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Molecular Characterization of *Echinococcus granulosus* sensu stricto Cysts of Domestic Ruminants in Jordan

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Abstract

The semi-nested PCR system and the mitochondrial cytochrome oxidase subunit 1 (*COX1*) partial gene sequencing were used to identify the species/genotypes of 139 hydatid cyst isolates collected from the livers and/or lungs of twenty-nine indigenous ruminant animals (seventeen sheep, three goats, six cattle and three camels) in Jordan. All the examined hydatid cyst isolates identity were *Echinococcus granulosus* sensu stricto (s.s.) which was known formerly as G1-G3 strains. *COX1* partial gene sequencing of twelve selected isolates of hydatid cysts from various intermediate hosts verified their identity as *E. granulosus* s.s. with sequences published in GenBank databases. A unique DNA sample showed two bands within the *COX1* partial gene PCR amplification. The first one was identical to an already published *E. granulosus* s.s. sequence, while the other band had 78 % identity to *E. granulosus* s.s., and 81 % to *E. vogeli COX1* partial gene sequence with two truncated regions (14 and 32 bp) in both species. The present molecular characterization and identification of the hydatid cyst isolates as *E. granulosus* s.s., from indigenous ruminant animals in Jordan confirmed earlier reports that used morphological, *in vitro* and *in vivo* techniques on the existence of *E. granulosus* s.s. G1 strain as the dominant species/genotype in Jordan.

Keywords: Echinococcosis, *Echinococcus granulosus* s.s., Semi-nested PCR, Mitochondrial *COX1* Gene Sequencing.

1. Introduction

Echinococcosis is a cosmopolitan zoonotic helminthic disease caused by a small tapeworm of the genus *Echinococcus* (Cestoda: Taeniidae) that cycles between canid or felid definitive hosts and various herbivores or rodents as intermediate hosts. Humans are accidental hosts that can be infected with the larval stage of the parasite in various internal organs rendering the disease as a major public health risk. The disease has been recently included by the WHO as part of the neglected zoonosis that warrants the implementation of control programs (Da Silva, 2010; Siracusano *et al.*, 2012; Nakao *et al.*, 2013a). Three different forms of echinococcosis are well-recognized in the intermediate hosts and humans: multilocular or alveolar echinococcosis caused by *E. multilocularis*, polycystic echinococcosis caused by *E. vogeli*, and unilocular or cystic echinococcosis (CE) caused by several *E. granulosus* species/strain genotype complex (Moro and Schantz, 2009; Torgerson, 2014).

Currently, several valid *Echinococcus* species are known to cause CE in various herbivorous animals. These include: *E. granulosus* sensu stricto (s.s.), *E. canadensis*, *E. equinus*, and *E. ortleppi* (Nakao *et al.*, 2007; Mcmanus, 2013; Romig *et al.*, 2015). *E. oligarthra* uses wild felids as definitive hosts and agoutis rodents as intermediate hosts and causes unilocular CE. Moreover, *E. shiquicus* has been verified as a distinct species causing unilocular CE in the Tibetan plateau of China (Xiao *et al.*, 2006), while the nature of cysts caused by *E. felidis* has not been elucidated yet. The latter species uses the lion as a definitive host, but its intermediate host is unknown (Huttner *et al.*, 2008).

Echinococcus granulosus manifests great intraspecific phenotypic variations in relation to the host specificity, adult and larval stage morphology, *in vitro* and *in vivo* development, biochemical composition, antigenicity and pathogenicity (Mcmanus, 2013). These variations are reflected by the genetic makeup of nucleic acid sequences that lead to the appearance and differentiation of several genotypes, genetic variants, strains within this species, some of which have now been raised to the level of new species (Mcmanus, 2013; Nakao *et al.*, 2013a; 2013b; Rojas *et al.*, 2014). The current knowledge of the *E. granulosus* sensu lato (s.l.) genotypes and/or species has been reviewed recently. It is well-established now that *E. granulosus* s.l. comprises ten genotypes (G1-G10) (Bowles *et al.*, 1992; Bowles and Mcmanus, 1993a, 1993b; Bowles *et al.*, 1994; Scott *et al.*, 1997; Lavikainen *et al.*, 2003). Accordingly, *E. granulosus* s.l. has been revised to include

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several species that correspond to the previously identified genotypes as follows: *E. granulosus* s.s. (G1- cosmopolitan sheep strain, G2- Tasmanian sheep strain, and G3- buffalo strain), *E. equinus* (G4- sporadic horse strain), *E. ortleppi* (G5- sporadic cattle strain), *E. canadensis* (G6- sporadic camel strain, G7- sporadic pig strain, G8- northern arctic cervid strain, G10- Fennoscandanian cervid strain). As for the G9 (Poland swine strain), its status is still uncertain (Mcmanus and Thompson, 2003; Mcmanus, 2013; Nakao *et al.*, 2013a; 2013b; Rojas *et al.*, 2014; Romig *et al.*, 2015).

