.org/10.1016/j.ijpara.2013.08.008 PMID: 24269720

14. Farhadi M, Fazaeli A, Haniloo A. Genetic characterization of livestock and human hydatid cyst isolates from northwest Iran, using the mitochondrial cox1 gene sequence. Parasitology research. 2015 Dec 1; 114(12):4363–70. https://doi.org/10.1007/s00436-015-4673-y PMID: 26280086

15. Latif AA, Toulah FH, El-Shafei AA, Alsolami MN. Prevalence of hydatidosis among slaughtered animals in Jeddah, Kingdom of Saudi Arabia. Journal of the Egyptian Society of Parasitology. 2012 Dec; 42(3):563–72. PMID: 23469631

16. McManus DP. Echinococcosis with particular reference to Southeast Asia. Advances in parasitology. 2010 Dec 31; 72:267–303. https://doi.org/10.1016/S0065-308X(10)72010-8 PMID: 20624535

17. Ibrahim MM. Study of cystic echinococcosis in slaughtered animals in Al Baha region, Saudi Arabia: interaction between some biotic and abiotic factors. ActaTropica. 2010 Jan 31; 113(1):26–33.

18. Pezeshki A, Akhlaghi L, Sharbatkhori M, Razmjou E, Oormazdi H, Mohebali M, et al. Genotyping of Echinococcus granulosus from domestic animals and humans from Ardabil Province, northwest Iran. Journal of helminthology. 2013 Dec; 87(4):387–91. https://doi.org/10.1017/S0022149X1200051X PMID: 23046636

19. Nikmanesh B, Mirhendi H, Ghavavand Z, Alebouyeh M, Sharbatkhori M, et al. Genotyping of Echinococcus granulosus isolates from human clinical samples based on sequencing of mitochondrial genes in Iran, Tehran. Iranian journal of parasitology. 2014 Mar; 9(1):20. PMID: 25642256
29. Utuk AE, Simsek S, Koroglu E, McManus DP. Molecular genetic characterization of different isolates of *Echinococcus granulosus* in east and southeast regions of Turkey. ActaTropica. 2008 Aug 31; 107 (2):192–4.

30. Casulli A, Manfredi MT, La Rosa G, Di Cerbo AR, Genchi C, Pozio E. *Echinococcus ortleppi* and *E. granulosus* G1, G2 and G3 genotypes in Italian bovines. Veterinary parasitology. 2008 Aug 1; 155 (1):168–72.

31. Simsek S, Kaplan M, Ozercan IH. A comprehensive molecular survey of *Echinococcus granulosus* in formalin-fixed paraffin-embedded tissues in human isolates in Turkey. Parasitology research. 2011 Aug 1; 109(2):411–6. https://doi.org/10.1007/s00436-011-2269-8 PMID: 21286751

32. Sharma M, Formda BA, Mazta S, Sehgal R, Singh BB, Malla N. Genetic diversity and population genetic structure analysis of *Echinococcus granulosus* sensu stricto complex based on mitochondrial DNA signature. PLoS One. 2013 Dec 9; 8(12):e82904. https://doi.org/10.1371/journal.pone.0082904 PMID: 24349394

33. Sharma M, Sehgal R, Formda BA, Malhotra A, Malla N. Molecular characterization of *Echinococcus granulosus* cysts in north Indian patients: identification of G1, G3, G5 and G6 genotypes. PLoS neglected tropical diseases. 2013 Jun 13; 7(6):e2262. https://doi.org/10.1371/journal.pntd.0002262 PMID: 23785531

34. Espinoza S, Salas AM, Vargas A, Freire V, Díaz E, Sánchez G, Venegas J. Detection of the G3 genotype of *Echinococcus granulosus* from hydatid cysts of Chilean cattle using COX1 and ND1 mitochondrial markers. Parasitology research. 2014 Jan 1; 113(1):139–47. https://doi.org/10.1007/s00436-013-3636-4 PMID: 24158646

35. Capuano F, Rinaldi L, Maurelli MP, Perugini AG, Veneziano V, Garippa G, et al. Cystic echinococcosis in water buffaloes: epidemiological survey and molecular evidence of ovine (G1) and buffalo (G3) strains. Veterinary parasitology. 2006 Apr 30; 137(3):262–8.

