Research Article

Jamila S. Al Malki, Nahed Ahmed Hussien*

Molecular characterization and phylogenetic studies of Echinococcus granulosus and Taenia multiceps coenurus cysts in slaughtered sheep in Saudi Arabia

https://doi.org/10.1515/biol-2021-0131
received August 07, 2021; accepted November 12, 2021

Abstract: Taeniids, consisting of two genera Echinococcus and Taenia, are obligatory tapeworms of mammals, and their pathogenicity was due to infection with larval stages. Hydatid (the larval stage of Echinococcus granulosus) and coenurus (the larval stage of Taenia multiceps) cysts are prevalent in domestic, wild ruminants, livestock, swine, and dogs, and accidentally they could also be found in humans. They lead to different clinical manifestations that cause economic loss in livestock and human morbidity. In Saudi Arabia, few studies were performed on hydatid and coenurus cystic genetic variations. The main goal of the present study was to identify E. granulosus and T. multiceps cyst isolates collected from slaughtered Harri sheep in Saudi Arabia by partial sequencing with PCR amplification of the cytochrome C oxidase 1 (COX1) gene. Molecular and phylogenetic evaluation based on COX1 sequences indicated that cyst isolates belong to E. granulosus and T. multiceps, respectively, successfully submitted in NCBI Genbank. Molecular characterization showed a low nucleotide diversity with two submitted isolates of coenurus with related isolates of Genbank. Conversely, E. granulosus isolates showed higher nucleotide diversity. The reported data could serve as a foundation for future molecular epidemiological and biological studies.

Keywords: Echinococcus granulosus, Taenia multiceps, coenurus, COX1, NAD1, Taif, Saudi Arabia

1 Introduction

Taenid tapeworms belong to subclass Eucestoda, order Cyclophyllidea, and family Taeniidae, and they represent zoonotic parasites of mammals and livestock. Family Taeniidae consists of two genera, Echinococcus and Taenia, and they are mainly focused on for their socioeconomic impact by causing human morbidity and loss of livestock [1,2].

Echinococcus granulosus causes hydatidosis disease due to its larval stage infection. Hydatidosis (cystic echinococcosis) is widely distributed, and its prevalence varies according to climate and their contact with livestock [3]. Hydatid cysts have been reported in different livestock, including sheep, camels, cattle, and goats, with various incidence rates, resulting in significant economic losses [4–6]. Recently, it was recorded that the monetary burden of surgically treated human cystic echinococcosis increased that differs from one country to another [7]. In addition, it was reported that about 2.5% of humans infected with hydatidosis died after resorting to surgery [8]. There are three possibilities for cystic echinococcosis management: medical treatment with albendazole, percutaneous procedures, and surgery that depends on cyst stage, state, and organ location [9].

Coenurus represents the infective larval stage of Taenia multiceps; they are also widely distributed mainly in the tropical countries in Africa, Asia, and the Middle East [10]. T. multiceps are found as an obligate intestinal tapeworm of dogs, also commonly found in sheep and goats, as an intermediate host for the parasite, but the infection was extended to cattle and humans [11]. Mainly, coenurus cysts present in the brain in either acute or chronic form also could be frequently found in intramuscular and subcutaneous tissues [12–14]. Coenurosis leads to different clinical manifestations according to the location and extent of the coenuri. Coenurus cysts in the brain cause fever, muscle tremors, hemorrhagic retinal lesions,
paralysis, ataxia, blindness, nystagmus, dysmetria, and scoliosis [15,16]. However, coenuri cysts in the subcutaneous and intramuscular tissues damage the functioning of normal organs and lead to muscular pain [11].

Due to the veterinary and medical significance of both *E. granulosus* and *T. multiceps*, there has been an intensive focus on their ecological, epidemiological, and taxonomic studies. Molecular characterization among many species found within Taeniidae, especially for the genus *Echinococcus*, has been well documented, but this was scarce with coenuri. However, there are very few documented studies on genetic variability regarding Taeniids in Saudi Arabia.

