Open Veterinary Journal, (2024), Vol. 14(3): 866-878
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Original Research
DOI: *10.5455/OVJ.2024.v14.i3.14*

Submitted: 30/12/2023 Accepted: 27/02/2024 Published: 31/03/2024

Echinococcus granulosus comparative genotyping in sheep in Saudi Arabia and Egypt

Abdulsalam A. M. Alkhaldi*

Department of Biology, College of Science, Jouf University, Sakaka, Saudi Arabia

Abstract

Background: Cystic echinococcosis (CE), which is triggered by the parasite *Echinococcus granulosus*, is a global zoonotic disease that is common in rural regions in which there are frequent encounters between dogs and other domestic animals. The disease can have devastating consequences, impacting the health of people and animals and leading to huge financial losses, especially in the agricultural industry. In the Kingdom of Saudi Arabia (KSA) and Egypt, despite the high incidence of disease, few investigations have been conducted into the genetic variation in species of the genus *Echinococcus*.

Aim: This study sought to compare the genetic features of the hydatid cysts carried in sheep in KSA with those found in Egypt.

Methods: DNA from the protoscolices was used in a PCR targeting the mitochondrial NADH dehydrogenase 1 (*NAD1*), cytochrome c oxidase subunit 1 (*COX1*), and nuclear actin II (*ACT II*) genes, and the resulting amplification products of 30 KSA and Egyptian isolates were sequenced and compared.

Results: Among the sheep in KSA, the overall prevalence of CE was 0.51%. Of the sheep cyst DNA samples, 95%, 100%, and 52% were positive for the *Cox1, nad1*, and *act II* genes, respectively. Targeting all three genes, all KSA samples belonged to the *E. granulosus* genotype (G1), whereas all Egyptian isolates belonged to *E. granulosus* (G1) and *E. canadensis* (G6).

Conclusion: We conclude that isolates of *E. granulosus* from the two countries shared a common origin in Arabic North Africa, with sheep and camels as common hosts.

Keywords: Sheep, Genetics, Saudi Arabia, Egypt, Echinococcosis.

Introduction

The cestode genus *Echinococcus* is extremely important in terms of zoonotic transmission causing the parasitic disease echinococcosis, which can be a major health problem in humans (Eckert and Deplazes, 2004). Cystic echinococcosis (CE; also known as hydatidosis) is characterized by the presence of hydatid cysts that are caused by the larval phase of *Echinococcus granulosus* sensu lato (s.l.). Alveolar cysts, which are caused by *Echinococcus multilocularis*, are fatal (Bonardi *et al.*, 2012).

Canids are definitive hosts of *E. granulosus*, whereas various herbivores and omnivores are intermediate hosts, including sheep, cattle, and camels. Infection of intermediate hosts occurs through the ingestion of adult parasite eggs in contaminated food, water, or soil; the eggs then develop into the larval phase, forming hydatid cysts in the intestine and internal organs. The definitive hosts ingest the cysts through the consumption of infected intermediate hosts. Humans are infected in the same way as intermediate hosts or via direct contact with animal hosts (Dowling *et al.*, 2000; Thompson and McManus, 2002).

With a total population of approximately 13,444,435, small ruminants, cattle, and camels are the predominant farm animal species in Saudi Arabia that are used to produce red meat. Sheep comprise the majority of the livestock (72%) and are imported in considerable numbers from Middle Eastern and African nations where CE is a serious problem (GASTAT, 2021). Consequently, there is a substantial risk of infected animals being brought into the country. The *Echinococcus* parasites have been reported to leak into resident circulation as a result of large-scale parasite importation via cattle (El-Ghareeb *et al.*, 2017; Toulah *et al.*, 2017; Fdaladdin *et al.*, 2018). This is pertinent for *E. granulosus* sensu stricto (s.s.), which is responsible for most of the human CE cases globally (Alvarez Rojas *et al.*, 2014).

According to estimates, hydatidosis causes annual economic losses of several billion dollars in the global livestock industry, including in the Kingdom of Saudi

***Corresponding Author:** Abdulsalam A. M. Alkhaldi. Department of Biology, College of Science, Jouf University, Sakaka, Saudi Arabia. Email: *abdulsalam@ju.edu.sa*

Arabia (KSA). These losses are the result of low productivity, for example in milk, meat, and wool, and the mortality and/or morbidity of diseased animals and the condemnation of infested tissues of slaughtered animals (Borji *et al.*, 2012; Singh *et al.*, 2014). Owing to the potential for human transmission, hydatidosis also has public health significance (El-Ghareeb *et al.*, 2017).

Echinococcus granulosus s.l. has been explored through biological and molecular studies, and shows great variety in its infectivity, host range, and genetic traits (Eckert *et al.*, 2001). Numerous strains (G1–G10) that comprise separate clades have been found (Nakao *et al.*, 2007; Nakao *et al.*, 2013). On the other hand, *E. equinus* (G4) and *E. ortleppi* (G5) are proposed as the new taxonomic designations for G1–G3 (Ito *et al.*, 2007), whereas G6–G10 are categorized as *E. canadensis* (Ito *et al.*, 2007). According to Carmen and Cardona (2014) and Eslami *et al.* (2016), these varied strains exhibit variations in lifecycle patterns, host specificity, geographic distribution, pathogenesis, transmission dynamics, and sensitivity to chemotherapy. G1, which is a sheep-specific strain, is frequently linked to infections in humans (Farhadi *et al.*, 2015). The presence of 10 genotypes is reinforced by mitochondrial DNA analysis, with G1–G2 representing ovine strains, G3–G5 representing bovine strains, G4 representing an equine strain, G6 representing a camel strain, and G7–G10 representing pig and cervid strains (Cardona and Carmena, 2013). The G1 sheep genotype is associated with human cases (Latif *et al.*, 2010), and *E. felidis* in the *E. granulosus* complex (Thompson and McManus, 2002; Nakao *et al.*, 2013).

The creation and implementation of prevention and control measures, diagnostic tests, and development of effective therapeutics are all significantly impacted by the identification and characterization of species of the genus *Echinococcus* (McManus, 2010). Advances in control measures, suggestive testing, and treatment choices can be facilitated by enhancing our understanding of species of the genus *Echinococcus* through improved characterization (McManus and Thompson, 2003). In addition, research on genetic variation in *E. granulosus* paves the way for the creation of disease management methods that are more successful, especially in endemic regions. This includes investigating vaccine resistance and examining progressive DNA vaccination utilizing recombinant DNA technology (Amini-Bavil-Olyaee *et al.*, 2006).

According to Sadjjadi (2006), CE is a severe public health issue across Arabic North Africa and the Middle East. The frequency of the G1 and G6 strains (*E. granulosus* s.s. and *E. canadensis,* respectively*)* has been shown by molecular research on *E. granulosus* s.l. from different final and intermediate hosts in Africa (Abushhewa *et al.*, 2010; Ibrahim *et al.*, 2011). In several regions of KSA, this zoonosis continues to have an impact on cattle (Haroun *et al.*, 2008; Ibrahim, 2010; Toulah *et al.*, 2012). Only the isolation of *E. granulosus* s.s. from sheep and camels allowed for the genetic identification of the accountable species of the genus *Echinococcus* in KSA (Al-Mutairi *et al.*, 2020). Epidemiological research on *E. granulosus* s.l. in KSA is limited, with most published studies concentrating on the prevalence over seasons and the level of fertility of hydatid cysts collected from animals (Ibrahim, 2010; Fdaladdin *et al.*, 2018; Hayajneh *et al.*, 2014; Almalki *et al.*, 2017; Amer *et al.*, 2018). The genetic variation in *E. granulosus* in KSA has received little attention (Abdel-Baki *et al.*, 2018; Metwally *et al.*, 2018).