36. Vural G, Baca AU, Gauci CG, Bagci O, Glic Y, Lightowers MW. Variability in the *Echinococcus granulosus* cytochrome C oxidase 1 mitochondrial gene sequence from livestock in Turkey and a re-appraisal of the G1–3 genotype cluster. Veterinary parasitology. 2008 Jul 4; 154(3):347–50.

37. M’rad S, Oudni-M’rad M, Filisetti D, Mekki M, Nouri A, Sayadi T, et al. Molecular identification of *Echinococcus granulosus* in Tunisia: first record of the Buffalo strain (G3) in human and bovine in the country. The Open Veterinary Science Journal. 2010 Aug 24; 4(1).

38. Mario L, Takan K, Brochado JF, Costa CV, Soares AG, Yamano K, et al. Infection of humans and animals with *Echinococcus granulosus* (G1 and G3 strains) and *E. ortleppi* in Southern Brazil. Veterinary parasitology. 2011 Apr 19; 177(1):97–103.

39. Hajialilo E, Harandi MF, Sharbatkhori M, Mirhendi H, Rostami S. Genetic characterization of *Echinococcus granulosus* in camels, cattle and sheep from the south-east of Iran indicates the presence of the G3 genotype. Journal of helmintology. 2012 Sep; 86(3):263–70. https://doi.org/10.1017/S0022149X11000320 PMID: 21749740

40. Pednekar RP, Gatne ML, Thompson RA, Traub RJ. Molecular and morphological characterisation of *Echinococcus* from food producing animals in India. Veterinary parasitology. 2009 Oct 28; 165(1):58–65.

41. Sharbatkhori M, Harandi MF, 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. Parasitology research. 2011 Mar 1; 108(3):521–7. https://doi.org/10.1007/s00436-010-2092-7 PMID: 20922416

42. Bardonnet K, Piarroux R, Dia L, Schneeberger F, Gocci T, Godot V, et al. Combined eco-epidemiological and molecular biology approaches to assess *Echinococcus granulosus* transmission to humans in Mauritania: occurrence of the ‘camel’ strain and human cystic echinococcosis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2002 Jul 1; 96(4):383–6. PMID: 12497974

43. Maillard S, Benchikh-Effegoun MC, Knapp J, Bart JM, Koski P, Gottstein B, Piarroux R. Taxonomic position and geographical distribution of the common sheep G1 and camel G6 strains of *Echinococcus granulosus* in three African countries. Parasitology research. 2007 Feb 1; 100(3):495–503. https://doi.org/10.1007/s00436-006-0286-9 PMID: 17016727

44. Khalifa NO, Khater HF, Fahmy HA, Radwan ME, Alfify JS. Genotyping and phylogenetic analysis of cystic echinococcosis isolated from camels and humans in Egypt. American Journal of Epidemiology and Infectious Disease. 2014 Jan 23; 2(3):74–82.

45. Kamenetzky L, Gutierrez AM, Canova SG, Haag KL, Guarnera EA, Parra A, et al. Several strains of *Echinococcus granulosus* infect livestock and humans in Argentina. Infection, Genetics and Evolution. 2002 Dec 31; 2(2):129–36. PMID: 12797989

46. Haag KL, Ayala FJ, Kamenetzky L, Gutierrez AM, Rosenzvit M. Livestock trade history, geography, and parasite strains: the mitochondrial genetic structure of *Echinococcus granulosus* in Argentina. Journal of Parasitology. 2004 Apr; 90(2):234–9. https://doi.org/10.1645/GE-173R PMID: 15165043
47. Nakao M, Li T, Han X, Ma X, Xiao N, Qiu J, et al. Genetic polymorphisms of Echinococcus tapeworms in China as determined by mitochondrial and nuclear DNA sequences. International journal for parasitology. 2010 Mar 1; 40(3):379–85. https://doi.org/10.1016/j.ijpara.2009.09.006 PMID: 19800346

48. Abushewa MH, Abushhiwa MH, Nolan MJ, Jex AR, Campbell BE, Jabbar A, Gasser RB. Genetic classification of Echinococcus granulosus cysts from humans, cattle and camels in Libya using mutation scanning-based analysis of mitochondrial loci. Molecular and cellular probes. 2010 Dec 31; 24(6):346–51. https://doi.org/10.1016/j.mcp.2010.07.005 PMID: 20659552