The present study aimed to (i) genetically characterize hydatid (larval stage of *E. granulosus*) and coenuri (larval stage of *T. multiceps*) cysts isolated from slaughtered Harri sheep in Taif, Saudi Arabia, by using mitochondrial cytochrome c oxidase 1 (COX1) and NADH dehydrogenase subunit 1 (NAD1) genes. Also, the study aimed to (ii) report the genetic relationship between selected genes loci of Taif isolates and other isolates present in other countries in Genbank.

## 2 Material and methods

### 2.1 Sample collection

The present study was carried out on Harri sheep from an animal slaughterhouse in Taif governorate (21.2819°N, 40.3841°E), Mecca Province, Saudi Arabia. During post-mortem veterinary examination, meat was systematically inspected for hydatid and coenuri cysts occurrence by applying procedures of the routine meat inspection (sampling period from January to October 2020). Hydatid cysts were found in muscles, liver, and viscera, but coenuri cysts were found only in muscles. Therefore, we have collected samples from 20 sheep (gender, female; age, 1–2 years), about 200–300 g of muscles from each animal, which contains either hydatid or coenuri cysts from slaughtered sheep under permission from the Ministry of Environment, Water and Agriculture (KSA). Each cyst from a different animal was labeled and handled as a different isolate before carrying out the examination.

### 2.2 Microscopic examination

Ten samples of hydatid cysts (of *E. granulosus*) and the other ten samples of coenuri cysts (of *T. multiceps*) were collected from animal meat and carefully opened with the help of a sterile scalpel. First, protoscolices of hydatid and coenuri cysts were scraped off the inner wall of their cysts. Then, protoscolices were loaded on glass slides, covered, and examined under a light microscope without staining.

### 2.3 DNA extraction of protoscolices

About 300 µL of lysis buffer TNES (10 mM Tris, 400 mM NaCl, 100 mM Na₂EDTA, 0.6% SDS, pH 7.5) was added to hydatid protoscolices in a microtube; then samples were frozen (in liquid nitrogen) and thawed (5×). To facilitate sample digestion, samples were ground before proteinase K (8 µL, 20 mg/mL) and incubated overnight at 37°C. Next, phenol/chloroform (100 µL) was added to the sample and centrifuged (16,500 g) for 5 min, and then, the clear upper phase was transferred into a new 1.5 mL Eppendorf. Next, for DNA precipitation, the same volume of absolute ethyl alcohol and sodium acetate (1%, 3 mol) was added to the sample and stored overnight (~20°C). Finally, samples were centrifuged (16,500 g) for 5 min, the supernatant was discarded, the pellet containing DNA was left to dry, and the pellet was finally dissolved in 100 µL sterile deionized water [17]. DNA was extracted from coenuri protoscolices with the same previous procedure using TNES lysis buffer and phenol/chloroform, but there was no need for freezing, thawing, or grinding.

### 2.4 PCR amplification

We have targeted two mitochondrial COX1 and NAD1 regions of *E. granulosus* and *T. multiceps* cysts by using two different primers JB3: 5′-TTTTGCGGATCTGAGGT TTAT-3′, JB4: 5′-TTTTTGGGCCATCTGAGGT TTAT-3′, JB4: 5′-TTTTTGGGCCATCTGAGGT TTAT-3′, and JB11 5′-AGATTCGTAAGGGCCTAATA-3′, JB12 5′-ACC ACTAATATTCCATTTC-3′, respectively [17]. The PCR reaction was set up with initial denaturation at 95°C (5 min), 40 cycles of denaturation at 94°C (45 s), primer annealing at 51°C (COX1) or 55°C (NAD1) (35 s), and then primer extension at 72°C (45 s). Final extension at 72°C (10 min) was necessary for complete amplification (Programmable Thermal Cycler, PTC-100TM thermal cycler, Model 96; MJ Research, Inc., Watertown, MA). PCR products were separated on 1% tris-borate/EDTA agarose gels and ethidium bromide staining and then visualized under gel documentation (Bio-Rad, USA) [18].