The goal of this study was to increase knowledge about the identity of *E. granulosus* s.l. cysts found in sheep in KSA and Egypt. To achieve this aim, partial mitochondrial NADH dehydrogenase 1 (*NAD1*), cytochrome C oxidase subunit 1 (*COX1*), and nuclear actin II (*ACT II*) gene sequences were analyzed using PCR amplification. To the best of our knowledge, this is the first attempt to detect and compare circulating sheep genetic strains of *E. granulosus* s.l. from the two countries.

Materials and Methods

This research used sheep in four slaughterhouses in the northern Saudi Arabian province of Al-Jouf. Over the course of a year, from January 2022 to December 2022, 153225 sheep that had been killed were evaluated for cystic hydatidosis. According to recommendations made by the World Health Organization, the Food and Agriculture Organization of the United Nations, and the United Nations Environment Programme (WHO/FAO/UNEP) (WHO/FAO/UNEP, 1994), animals that had been killed were inspected by visual examination and palpation for hydatid cysts in their organs.

For the Egyptian samples, hydatid cysts that had previously been identified from sheep at a slaughterhouse in the Egyptian governorates of Cairo and Giza were employed.

Sampling

One hundred sheep-derived hydatid cysts were selected for PCR testing using a simple random sampling method. A computer-generated list of 100 random numbers was used to ensure equal chances of selection from the total cyst population. Thereafter, the selected cysts were rinsed with a physiological saline solution. The external surface underwent sterilization, and the hydatid fluid was examined under a microscope to determine cyst fertility based on the presence or absence of protoscolices. Each sample yielded protoscolices from a single cyst, which were then placed into sterile test tubes, preserved in 70% ethanol, and stored at −20°C for DNA extraction.

Extraction of DNA

DNA extraction from 25 mg sediment of protoscolices from each hydatid cyst sample was performed following the manufacturer's instructions with slight modifications. The commercially available DNeasy

Blood & Tissue Kit (Qiagen Inc., Germany) was utilized for DNA extraction, as previously described (Alvi *et al.*, 2023).

PCR amplification

A 25-µl reaction mixture comprising 12.5 µl of 2 × MyTaq™ Red Mix (Cat. BIO-25043, Meridian Life Science Inc., USA), 0.5 µl of each primer (10 µM), 10 µl nuclease-free water, and 2 µl of target DNA was used to amplify the genes *COX1, NAD1*, and *ACT II*. In addition, positive and negative controls were incorporated into the PCR assay. Positive control DNAs for three genes (*COX1, NAD1, and ACT II*) previously identified were obtained from the National Research Centre (NRC) in Cairo, Egypt. Negative controls lacking DNA were also included. PCR primers and conditions are shown in Tables 1 and 2, respectively. The PCR results were inspected using the InGenius3 gel documentation system (Syngene, UK), 1.5% agarose gel electrophoresis, ethidium bromide staining, and the 100 bp marker plus.

DNA analysis and phylogenetic tree reconstruction

A GeneJETTM Gel Extraction Kit (K0691, Thermo Fisher Scientific, USA) was used to clean 30 of the positive PCR products from each of the KSA and Egyptian isolates (15 from KSA and 15 from Egypt) that targeted the *COX1*, *NAD1*, and *ACT II* genes. Sequencing was performed by Macrogen Company (Korea). Two-way sequencing using the specific primers used in PCR (NL1401, Vivantis Co, Malaysia) served as a confirmation of the accuracy of the data. The programs BioEdit 7.0.4.1 and MUSCLE were used to examine the acquired nucleotide sequences. Using the neighbor-joining technique in the application of CLC 6, the obtained sequences were aligned with reference sequences for the three genes of species of the genus Echinococcus available in the GenBank database (Tables 3–5).

A total of 15 nucleotide sequences for the KSA isolates in this study were deposited in the GenBank database under the accession numbers OQ970593– OQ970597 for the *COX1* gene, OQ844076–OQ844080 for the *NAD1* gene, and OQ791975–OQ791979 for the *ACT II* gene.

Fifteen nucleotide sequences produced in this investigation for the Egyptian isolates were deposited in the GenBank database under the accession numbers OQ970598–OQ970602 for the *COX1* gene, OQ844081–OQ844085 for the *NAD1* gene, and OQ791980–OQ791984 for the *ACT II* gene.

Ethical approval

Not needed for this study.

Results

Over the course of a year, from January 2022 to December 2022, a total of 153225 sheep that were killed in four slaughterhouses in the Al-Jouf Province of northern Saudi Arabia were tested for cystic hydatidosis. Among the sheep that were investigated, the overall prevalence of cystic hydatidosis was 0.51% (784/153225).

Table 1. PCR primers and probes used in the study.

Gene	Sequence (5′–3′)	Amplicon size (bp)	Reference
COX1	TTTTTTGGGCATCCTGAGGTTTAT TAAAGAAAGAACATAATGAAAATG	450	Bowles *et al.*, 1992
NAD1	AGATTCGTAAGGGGCCTAATA ACCACTAACTAATTCACTTTC	550	Bowles and McManus, 1993
ACT II	GTCTTCCCCTCTATCGTGGG CTAATGAAATTAGTGCTTTGTGCGC	266	da Silva *et al.*, 1993

Table 2. Cycling conditions for the detection of genes in this study.

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles
Cox1	95°C 5 minutes	95°C 20 seconds	55°C 30 seconds	72°C 45 seconds	72°C 10 minutes	40
nad1	95°C 5 minutes	95°C 30 seconds	51°C 30 seconds	72 °C 45 seconds	72°C 10 minutes	40
actII	95°C 5 minutes	95°C 20 seconds	60°C 30 seconds	72°C 75 seconds	72°C 10 minutes	40

Table 3. *Cox1* gene sequences from the GenBank database used for phylogenetic tree reconstruction.

GenBank accession no.	Species	Host	Country	Genotype
AB921054	*E. granulosus*	Camel	Egypt	G1
AB921090	*E. granulosus*	Sheep	Egypt	G1
DQ341566	*E. granulosus*	Sheep	Algeria	G1
AB921055	*E. ortleppi*	Camel	Egypt	G5
AB921058	*E. canadensis*	Camel	Egypt	G6
HM636639	*E. granulosus*	Cattle	Libya	G1–3
HM636641	*E. granulosus*	Human	Libya	G1–3 (G1)
FJ796205	*E. granulosus*	Cattle, camel	Iran	G1–3 (G1)
HQ717150	*E. granulosus*	Cattle	Turkey	G1–3 (G1)
HQ717148	*E. granulosus*	Human	Turkey	G1–3 (G1)
KF612390	*E. granulosus*	Human	Iran	G1
DQ856467	*E. granulosus*	Sheep	Greece	G1
M84662	*E. granulosus*	Sheep	Australia	G2
DQ341574	*E. granulosus*	Sheep	Algeria	G2
FJ796206	*E. granulosus*	Camel	Iran	G1–3 (G3)
M84663	*E. granulosus*	Buffalo	Iran	G3
KF612397	*E. granulosus*	Human	Iran	G3
DQ856466	*E. granulosus*	Sheep	Greece	G3
M84664	*E. granulosus*	Horse	India	G4
M84665	*E. granulosus*	Cattle	Netherlands	G5
FJ796207	*E. granulosus*	Camel	Iran	G6–10 (G6)
M84666	*E. granulosus*	Camel	Africa	G6
DQ341581	*E. canadensis*	camel	Algeria	G6
HM636638	*E. granulosus*	Cattle, camel	Libya	G6
KF612400	*E. granulosus*	Human	Iran	G6
HQ717155	*E. canadensis*	Human	Turkey	G6–10 (G7)
DQ856468	*E. canadensis*	Goat	Greece	G7
AB235848	*E. canadensis*	Moose	USA	G8
AF525457	*E. canadensis*	Reindeer	Finland	G10
EF558356	*E. felidis*	Lion	Uganda	
M84670	*E. vogeli*	Rodent	South Africa	
M84668	*E. multilocularis*	Human	Alaska, China	
M84669	*E. multilocularis*	Rodent	Germany	
DQ341575	*E. granulosus*	Camel	Algeria	G2
DQ341568	*E. granulosus*	Camel	Algeria	G1
DQ341567	*E. granulosus*	Sheep	Ethiopia	G1
MZ350810	*E. granulosus*	Sheep	Taif–Saudi Arabia	
MW051127	*E. granulosus*	Camel	Saudi Arabia	
MN720282	*E. granulosus*	Sheep	Al-Madinah–Saudi Arabia	
MN720281	*E. granulosus*	Camel	Al-Madinah–Saudi Arabia	

Table 4. *Nad1* gene sequences from the GenBank database used for phylogenetic tree reconstruction.

GenBank accession no.	Species	Host	Country	Genotype
AB921120	*E. canadensis*	Camel	Egypt	G6
AB921111	*E. canadensis*	Camel	Egypt	G6
AB921119	*E. canadensis*	Camel	Egypt	G6
AB921121	*E. canadensis*	Camel	Egypt	G6
DQ856471	*E. canadensis*	Goat	Greece	G7
AJ237638	*E. canadensis*	Pig	Poland	G7
AB921095	*E. canadensis*	Camel	Egypt	G6
KF612372	*E. granulosus*	Human	Iran	G6
HM636642	*E. granulosus*	Cattle	Libya	G6
FJ796216	*E. granulosus*	Camel	Iran	G6
HQ717154	*E. granulosus*	Human	Turkey	G7
AF525297	*E. canadensis*	Reindeer	Finland	G10
AB921092	*E. ortleppi*	Camel	Egypt	G5
AJ237636	*E. ortleppi*	Cattle	Netherlands	G5
AB921125	*E. granulosus*	Sheep	Egypt	G1
AB921091	*E. granulosus*	Camel	Egypt	G1
DQ856470	*E. granulosus*	Sheep	Greece	G1
FJ796214	*E. granulosus*	Camel	Iran	G3
KF612369	*E. granulosus*	Human	Iran	G3
HM636643	*E. granulosus*	Cattle	Libya	
HM636644	*E. granulosus*	Human	Libya	
FJ796208	*E. granulosus*	Sheep	Iran	G1
HQ717153	*E. granulosus*	Human	Turkey	G1
HQ717151	*E. granulosus*	Cattle	Turkey	G1
KF612360	*E. granulosus*	---	Iran	G1
KF612349	*E. granulosus*	---	Iran	G1
DQ856469	*E. granulosus*	Sheep	Greece	G3
AJ237634	*E. granulosus*	Buffalo	India	G3
AJ237633	*E. granulosus*	Sheep	---	G2
AB208064	*E. shiquicus*	Ochotona curzoniae	China	
AB235848	*E. canadensis*	---	Japan	G8
NC011121	*E. canadensis*	Camel	Kazakhstan	G6
AF297617	*E. granulosus*	---	China	G1
AJ237639	*E. multilocularis*	---	---	
AJ237640	*E. multilocularis*	Rodents	Germany	
AJ237642	*E. oligarthrus*	---	Panama	
AJ237641	*E. vogeli*	---	---	
EF558357	*E. felidis*	African lion	---	
NC009938	*T. saginata*	---	---	
AJ237635	*E. equinus*	Horse	India	G4
AJ237637	*E. canadensis*	Camel	---	G6
AB921105	*E. canadensis*	Camel	Egypt	G6

Table 5. *Act II* gene sequences from the GenBank database used for phylogenetic tree reconstruction.

GenBank accession no.	Species	Host	Country	Genotype
AB921019	*E. granulosus*	Camel	Egypt	G1
AB921052	*E. granulosus*	Sheep	Egypt	G1
AB921053	*E. granulosus*	Sheep	Egypt	G1
AF528498	*E. granulosus*	Human	Algeria	G1
AF528500	*E. granulosus*	Camel	Algeria	G6
DQ341545	*E. granulosus*	Sheep	Algeria	G1
DQ341548	*E. granulosus*	Cattle	Mauritania	G6

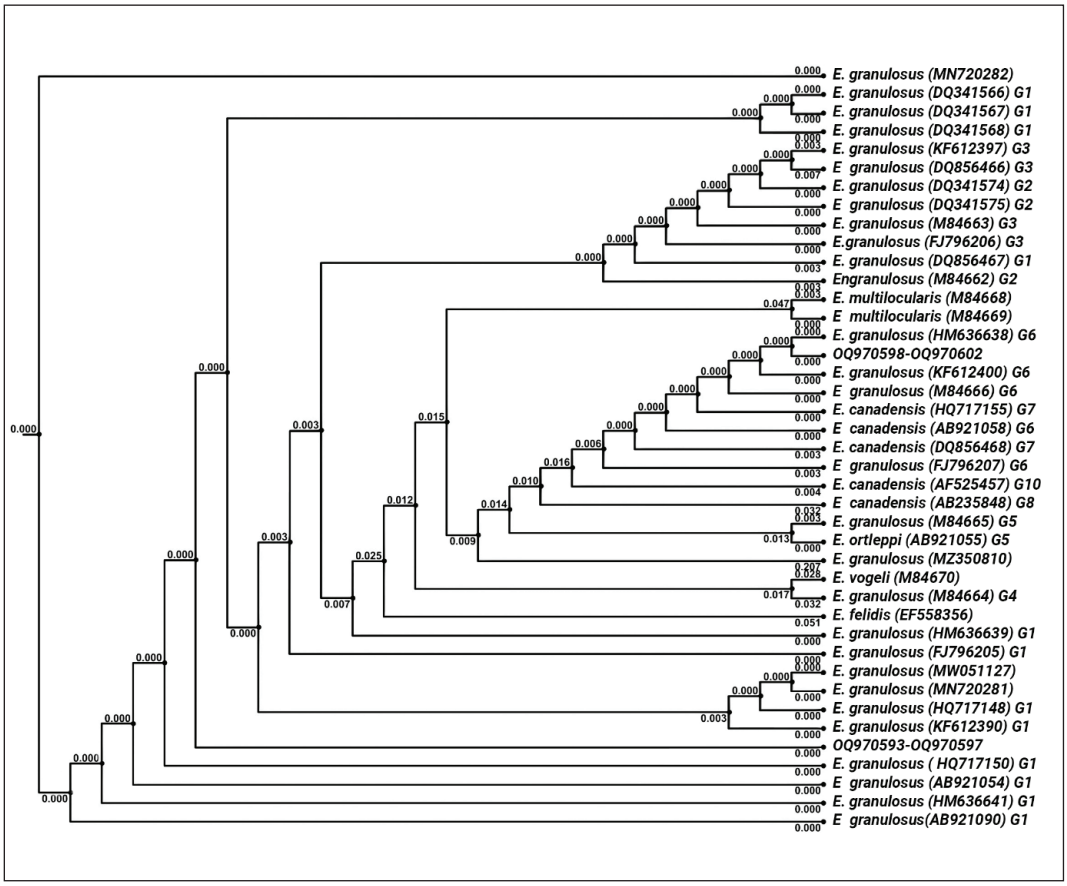

Fig. 1. Neighbor-joining phylogenetic tree of representative *COX1* nucleotide sequences of *E. granulosus* isolates (OQ970593–OQ970602) and reference sequences. GenBank accession numbers are shown in parentheses.

Amplification of act II, nad1, and cox1 genes

Fragments of approximately 450, 550, and 266 bp were amplified using PCR on sheep cyst DNA samples to target the *COX1*, *NAD1*, and *ACT II* genes, respectively. Of the 100 samples tested, 95%, 100%, and 52% were positive for these genes, respectively, but only 24% of samples were positive for all three genes.