### 2.5 Sequencing and phylogenetic evaluation

Different PCR products of COX1 and NAD1 of *E. granulosus* and *T. multiceps* cysts were randomly selected and
subjected to sequencing using an ABI Prism 3730 Genetic Analyzer automated sequencer. Four sequences of COX1 regions (two of each hydatid and coenurus cysts) were directly submitted in NCBI Genbank to have accession numbers. Sequences of COX1 and NAD1 were aligned with different sequences in Genbank using online NCBI BLAST, phylogenetically estimated, and finally viewed as rectangular cladogram in the phylogenetic Tree View [19].

3 Results

3.1 Morphological and microscopic examination of cysts

Hydatid and coenurus cysts appeared as fluid-filled round/oval sacs with different sizes embedded in the muscles of sheep. Protoscolices of both cysts were carefully scraped off the inner wall of their cyst rather than collecting them from cysts’ inner fluid. E. granulosus protoscolices appeared as an oval body with an invaginated scolex provided with several hooks (Figure 1). Visual examination showed numerous scolices in the inner wall of coenurus cysts. Microscopically, the isolated protoscolex showed both large and small rostellar taeniid hooks and four scup-shaped suckers (Figure 2).

3.2 PCR, sequencing, and phylogenetic analysis

Mitochondrial DNA was used to amplify two separate gene loci, COX1 and NAD1, for both E. granulosus and T. multiceps cysts to yield amplicons of 446 bp and 520 bp, respectively. The present results report the success of PCR amplification for both selected portions (Figures 3 and 4). However, NADI amplification shows other lower nonspecific bands.

Randomly selected PCR products from the two loci were sequenced by using their forward/reverse primers. Sequences of COX1 were deposited in Genbank and have been assigned different accession numbers: E. granulosus (COX1) MZ345697.1 and MZ350810.1; and T. multiceps coenurus cyst (COX1) MZ346598.1 and MZ348363.1, respectively. MZ345697.1, MZ350810.1, MZ346598.1, and MZ348363.1 were blasted with other related sequences in different countries. Phylogenetic trees were constructed from those sequences according to higher percentage identity and query coverage range, as shown in Figures 5 and 6. Sequences of NADI could not be blasted with other sequences nor submitted in Genbank due to the presence of nonspecific bands in PCR products that lead to the high noisy background in FASTA sequence (data not shown).

For E. granulosus (COX1): The BLAST analysis showed that Taif sheep isolate of COX1 sequence exhibited percentage identity 84–95.67% with Query coverage ranging from 94 to 100% of E. granulosus in GenBank isolates that were collected from other different countries. Alignment of MZ345697.1 with the other isolate MZ350810.1 showed identity with 94.95%. MZ345697.1 showed a higher alignment identity (95.67%) with E. granulosus KX017221.1 from dog stool in Palestine. MZ345697.1 showed 85% homology with Iran isolates KU359037.1, MW549010.1, MW567459.1, MW315464.1, MW549013.1, KU220240.1, and KU376099.1 from different hosts such as camel, sheep, and goat. MZ345697.1 showed 85% identity with E. granulosus

Figure 1: Echinococcus granulosus protoscolices at 10× (a), and 40× (b) magnifications. Scale-bars: 100 µm.
isolates from Egypt MZ348909.1 and MZ348904.1 in camel and *Homo sapiens*, respectively. In addition, the COX is late of Taif sheep showed the same identity percentage with other isolates from buffalos (MH428014.1 and MH428013.1) in India, cattle (HF947565.1) in Portugal, goat (KX379145.1) in Italy, pig (JF82338.1) in Brazil, and sheep (GQ502208.1) in Chile (Figure 5). MZ345697.1 showed lower homology (84%) with isolates from cattle in Turkey (KM100577.1, KM100574.1, MT380278.1, MT380277.1, and MT380265.1), Portugal (HF947576.1), *Homo sapiens* in India (JX854033.1), Iran (MK291233.1), and *Homo sapiens* in Poland (MH301007.1).