Phylogenetic analysis

Targeting the *COX1* gene, all the selected KSA hydatid cyst isolates from sheep (GenBank accession numbers OQ970593–OQ970597) showed high sequence similarity with *E. granulosus* s.s. G1 isolated from sheep and camels (AB921090 and AB921054, respectively) in Egypt, humans (HM636641) in Libya, and cattle in Turkey (HQ717150) (Fig. 1).

On the contrary, all *COX1* sequences of the Egyptian isolates from sheep (OQ970598–OQ970602) exhibited high similarity with *E. canadensis* genotype G6 isolated from camels in Africa (M84666), camels and cattle (HM636638) in Libya, and humans (KF612400) in Iran (Fig. 1).

Concerning the *NAD1* gene, both the KSA hydatid cyst isolates (OQ844076–OQ844080) and the Egyptian isolates (OQ844081–OQ844085) showed high sequence similarity with *E. granulosus* s.s. genotype G1 isolated from sheep and camels (AB921125 and AB921091, respectively) in Egypt and sheep (DQ856470) in Greece (Fig. 2).

Regarding the *ACT II* gene, both the KSA hydatid cyst isolates (OQ791975–OQ791979) and the Egyptian isolates (OQ791980–OQ791984) showed high sequence similarity with *E. granulosus* s.s. genotype G1 isolated from sheep (AB921052 and DQ341545) in Egypt and Algeria, respectively (Fig. 3).

Discussion

The inclusion of CE in the WHO's strategy to battle neglected diseases reflects the significance of this disease for both public health and economic stability. According to Eckert *et al.* (2001) and Almalki *et al.* (2017), this zoonotic infection caused by *Echinococcus granulosus*, is a public health concern in many areas, especially among populations that keep sheep, like those in KSA. In intermediate hosts and humans, the illness is frequently asymptomatic for a long time (Alsulami, 2019).

Hydatid disease is endemic in KSA, with dogs having a significant role in the distribution and transmission of the disease, particularly in rural regions (Almalki *et al.*, 2017). Studies conducted in KSA have emphasized the direct causes of human infection, such as the domestic slaughter of sheep and camels (Al-Malki and Degheidy, 2013; Toulah *et al.*, 2017;). Similar circumstances exist in Egypt for the development of the dog–livestock cycle for the spread of species of the genus *Echinococcus*. Because dogs are frequently utilized as guard dogs on livestock farms, there is a high infection rate of *Echinococcus* among homeless dogs in Egypt and frequent animal–dog contact. Dogs can also access slaughterhouses and eat the offal of dead animals, leading to infection (Mazyad *et al.*, 2007).

Meanwhile, Hayajneh *et al.* (2014) conducted an abattoir survey of sheep and goats in KSA and attributed

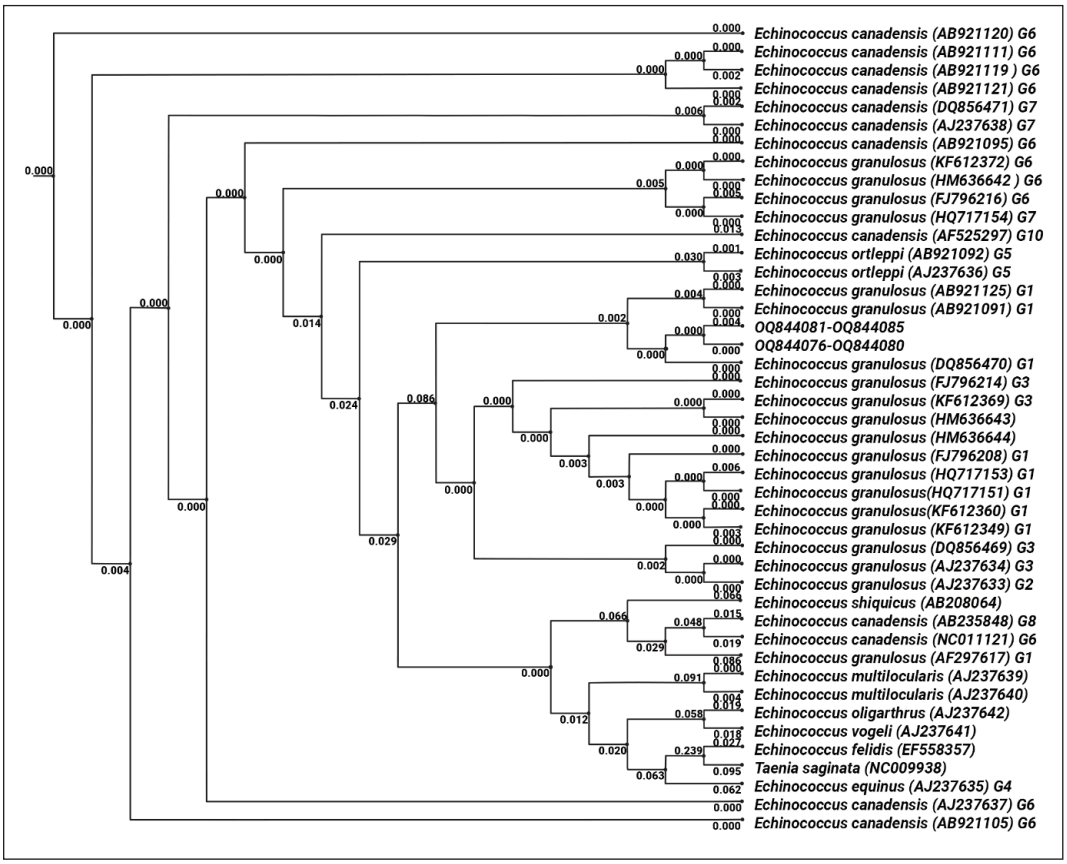

Fig. 2. Neighbor-joining phylogenetic tree of representative *NAD1* nucleotide sequences of *E. granulosus* isolates (OQ844076–OQ844085) and reference sequences. GenBank accession numbers are shown in parentheses.

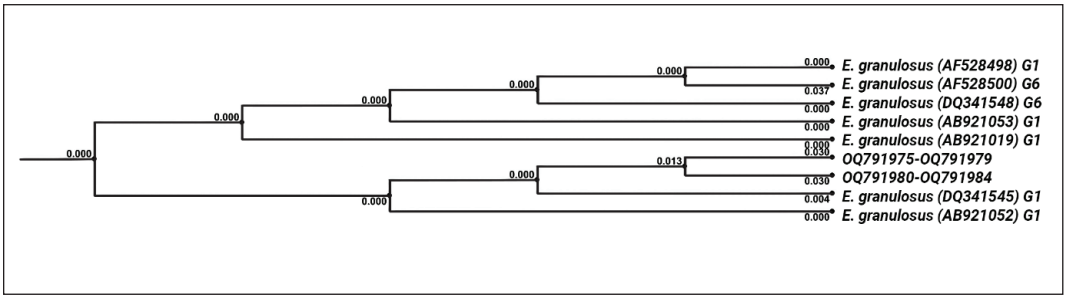

Fig. 3. Neighbor-joining phylogenetic tree of representative *ACT II* nucleotide sequences of *E. granulosus* isolates (OQ791975–OQ791984) and reference sequences. GenBank accession numbers are shown in parentheses.

the deviations in the prevalence of hydatidosis to variations in the sheep strain and differences in the quantity, age, and source of the tested sheep within and outside KSA, as well as cultural variations, community activities, and the availability of dogs.

In this study, we compared the genetic characteristics of hydatid cysts carried by sheep in KSA with those that were recovered from sheep in Egypt. Our results from PCR assays targeting the *COX1, NAD1*, and *ACT II* genes in hydatid cyst DNA samples from sheep in KSA and Egypt were congruent with the findings of Barazesh *et al.* (2019), who compared the genotypic diversity of *E. granulosus* isolates from livestock in Turkey and Iran.

Mitochondrial DNA (mtDNA) sequencing has been effective for the molecular characterization and identification of taeniid tapeworms, notably at the *COX1* and *NAD1* loci (Heidari *et al.*, 2019). For phylogenetic research and the evolution of helminth parasites to detect intraspecific and/or interspecific variation, the *COX1* gene represents the most prevalent mtDNA gene (Paoletti *et al.*, 2019). The mitochondrial *COX1* gene is ideal for identifying genetic variation because the evolutionary change rate of this gene is fast enough to differentiate between various species while remaining slow enough for the same species. Therefore, to create DNA barcodes and differentiate between different species of helminths in the current investigation, the mitochondrial *COX1* gene was selected (Gunyakti Kilinc *et al.*, 2020).

Through targeting the *COX1* gene, all the KSA hydatid cyst isolates from sheep (GenBank accession numbers OQ970593–OQ970597) demonstrated high sequence similarity with the genotype G1 of *E. granulosus* s.s. isolated from sheep and camels (AB921090 and AB921054, respectively) in Egypt, humans (HM636641) in Libya, and cattle in Turkey (HQ717150). Moreover, our results show a substantial degree of resemblance for both sequences from KSA and Egypt with *E. granulosus COX1* recovered from sheep in Taif, KSA (MZ345697) in a previous study by Al Malki and Hussien (2021). These authors, who also used the *COX1* gene sequence for molecular characterization of *E. granulosus* isolated from hydatid cysts in sheep in Taif, KSA, found nucleotide diversity between their isolates and those in the GenBank database that was collected from other countries. BLAST analysis revealed that one of the Taif sheep isolates exhibited the highest sequence alignment identity with *E. granulosus* from Palestinian dog feces (95.67%) and lower sequence identities of 84%–85% with isolates from camels and humans in Egypt, cattle in Turkey, and camels, sheep, and goats in Iran.

Our findings are consistent with those of Metwally *et al.* (2018) in Riyadh (KSA), who reported that sequencing of the *cox1* gene confirmed the presence of *E. granulosus* s.s. (genotypes G1–G3) in 16 out of 17 sheep cysts and 2 out of 27 camel cysts. Moreover, the findings of this study concur with those of Al-Mutairi *et al.* (2020) in Al-Madinah (KSA), who recognized the native sheep cysts as being caused by *E. granulosus s.s.* (G1–G3 complex) owing to their striking resemblance to human cases in Turkey and Iran. For example, the alignment of the KSA hydatid cyst isolate (OQ970593) in this study showed high identity with the isolate of *E. granulosus* (MN720282) in Al-Madinah (KSA). All *E. granulosus* s.s. isolates, including those from camels killed in abattoirs in Al-Ahsa (KSA) were related to the G1 cluster, despite the fact that the G3 genotype had previously been described from the Middle East by Al-Hizab *et al.* (2018). Accordingly, among animal isolates in KSA, the G1–G3 cryptic species are more common.

Similar trends with different G1 and G3 fractions have been described in other studies. For example, in a Tunisian study of 30 cysts from sheep, cattle, and humans, G1 constituted 93.3% of these CE cases and G3 constituted 6.7% of isolates (M'rad *et al.*, 2010); in a Turkish study of 112 sheep and cattle, 95.5% and 4.5% of CE were G1 and G3, respectively (Vural *et al.*, 2008); and in a study performed on animals in southeastern Iran, 73.7% and 13.2% of CE cases were G1 and G3, respectively (de la Rue *et al.*, 2011).

G1 is also the common genotype in different hosts in Palestine (Adwan *et al.*, 2013), Ethiopia (Hailemariam *et al.*, 2012), India (Sharma *et al.*, 2013), Tunisia (Farjallah *et al.*, 2007), China (Yan *et al.*, 2013), and Iran (Rostami Nejad *et al.*, 2012; Pezeshki *et al.*, 2013; Nikmanesh *et al.*, 2014). Furthermore, in several Latin American and European nations, *E. granulosus* s.s. (G1–G3 complex) is the predominant genotype in humans, sheep, goats, and cattle (Beato *et al.*, 2010; Piccoli *et al.*, 2013).

No G6 isolates were found among the KSA isolates in our study; while the G6 genotype has been reported in different countries such as Egypt (Aaty *et al.*, 2012), Sudan (Omer *et al.*, 2010), Iran (Karamian *et al.*, 2017; Mohaghegh *et al.*, 2019), Turkey (Mehmood *et al.*, 2020), and Argentina (Debiaggi *et al.*, 2023). This finding was concordant with research conducted in KSA by Metwally *et al.* (2018). According to Omar *et al.* (2013), the one-humped camel performs a significant role in the epidemiology of *Echinococcus* and the camel genotype (G6) is the most dominant genotype of *E. granulosus* in Egypt. However, further research is necessary (Abdel-Aziz and El-Meghanawy, 2016). According to several studies, the G6 genotype is not thought to play a vital role in public health (Santivañez *et al.*, 2008; Casulli *et al.*, 2010), although it was recently reported that this genotype occurs more frequently than was indicated in earlier studies (M'Rad *et al.*, 2005).

When DNA sequencing was used to target the *Cox1* gene, all of the Egyptian sheep isolates in this study (OQ970598–OQ970602) revealed a high similarity with *E. canadensis* genotype G6 recovered from camels in Africa (M84666), camels and cattle in Libya (HM636638), and humans in Iran (KF612400). This finding is in line with those of Barghash *et al.* (2017), who confirmed the prevalence of *E. canadensis* G6 (camel strain) in sheep in Great Cairo, the West Delta, and Upper Egypt with the potential for human infection. The prevalent genotype among humans, sheep, goats, cattle, and camels in Sudan is G6 (Ibrahim *et al.*, 2011; Ahmed *et al.*, 2013).

In summary, the results of this study suggest that humans and livestock animals may share the G6 genotype. This finding is congruent with those of Barghash *et al.* (2017), Azab *et al.* (2004), and Derbala (2004), who discovered that camel strain G6 was responsible for human instances of CE.

For *nad1* sequencing, the KSA isolates (OQ844076–OQ844080) and the Egyptian isolates from hydatid cysts (OQ844081–OQ844085) revealed a high similarity with *E. granulosus* s.s. genotype G1 recovered from sheep and camels in Egypt (AB921125 and AB921091) and sheep in Greece (DQ856470). This agreed with the *Cox1* gene sequencing studies stated above and the findings of Al-Hizab *et al.* (2018) in KSA, Abd El Baki *et al.* (2009), and Barghash *et al.* (2017) in Egypt, and Barazesh *et al.* (2019) in Iran, demonstrating that genotype G1 is widespread in humans, sheep, and camels.

G1 is the only genotype found exclusively in humans in Libya, with nearly no evidence of G1 in cattle (Abushhewa *et al.*, 2010). Previous molecular studies in Iran on sequence variation in the *nad1* and *cox1* genes not only demonstrated the presence of G1 strains, but also G6 strains of *E. granulosus* in humans and several intermediate hosts such as sheep, camels, and cattle.

It is generally recognized that the *E. granulosus* sheep strain (G1) is the predominant species of the genus *Echinococcus* involved in human CE and that the camel strain (G6) also plays a role (Torgerson and Budke, 2003; Magambo *et al.*, 2006). According to Barghash *et al.* (2017), the G1 strain of *E. granulosus* could infrequently infect sheep, goats, cattle, and occasionally camels in Upper Egypt and the West Delta, whereas the camel strain (G6) was dominant in the Great Cairo. Al-Hizab *et al.* (2018) reported work on native camels in the east of KSA targeting the *nad1* gene sequence, and revealed that the mainstream belonged to *E. granulosus* s.s. and very few to *E. canadensis* G6/7. Our results demonstrate the high likelihood of movement of genotypes G1 and G6 between humans, camels, and sheep.

The *act II* gene sequences in the KSA hydatid cyst isolates (OQ791975–OQ791979) and the Egyptian isolates (OQ791980–OQ791984) revealed a significant degree of similarity with *E. granulosus* s.s. genotype G1 recovered from sheep in Egypt (AB921052) and Algeria (DQ341545), respectively.