For larval stage (coenurus) of *T. multiceps* (COX1): The BLAST analysis showed that COX1 sequence (Taif sheep isolate) exhibited a higher percentage identity 99–98% (Query coverage 100–97%) with that of *T. multiceps* COX1 in GenBank isolates that were collected from other different countries. Taif isolate 1, MZ346598.1, showed 97% homology with our second isolate, MZ348363.1, in the present study. MZ346598.1 showed a higher alignment identity (99%) with coenurus cyst isolates found in brains of sheep in Egypt (LC271737.1 and LC271703.1), is late from sheep and humans in China (JX535575.1, FJ495086.1, and KT258024.1), Greece (KR604810.1), and Turkey (EF393620.1).

**Figure 2:** (a) Coenurus cyst attached to a muscle. (b) Multiple scolices appeared after cyst opening. (c) Isolated protoscolex large and small rostellar hooks: blue arrow and 4 suckers: red arrows. (d) Isolated rostellar hooks. Scale bars: 100 µm.

**Figure 3:** Representative agarose gel stained with ethidium bromide (1.5%) showing PCR product (446 bp) of COX1 of *E. granulosus* (lanes 1 and 2) and *T. multiceps* (lanes 3 and 4) cysts. Lane M: low-molecular-weight marker (50–1,500 bp).
Cystic echinococcosis and coenurosis are zoonotic diseases that affect humans and livestock due to infection with the larval stages of *Echinococcus granulosus* and of *Taenia multiceps* cysts, leading to economic loss that increases annually [4,9,11]. Dogs, especially stray unvaccinated dogs, are definitive hosts for different cestodes of Taeniidae family, including *E. granulosus* and *T. multiceps*. Dogs were commonly associated with extensive livestock herding to protect domestic animals against predators that could lead to their infection [20,21]. For the first time, the present study reports the genetic characterization of two different parasite cysts, hydatid (the larval stage of *E. granulosus*) and coenurus (the larval stage of *T. multiceps*), in Taif governorate, Makkah Province, Saudi Arabia.

Sheep, goats, cattle, and camels represent the primary livestock species producing red meat in Saudi Arabia, with an estimated total population of 13,444,435 heads [22]. Sheep represent the significant population (72%) of the livestock, and they import large numbers of sheep to satisfy the needs of Saudi citizens. Awassi, Harri, and Najdi are the most available sheep species in Saudi Arabia [23]. Any endemic infection for livestock, such as hydatidosis and coenurosis, could lead to economic setbacks; therefore, there is a need to highlight their study, especially in KSA.

Hydatidosis and coenurosis due to *E. granulosus* and *T. multiceps* larval infection, respectively, cause significant damage in livestock that leads to production losses, especially in endemic areas [2,24]. Moreover, these parasites were distributed worldwide in different intermediate hosts, especially in sheep and goats, accidentally humans, reflecting their medical and veterinary importance [25].

The present study aims to identify two Taeniids parasites found in slaughtered Harri sheep meat based on COX1 and NAD1 partial sequencing and phylogenetic relationship. It was reported that the development of molecular techniques rather than traditional morphological criteria has provided improved tools for taeniid species identification and investigating relationships among them. Sequencing of mitochondrial DNA, especially COX1 and NAD1 loci, has been successfully used for molecular characterization and identification of taeniids tapeworm [26,27].