Partial sequencing of the *ACT II* gene in sheep in KSA and Egypt has yielded limited data. Only Amer *et al.* (2015) in Egypt verified that three out of seven sheep cysts had the G6 genotype of *E. canadensis*, as demonstrated by direct sequencing of the *ACT II* gene; in contrast, four of seven cysts from sheep had the *E. granulosus* s.s. G1 genotype.

Conclusion

E. granulosus s.s. (G1) and *E. canadensis* (G6) were the genotypes with the greatest zoonotic significance in sheep in KSA and Egypt in this study. The determination of the precise genotypes present in sheep in our study provides vital data and paves the way for the implementation of effective preventive measures and therapeutic approaches in the many animal populations impacted by CE. The epidemiology of the isolates in KSA and Egypt can be determined with the use of further research, *E. granulosus* strain genotyping, and phylogeny. Having more data available will help public health authorities to better manage and prevent infection.

Acknowledgment

The authors would like to thank Dr. Khaled Abd EL-Hamid (Prof. of Veterinary Diseases (Biotechnology)-National Research Center [NRC], Cairo, Egypt) for his

assistance in analyzing the results of the research and for providing the samples of *Echinococcus granulosus* from Egypt.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work was supported by the Deanship of Scientific Research at Jouf University [grant number 39/825].

Data availability

The data of the current study are available.

References

Aaty, H.A., Abdel-Hameed, D.M., Alam-Eldin, Y.H., El-Shennawy, S.F., Aminou, H.A., Makled, S.S. and Darweesh, S.K. (2012). Molecular genotyping of *Echinococcus granulosus* in animal and human isolates from Egypt. Acta Trop. 121(2), 125–128.

Abdel-Aziz, A.R. and El-Meghanawy, R.A. (2016). Molecular characterization of hydatid cyst from Egyptian one humped camels (*Camelus dromedaries*). PSM Vet. Res. 01, 13–16.

Abdel-Baki, A.S., Almalki, E., Al-Quarishy, S. (2018). Prevalence and characterization of hydatidosis in Najdi sheep slaughtered in Riyadh city, Saudi Arabia. Saudi J. Biol. Sci. 25, 1375–1379.

Abd El Baki, M.H., El Missiry, A.M., Abd El Aaty, H.E., Mohamad, A.A. and Aminou, H.A. (2009). Detection of G1 genotype of human cystic echinococcosis in Egypt. J. Egypt. Soc. Parasitol. 39, 711–721.

Abushhewa, M.H., Abushhiwa, M.H., Nolan, M.J., Jex, A.R., Campbell, B.E., Jabbar, A. and Gasser, R.B., 2010. Genetic classification of *Echinococcus granulosus* cysts from humans, cattle and camels in Libya using mutation scanning-based analysis of mitochondrial loci. Mol. Cell. Probes. 24, 346–351.

Adwan, G., Adwan, K., Bdir, S. and Abuseir, S. (2013). Molecular characterization of *Echinococcus granulosus* isolated from sheep in Palestine. Exp. Parasitol. 134, 195–199.

Ahmed, M.E., Eltom, K.H., Musa, N.O., Ali, I.A., Elamin, F.M., Grobusch, M.P. and Aradaib, I.E. (2013). First report on circulation of *Echinococcus ortleppi* in the one humped camel (*Camelus dromedaries*), Sudan. BMC Vet. Res. 9, 127.

Al-Hizab, F.A., Hamouda, M.A., Amer, O.H., Edris, A.M., Ibrahim, A.M., Abdel-Raheem, S.M. and El-Ghareeb, W.R. (2018). Hepatic cystic echinococcosis in camels of Saudi Arabia: prevalence, risk factors and economic loss. J. Camel Pract. Res. 25, 295–302.

Almalki, E., Al-Quarishy, S. and Abdel-Baki, A.S. (2017). Assessment of prevalence of hydatidosis in slaughtered Sawakny sheep in Riyadh city, Saudi Arabia. Saudi J. Biol. Sci. 24, 1534–1537.

Al-Malki, J.S. and Degheidy, N.S., 2013. Epidemiological studies of hydatidosis among slaughtered sheep and human in Taif, Saudi Arabia. AVMJ. 59, 45–50.

Al Malki, J.S. and Hussien, N.A. (2021). Molecular characterization and phylogenetic studies of *Echinococcus granulosus* and *Taenia multiceps coenurus* cysts in slaughtered sheep in Saudi Arabia. Open Life Sci. 16, 1252–1260.

Al-Mutairi, N.M., Taha, H.A. and Nigm, A.H. (2020). Molecular characterization of *Echinococcus granulosus* in livestock of Al-Madinah (Saudi Arabia). J. Helminthol. 94, e157.

Alsulami, M. 2019. Prevalence and histopathological study on cystic hydatidosis in heart and spleen of goat slaughtered at Makkah, Saudi Arabia. Ann. Parasitol. 65, 225–236.

Alvarez Rojas, C.A., Romig, T. and Lightowlers, M.W. (2014). *Echinococcus granulosus* sensu lato genotypes infecting humans—review of current knowledge. Int. J. Parasitol. 44, 9–18.

Alvi, M.A., Alshammari, A., Ali, R.M.A., Ul Haq, S., Bashir, R., Li, L., Saqib, M., Sajid, M.S., Ghafoor, M., Imran, M. and Ijaz, M.U. (2023). Revealing novel cytb and nad5 genes-based population diversity and benzimidazole resistance in *Echinococcus granulosus* of bovine origin. Front. Vet. Sci. 10, 1191271.

Amer, O.H., Haouas, N., Al-Hathal, E.A.R., El-Shikh, I. and Ashankyty, I. (2018). Cystic echinococcosis in slaughtered animals in Ha'il, Northwestern Saudi Arabia. Jpn. J. Vet. Res. 66, 289–296.

Amer, S., Helal, I.B., Kamau, E., Feng, Y. and Xiao, L. (2015). Molecular characterization of *Echinococcus granulosus* sensu lato from farm animals in Egypt. PLoS One 10, e0118509.

Amini-Bavil-Olyaee, S., Alavian, S.M., Adeli, A., Sarrami-Forooshani, R., Sabahi, F., Sabouri, E., Tavangar, H.R., Azizi, M. and Mahboudi, F. (2006). Hepatitis B virus genotyping, core promoter, and precore/core mutations among Afghan patients infected with hepatitis B: a preliminary report. J. Med. Virol. 78, 358–364.

Azab, M.E., Bishara, S.A., Helmy, H., Oteifa, N.M., El-Hoseiny, L.M., Ramzy, R.M. and Ahmed, M.A.B. (2004). Molecular characterization of Egyptian human and animal *Echinococcus granulosus* isolates by RAPD-PCR technique. J. Egypt. Soc. Parasitol. 34, 83–96.

Barazesh, A., Sarkari, B., Sarısu, G., Hami, M., Mikaeili, F., Aydın, A., Ekici, A., Ebrahimi, S. (2019). Comparative genotyping of *Echinococcus granulosus* infecting livestock in Turkey and Iran. Turkiye Parazitol. Derg. 43, 123–129.

Barghash, S.M., El Sayed, R.A., El-Alfy, N.M., Abou-Elnour, B.M., El-Kattan, A.M. and Sadek, A.M. (2017). Prevalence and molecular identification of *Echinococcus granulosus* in humans and slaughtered animals in Egypt. Eur. J. Biomed. Pharm. Sci. 4, 34–42.

Beato, S., Parreira, R., Calado, M. and Grácio, M.A. (2010). Apparent dominance of the G1–G3 genetic cluster of *Echinococcus granulosus* strains in the central inland region of Portugal. Parasitol. Int. 59, 638–642.