Our choice to use mitochondrial DNA (mtDNA) sequence, especially COX1 and NAD1, for molecular characterization of *E. granulosus* and *T. multiceps* larva was based on the previous assessment. mtDNA is widely used in molecular characterization and phylogenetic evaluation studies. COX1 gene represents the most common gene of mtDNA for phylogenetic analysis and evolutionary biology of helminth parasites to determine interspecific and/or intraspecific variation [8,28-30]. The mitochondrial COX1 gene is very suitable for genetic diversity detection and haplotype analysis. Its evolutionary change rate is slow enough for the same species but fast enough for differentiation between different species. Therefore, the mitochondrial COX1 gene has been selected for use in the creation of DNA barcodes and species differentiation of different helminths as our present study [31,32].

Worldwide, there have been well-documented research studies about the genetic variability among many species found within the Taeniidae, especially for the genus...
Echinococcus. Still, there are a few related species of Taenia, especially for *T. multiceps* [33]. However, there is a scarcity of studies related to the genetic characterization of *E. granulosus* and *T. multiceps* larval stages in KSA and their phylogenetic relationship.

In the current study, a genetically characterized sample based on COX1 isolated from a hydatid cyst of MZ345697.1 showed identity about 94.95% with the other isolate MZ350810.1. For coenurus cyst, isolate MZ346598.1 showed 97% homology with the second isolate MZ348363.1. In addition, comparing partial sequences of Taif COX1 isolates from both cysts showed different identities with other isolates from organs present in various hosts found in other countries. The present results are inconsistent with previous studies, in which Al-Hizab et al. [34] and AL-Mutairi et al. [35] have reported *E. granulosus* species variation based on molecular characterization in infected sheep and camels in the Arabian Peninsula and KSA. AL-Mutairi et al. [35] have reported the phenotypic and genetic characterization of hydatid cysts isolated from sheep and camel meat in Al-Madinah, KSA. Genetic characterization of *E. granulosus* was based on random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for the whole genome, PCR amplification of COX1 and 12S rRNA genes followed by single-stranded conformation polymorphism (SSCP), and then sequencing. They have investigated that cyst isolates slightly vary from each other and other isolates found in Genbank. They have concluded that there is an intraspecific variation in *E. granulosus* found in camels and local sheep.

In addition, Christodouloupolous et al. [36] have investigated COX1 nucleotide diversity of coenurus cyst isolates...
from sheep and goats in Pakistan, and their phylogenetic analysis shows high related homology with China isolates but highly different from other isolates from different countries. They have reported that coenurus cerebralis commonly found in the brain (cerebral form) could also be found in other non-cerebral tissue such as intramuscular and subcutaneous tissues.

Diversity in the nucleotide sequences of the presently studied isolates was recorded before within the same region, such as in Iran [5,13], China [26], and Italy [37,38]. Most of their phylogenetic analysis depends on one or more mtDNA sequences such as nad1, cox1, and 12S rRNA. They have concluded that there are no monophyletic groups based on the intermediate host species, the organ from which the parasite was isolated, and geographical origin [33,36]. However, reporting parasite characterization and genetic identification will be crucial for controlling and preventing parasitic infections [8].

The limitation of this study is the use of the limited sample size, and only partial sequencing was done for the COX1 gene that could have implications on data interpretation. Thus, we recommend future studies considering a larger sample size, complete sequencing COX1, and other characterization-related genes in a massive epidemiological survey for further evaluation.

5 Conclusion

The present study reports the molecular characterization of hydatid (the larval stage of E. granulosus) and coenurus (the larval stage of T. multiceps) cysts present in slaughtered sheep meat in Taif, KSA. We have determined sequences differences within isolates of the same species that we submitted in GenBank and other isolates.
found in Genbank from different host organs in different countries. For a more precise identification and characterization of Taeniids in KSA, additional isolates from other hosts, other geographic areas, different molecular protocols, long-sequenced genes in the mitochondrial and nuclear genome, and further information concerning biological characteristics may be necessary to increase our understanding of the epidemiological distribution in KSA.

Acknowledgments: This work was supported by Taif University Researchers Supporting Project number (TURSP-2020/299), Taif University, Taif, Saudi Arabia. The authors would like to thank the Ministry of Environment, Water and Agriculture in Taif governorate (KSA) for facilitating data and sample collections.