Bonardi, M., Dellabianca, C., Della Valle, V., Valentini, A., Raineri, C. and Dore, R. (2012). Hydatid cyst of the cardiac interventricular septum. Int. J. Cardiol. 158, e45–e46.

Borji, H., Azizzadeh, M. and Kamelli, M. (2012). A retrospective study of abattoir condemnation due to parasitic infections: economic importance in Ahwaz, southwestern Iran. J. Parasitol. 98, 954–957.

Bowles, J., Blair, D. and McManus, D.P. (1992). Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol. Biochem. Parasitol. 54, 165–173.

Bowles, J. and McManus, D.P. (1993). NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. Int. J. Parasitol. 23, 969–972.

Cardona, G.A. and Carmena, D. (2013). A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. Vet. Parasitol. 192, 10–32.

Carmena, D. and Cardona, G.A. (2014). Echinococcosis in wild carnivorous species: epidemiology, genotypic diversity, and implications for veterinary public health. Vet. Parasitol. 202, 69–94.

Casulli, A., Zeyhle, E., Brunetti, E., Pozio, E., Meroni, V., Genco, F. and Filice, C. (2010). Molecular evidence of the camel strain (G6 genotype) of *Echinococcus granulosus* in humans from Turkana, Kenya. Trans. R. Soc. Trop. Med. Hyg. 104, 29–32.

da Silva, C.M., Henrique, H.B., Ferreira, B., Picón, M., Gorfinkiel, N., Ehrlich, R. and Zaha, A. (1993). Molecular cloning and characterization of actin genes from *Echinococcus granulosus*. Mol. Biochem. Parasitol. 60, 209–219.

Debiaggi, M.F., Rojas, C.A.A., Lazzarini, L.E., Calfunao, D., Titanti, P., Calanni, L., Iacono, M., Soriano, S.V., Deplazes, P. and Pierangeli, N.B. (2023). Differences in clinical aspects of human cystic echinococcosis caused by *Echinococcus granulosus sensu stricto* and the G6 genotype in Neuquén, Argentina. Parasitology 150(7), 599–605.

de la Rue, M.L., Takano, K., Brochado, J.F., Costa, C.V., Soares, A.G., Yamano, K., Yagi, K., Katoh, Y. and Takahashi, K. (2011). Infection of humans and animals with *Echinococcus granulosus* (G1 and G3 strains) and *E. ortleppi* in Southern Brazil. Vet. Parasitol. 177, 97–103.

Derbala, A.A. Current status of hydatidosis/ echinococcosis: guidelines for surveillance, siology, diagnosis and recommendations for prevention and control in Egypt. In: 29th World Congress of the World Small Animal Vet. Ass., Rhodes, Greece, 2004, p 6–9.

Dowling, P.M., Abo-Shehada, M.N. and Torgerson, P.R. (2000). Risk factors associated with human cystic echinococcosis in Jordan: results of a case-control study. Ann. Trop. Med. Parasitol. 94, 69–75.

Eckert, J. and Deplazes, P. (2004). Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin. Microbiol. Rev. 17, 107–135.

Eckert, J., Gemmell, M.A., Meslin, F., Pawłowski, Z.S., WHO/OIE. Manual on echinococcosis in humans and animals: a public health problem of global concern. World Organisation for Animal Health (Office International des Épizooties) and World Health Organization, Geneva, Switzer, pp 1–250, 2001.

El-Ghareeb, W.R., Edris, A.M., Alfifi, A.E. and Ibrahim, A.M. (2017). Prevalence and histopathological studies on hydatidosis among sheep carcasses at Al-Ahsa, Saudi Arabia. AJVS. 55, 146–153.

Eslami, A., Meshgi, B., Jalousian, F., Rahmani, S. and Salari, M.A. (2016). Genotype and phenotype of *Echinococcus granulosus* derived from wild sheep (*Ovis orientalis*) in Iran. Korean J. Parasitol. 54, 55–60.

Farhadi, M., Fazaeli, A. and Haniloo, A. (2015). Genetic characterization of livestock and human hydatid cyst isolates from northwest Iran, using the mitochondrial *cox1* gene sequence. Parasitol. Res. 114, 4363–4370.

Farjallah, S., Busi, M., Mahjoub, M.O., Slimane, B.B., Said, K. and D'Amelio, S. (2007). Molecular characterization of *Echinococcus granulosus* in Tunisia and Mauritania by mitochondrial rrnS gene sequencing. Parassitologia. 49, 239–246.

Fdaladdin, Y.A.J., Alsaggaf, A.I. and Wakid, M.H. (2018). Comparative epidemiological studies on echinococcosis of local and imported livestock in Al-Madina Al-Munawwarah in Saudi Arabia. Egypt. J. Hosp. Med. 50, 108–126.

GASTAT. (2021). Agriculture, water, and environment. In: General authority for statistics. Statistical yearbook of 2018. Available via https://www.stats.gov.sa/en/1011 (Accessed 3 March 2021),

Gunyakti Kilinc, S., Celik, F., Kesik, H.K. and Simsek, S. (2020). *In silico* analysis of the biodiversity and conservation status of mitochondrial cytochrome C oxidase subunit 1 (*CO1*) gene of *Taenia multiceps*. Acta Parasitol. 65, 852–858.

Hailemariam, Z., Nakao, M., Menkir, S., Lavikainen, A., Yanagida, T., Okamoto, M. and Ito, A. (2012). Molecular identification of unilocular hydatid cysts from domestic ungulates in Ethiopia: implications for human infections. Parasitol. Int. 61, 375–377.

Haroun, E.M., Omer, O.H., Mahmoud, O.M. and Draz, A. (2008). Serological studies on cystic

echinococcosis in camels in Saudi Arabia. Res. J. Vet. Sci. 1, 71–73.

Hayajneh, F.M.F., Althomali, A.M.H. and Nasr, A.T.M. (2014). Prevalence and characterization of hydatidosis in animals slaughtered at Al Taif abattoir, Kingdom of Saudi Arabia. Open J. Anim. Sci. 4, 38–41.

Heidari, Z., Sharbatkhori, M., Mobedi, I., Mirhendi, S.H., Nikmanesh, B., Sharifdini, M., Mohebali, M., Zarei, Z., Arzamani, K. and Kia, E.B. (2019). *Echinococcus multilocularis* and *Echinococcus granulosus* in canines in North-Khorasan Province, northeastern Iran, identified using morphology and genetic characterization of mitochondrial DNA. Parasit. Vectors. 12, 606.

Ibrahim, M.M. (2010). Study of cystic echinococcosis in slaughtered animals in Al Baha region, Saudi Arabia: interaction between some biotic and abiotic factors. Acta Trop. 113, 26–33.

Ibrahim, K., Thomas, R., Peter, K. and Omer, R.A. (2011). A molecular survey on cystic echinococcosis in Sinnar area, Blue Nile state (Sudan). Chin. Med. J. (Engl.). 124, 2829–2833.

Ito, A., Nakao, M. and Sako, Y. (2007). Echinococcosis: serological detection of patients and molecular identification of parasites. Future Microbiol. 2, 439–449.

Karamian, M., Haghighi, F., Hemmati, M., Taylor, W.R., Salehabadi, A. and Ghatee, M.A. (2017). Heterogenity of *Echinococcus canadensis* genotype 6− the main causative agent of cystic echinococcosis in Birjand, Eastern Iran. Vet. Parasitol. 245, 78–85.

Latif, A.A., Tanveer, A., Maqbool, A., Siddiqi, N., Kyaw-Tanner, M.K. and Traub, R.J. (2010). Morphological and molecular characterisation of *Echinococcus granulosus* in livestock and humans in Punjab, Pakistan. Vet. Parasitol. 170, 44–49.

Magambo, J., Njoroge, E. and Zeyhle, E. (2006). Epidemiology and control of echinococcosis in sub-Saharan Africa. Parasitol. Int. 55, S193–S195.

Mazyad, S.A., Mahmoud, L.H. and Hegazy, M.M. (2007). *Echinococcosis granulosus* in stray dogs and Echino-IHAT in the hunters in Cairo, Egypt. J. Egypt. Soc. Parasitol. 37, 523–532.

McManus, D.P. (2010). Echinococcosis with particular reference to Southeast Asia. Adv. Parasitol. 72, 267–303.

McManus, D.P. and Thompson, R.C.A. (2003). Molecular epidemiology and taxonomy of cystic echinococcosis. Parasitology. 127, S37–S51.

Mehmood, S., Simsek, S., Celik, F., Kesik, H.K., Kilinc, S.G. and Ahmed, H. (2020). Molecular survey on cattle and sheep hydatidosis and first detection of *Echinococcus canadensis* (G6/G7) in sheep in Turkey. Parasitology 147(9), 1055–1062.

Metwally, D.M., Qassim, L.E., Al-Turaiki, I.M., Almeer, R.S., El-Khadragy, M.F., (2018). Gene-based molecular analysis of *Cox1* in *Echinococcus granulosus* cysts isolated from naturally infected livestock in Riyadh, Saudi Arabia. PLoS One. 13, e0195016.

Mohaghegh, M.A., Yousofi-Darani, H., Jafarian, A.H., Mirbadie, S.R., Fasihi-Harandi, M., Ghavimi, R., Jabalameli, Z., Azami, M., Mohammadi, M. and Hejazi, S.H. (2019). Isolated human and livestock *Echinococcus granulosus* genotypes using real-time PCR of *cox1* gene in Northeast Iran. Acta Parasitologica. 64, 679–685.

M'Rad, S., Filisetti, D., Oudni, M., Mekki, M., Belguith, M., Nouri, A., Sayadi, T., Lahmar, S., Candolfi, E., Azaiez, R., Mezhoud, H. and Babba, H. (2005). Molecular evidence of ovine (G1) and camel (G6) strains of *Echinococcus granulosus* in Tunisia and putative role of cattle in human contamination. Vet. Parasitol. 129, 267–272.

M'rad, S., Oudni-M'rad, M., Filisetti, D., Mekki, M., Nouri, A. and Sayadi, T. (2010). Molecular identification of *Echinococcus granulosus* in Tunisia: first record of the Buffalo strain (G3) in human and bovine in the country. Open Vet. Sci. J. 4, 27–30.

Nakao, M., Lavikainen, A., Yanagida, T. and Ito, A. (2013). Phylogenetic systematics of the genus *Echinococcus* (*Cestoda: Taeniidae*). Int. J. Parasitol. 43, 1017–1029.

Nakao, M., McManus, D.P., Schantz, P.M., Craig, P.S. and Ito, A. (2007). A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. Parasitology. 134, 713–722.

Nikmanesh, B., Mirhendi, H., Ghalavand, Z., Alebouyeh, M., Sharbatkhori, M., Kia, E., Mohebali, M., Eghbali, M. and Rokni, M.B. (2014). Genotyping of *Echinococcus granulosus* isolates from human clinical samples based on sequencing of mitochondrial genes in Iran, Tehran, Iran. J. Parasitol. 9, 20–27.

Omer, R.A., Dinkel, A., Romig, T., Mackenstedt, U., Elnahas, A.A., Aradaib, I.E., Ahmed, M.E., Elmalik, K.H. and Adam, A. (2010). A molecular survey of cystic echinococcosis in Sudan. Vet. Parasitol. 169(3-4), 340–346.

Omar, M., Sultan, K., Haridy, M. and Omran, A. (2013). Prevalence of cystic *echinococcosis* in slaughtered ruminants in different abattoirs, Upper Egypt. Am. J. Anim. Vet. Sci. 8, 117–121.

Paoletti, B., Della Salda, L., Di Cesare, A., Iorio, R., Vergara, A., Fava, C., Olivastri, A., Dessì, G., Scala, A. and Varcasia, A. (2019). Epidemiological survey on cystic echinococcosis in wild boar from Central Italy. Parasitol. Res. 118, 43–46.

Pezeshki, A., Akhlaghi, L., Sharbatkhori, M., Razmjou, E., Oormazdi, H., Mohebali, M. and Meamar, A.R. (2013). Genotyping of *Echinococcus granulosus* from domestic animals and humans from Ardabil

Province, northwest Iran. J. Helminthol. 87, 387–391.

Piccoli, L., Bazzocchi, C., Brunetti, E., Mihailescu, P., Bandi, C., Mastalier, B., Cordos, I., Beuran, M., Popa, L.G., Meroni, V., Genco, F. and Cretu, C. (2013). Molecular characterization of *Echinococcus granulosus* in south-eastern Romania: evidence of G1–G3 and G6–G10 complexes in humans. Clin. Microbiol. Infect. 19, 578–582.

Rostami Nejad, M.R., Taghipour, N., Nochi, Z., Mojarad, E.N., Mohebbi, S.R., Harandi, M.F. and Zali, M.R. (2012). Molecular identification of animal isolates of *Echinococcus granulosus* from Iran using four mitochondrial genes. J. Helminthol. 86, 485–492.

Sadjjadi, S.M. (2006). Present situation of echinococcosis in the Middle East and Arabic North Africa. Parasitol. Int. 55, S197–S202.

Santivañez, S.J., Gutierrez, A.M., Rosenzvit, M.C., Muzulin, P.M., Rodriguez, M.L., Vasquez, J.C., Rodriguez, S., Gonzalez, A.E., Gilman, R.H., Garcia, H.H., Cysticercosis Working Group in Peru. (2008). Human hydatid disease in Peru is basically restricted to *Echinococcus granulosus* Genotype G1. Am. J. Trop. Med. Hyg. 79, 89–92.

Sharma, M., Fomda, B.A., Mazta, S., Sehgal, R., Singh, B.B. and Malla, N. (2013). Genetic diversity and population genetic structure analysis of *Echinococcus granulosus* sensu stricto complex based on mitochondrial DNA signature. PLoS One 8, e82904.

Singh, B.B., Dhand, N.K., Ghatak, S. and Gill, J.P. (2014). Economic losses due to cystic echinococcosis in India: need for urgent action to control the disease. Prev. Vet. Med. 113, 1–12.

Thompson, R.C. and McManus, D.P. (2002). Towards a taxonomic revision of the genus *Echinococcus*. Trends Parasitol. 18, 452–457.

Torgerson, P.R. and Budke, C.M. (2003). Echinococcosis–an international public health challenge. Res. Vet. Sci. 74, 191–202.

Toulah, F.H., El Shafei, A.A. and Alsolami, M.N. (2012). Prevalence of hydatidosis among slaughtered animals in Jeddah, Kingdom of Saudi Arabia. J. Egypt. Soc. Parasitol. 42, 563–572.

Toulah, F.H., El Shafi, A.A., Alsolami, M. and Wakid, M. (2017). Hydatidosis among imported animals in Jeddah, Saudi Arabia. J. Liver Clin. Res. 4, 1031.

Vural, G., Baca, A.U., Gauci, C.G., Bagci, O., Gicik, Y. and Lightowlers, M.W. (2008). 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. Vet. Parasitol. 154, 347–350.

WHO/FAO/UNEP Guidelines for surveillance, prevention and control of echinococcosis, / Hydatidosis Document No. VPH/81.28. World Health Organization, Geneva, Switzerland, 1994.

Yan, N., Nie, H.M., Jiang, Z.R., Yang, A.G., Deng, S.J., Guo, L., Yu, H., Yan, Y.B., Tsering, D., Kong, W.S., Wang, N., Wang, J.H., Xie, Y., Fu, Y., Yang, D.Y., Wang, S.X., Gu, X.B., Peng, X.R. and Yang, G.Y. (2013). Genetic variability of *Echinococcus granulosus* from the Tibetan Plateau inferred by mitochondrial DNA sequences. Vet. Parasitol. 196, 179–183.