Funding information: Taif University Researchers Supporting Project number (TURSP-2020/299).

Author contributions: J.M., and N.H. participated in the design of the study. J.M. collected samples and performed parasitological assay. J.M. and N.H. carried out the molecular evaluation. N.H. performed data analysis and wrote the article draft. J.M., and N.H. read and approved the final manuscript.

Conflict of interest: The authors state no conflict of interest.

Ethics approval: Data and samples were collected from veterinarians as per their periodic examination according to the ministerial recommendation. We have a permission letter from the Ministry of Environment, Water, and Agriculture (KSA) to collect cattle samples from different locations in Taif for our research study with the number 106082/1074/1442, dated 21-02-1442H. Sample collection and all experimental procedures were performed in accordance with a national ethical requirement. National Committee of Bioethics (NCBE) at King Abdulaziz City for Science and Technology (KACST) with number: 10023117, valid till October 1, 2023.

Data availability statement: The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Eckert J, Schantz PM, Gasser RB, Torgerson PR, Bessonov AS, Movsessian SO, et al. Geographic distribution and prevalence. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, editors. WHO/OIE manual on Echinococcosis in humans and animals: a public health problem of global concern. Geneva: World Health Organisation; 2001. p. 100–42.

[2] Hoberg EP. Taenia tapeworms: their biology, evolution and socioeconomic significance. Microbes Infect. 2002;4(8):859–66. doi: 10.1016/s1286-4579(02)01606-4.

[3] 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):1425–37. doi: 10.3748/wjg.v18.i13.1425.

[4] Dalimi A, Motamedi G, Hosseini M, Mohammadian B, Malaki H, Ghamari Z, et al. Echinococcosis/hydatidosis in western Iran. Vet Parasitol. 2002;105:161–71. doi: 10.1016/s0304-4017(02)00005-5.

[5] Harandi MF, 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. Parasitology. 2002;125:367–73. doi: 10.1017/ S0031182002002172.

[6] Rokni M. Echinococcosis/hydatidosis in Iran. Iran J Parasitol. 2009;4(2):1–16.

[7] Basinger SC, Khan A, Ahmed H, Afzal MS, Simsek S, Budke CM. Estimation of the monetary burden of treated human cystic echinococcosis in Pakistan. Acta tropica. 2021;222:106026. doi: 10.1016/j.actatropica.2021.106026.

[8] Harandi MF, Budke CM, Rostami S. The monetary burden of cystic echinococcosis in Iran. Plos Negl Trop Dis. 2012;6:e1915. doi: 10.1371/journal.pntd.0000195.

[9] Khan A, Ahmed H, Khan H, Saleem S, Simsek S, Brunetti E, et al. Cystic Echinococcosis in Pakistan: a review of reported cases, diagnosis, and management. Acta tropica. 2020;212:105709. doi: 10.1016/j.actatropica.2020.105709.

[10] Amrabadi O, Oryan A, Moazeni M, Shari-Fiyazdi H, Akbari M. Histopathological and molecular evaluation of the experimentally infected goats by the larval forms of Taenia multiceps. Iran J Parasitol. 2019;14:95–105. doi: 10.18502/ijp.v14i1.722.

[11] Sharma DK, Chauhan PP. Coenurus status in Afro-Asian region: a review. Small Ruminant Res. 2006;64:197–202. doi: 10.1016/j.smallrumres.2005.05.021.

[12] Christodouloupolou G, Theodoropoulo G, Petakos G. Epidemiological survey of cestode-larva disease in Greek sheep flocks. Vet Parasitol. 2008;153:368–73. doi: 10.1016/j.vetpar.2008.02.002.

[13] Oryan A, Nazifi S, Shari-Fiyazdi H, Ahmadnia S. Pathological, molecular, and biochemical characterization of Coenurus gaigeri in Iranian native goats. J Parasitol. 2010;96:961–7. doi: 10.1645/GE-2399.1.

[14] Schuster RK, Sivakumar S, Wieckowsky T. Non-cerebral coenurosis in goats. Parasitol Res. 2010;107:721–6. doi: 10.1007/s00436-010-1919-6.

[15] Giadinis ND, Psychas V, Polizopoulou Z, Papadopoulos E, Papaioannou N, Komenou A, et al. Acute coenurosis of dairy sheep from 11 flocks in Greece. N Z Vet J. 2012;60:247–53. doi: 10.1080/00480169.2012.665343.

[16] Akbari M, Moazeni M, Oryan A, Shari-Fiyazdi H, Amrabadi O. Experimental cerebral and non-cerebral coenurosis in goats: a comparative study on the morphological and molecular characteristics of the parasite. Vet Parasitol. 2015;211:201–7. doi: 10.1016/j.vetpar.2015.06.013.
Barazesh A, Sarkari B, Ebrahimi S, Hami M. DNA extraction from hydatid cyst protoscolices: Comparison of five different methods. Vet. World. 2018;11(2):231–4.

Sambrook J, Fritsch EF, Maniatis T. In: Sambrook J, Fritsch EF, Maniatis T, editors. Molecular cloning: a laboratory manual. 2nd edn. New York, NY, USA: CSH Cold Spring Harbor Press; 1989.

Kuznetsov A, Bollin CJ. NCBI genome workbench: desktop software for comparative genomics, visualization, and GenBank data submission. Methods Mol Biol. 2021;2231:261–95.

Abdi J, Asadolahi KH, Maleki MH, Ashrafi-Hafez A. Prevalence of helminthes infection of stray dogs in ilam province. J Paramed Sci. 2013;4(2):58–61.

Khorashad A, et al. Molecular characterization and phylogeny of Taenia multiceps isolates from sheep and goats in faisalabad, Pakistan. Front. Vet. Sci. 2017;4(1):1202. doi: 10.3389/fvets.2017.01202.

Suliman GM, Al-Owamer AN, El-Waziry AM, Hussein E, Abuellatah K, Sowelam A. A comparative study of sheep breeds: fattening performance, carcass characteristics, meat chemical composition and quality attributes. Front. Vet. Sci. 2021;8:647192. doi: 10.3389/fvets.2021.647192.

Raissi V, Etemadi S, Sohrabi N, Raiesi O, Shahrami K, Salimi-Khorashad A, et al. Molecular characterization and phylogeny of Taenia hydatigena and Echinococcus granulosus from Iranian sheep and cattle based on COX1 gene. Curr. Microbiol. 2021;78(4):1202–7. doi: 10.1007/s00284-021-02377-0.

Craig P, Pawlowski Z, editors. Cestode zoonoses: echinococcosis and cysticercosis an emergent and global problem. Amsterdam: Ios Press; 2002.

Zhang Y, Zhao W, Yang D, Tian Y, Zhang W, Liu A. Genetic characterization of three mitochondrial gene sequences of goat/sheep-derived Coenurus cerebralis and Cysticercus tenuicollis isolates in Inner Mongolia, China. Parasite. 2018;25(3–4):1. doi: 10.1051/parasite/20180002.

Heidari Z, Sharbatkhori M, Mobedi M, Mirhendi SH, Nikmanesh B, Sharifdini M, et al. Echinococcus multilocularis and Echinococcus granulosus in canines in North-Khorasan Province, northeastern Iran, identified using morphology and genetic characterization of mitochondrial DNA. Parasite Vectors. 2019;12(1):1–13. doi: 10.1186/s13071-019-3859-z.

Mohaghegh MA, Yousoff-Darani H, Jafarian AH, Mirbadie SR, Fasihi-Harandi M, Ghavimi R, et al. Isolated human and Livestock Echinococcus granulosus genotypes using real-time PCR of cox1 gene in Northeast Iran. Acta Parasitol. 2019;64(3):679–85. doi: 10.2478/s11686-019-00117-w.