Several previous studies indicated that the cosmopolitan common sheep/dog strain (G1 genotype) is the dominant strain in Jordan (Al-Qaoud *et al.*, 2003a; Yanagida *et al.*, 2012). However, other *E. granulosus* strains were reported in Jordan. These include: the horse and camel strains. The horse strain was identified in donkeys using *in vitro* parasite development (Hijjawi *et al.*, 1992), *in vivo* secondary hydatid cyst development in mice (Al-Abbasi and Abdel-Hafez, unpublished data), as well as the RAPD-PCR and RFLP-PCR techniques (Al-Qaoud *et al.*, 2003a). The existence of a camel strain was indicated using morphological criteria of metacestode protoscolices (PSCs) (Said *et al.*, 1988). The present study aimed at the identification and molecular characterization of *E. granulosus* metacestode stage infecting indigenous domestic ruminants in Jordan using semi-nested PCR and partial sequencing of the mitochondrial *COX1* gene.

2. Materials and Methods

2.1. Sample Collection and PSCs Isolation

Some 139 unilocular hydatid cysts were collected from the livers (L) and/or lungs (g) of twenty-nine indigenous livestock animals slaughtered in four different abattoirs located in the cities of Irbid, Ramtha, Jerash (northern Jordan) and Amman (central Jordan) (Table 1). Both fertile and sterile hydatid cysts were isolated from the livers and/or the lungs of indigenous sheep, goats, cattle and camels. Protoscolices (PSCs) and/ or the germinal layer tissues (GL) were isolated under aseptic conditions from each single cyst within six hours after the death of the host as described previously (Nasrieh and Abdel-Hafez, 2004).

2.2. DNA Isolation

DNA was isolated from eighty-four PSCs of fertile cysts and fifty-five germinal layer tissues of sterile cysts (Table 1). PSCs DNA was isolated according to Dinkel *et al.* (1998) using phenol-chloroform-isoamyl alcohol (25:24:1) and ethanol precipitation protocol. Precipitated DNA was re-suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) and stored at -20°C for further use. However, the GL tissue DNA isolation was performed using the Cetyl Trimethyl Ammonium Bromide (CTAB) protocol with minor modifications. Primarily, 0.3g GL tissue was digested in liquid nitrogen using small mortar and pestle, and then the mixture was re-suspended in warmed 2-mercaptoethanol/CTAB extraction buffer, and then the plant extraction protocol was followed (Richards *et al.*, 2001).

Table 1. Number of infected animal hosts and hydatid cyst isolates used in the present study, with information on their location and type. DNA samples were extracted from the PSCs of fertile cysts and the germinal membrane of sterile cysts.

Total No. of cysts	No. of sterile cysts	No. of fertile cysts	No. of cyst isolates from		No. of animals	Animal type
			Lung	Liver		
91	15	76	26	65	17	Sheep
22	18	4	1	21	3	Goats
13	13	0	12	1	6	Cattle
13	9	4	10	3	3	Camels
139	55	84	49	90	29	All

2.3. Semi-nested Polymerase Chain Reaction (PCR)

To genotype the prepared DNA samples from hydatid cyst isolates, the semi-nested PCR technique as described by Dinkel *et al.* (2004) was adopted with a minor modification (Figure 1). All PCRs target sequences in this system belong to the mitochondrial 12S rRNA gene, but another set of primers specific to the cytochrome b (Cytb) gene were added to each reaction (as multiplex) to serve as control for the DNA and PCR system.

Initially, the PCR reaction was performed to all extracted DNA samples in order to discriminate *E. granulosus* s.s. from the *E. ortleppi* and *E. canadensis* species. The reaction was performed in a 25µl volume containing 1µl DNA template, 1X green GoTaq reaction buffer, 0.2mM of each dNTP, two units GoTaq DNA Polymerase (all from Promega, USA), 0.4 µM of E.g.ss1for. (5´GTA TTT TGT AAA GTT GTT CTA 3´) and E.g.ss1rev. (5´CTA AAT CAC ATC ATC TTA CAA T 3´) primers, and 0.2 µM of F/Cytb (5´GTC AGA TGT CTT ATT GGG CTG C 3´) and R/Cytb (5´TCT GGG TGA CAC CCA CCT AAA TA 3´) primers. The amplification was carried out after initial denaturation step for four minutes at 94 ºC, for thirty-five cycles as follows: denaturation step for thirty seconds at 94 ºC, annealing step for forty seconds at 54ºC, elongation step for forty seconds at 72 ºC and a final elongation step for five minutes at 72 ºC using TRIO thermoblock (Biometra, Germany).

Samples with the PCR amplification product were considered as *E. granulosus* s.s. species, but for the negative samples, another PCR was carried out using the E.g.cs1for. (5′ ATT TTT AAA ATG TTC GTC CTG 3′) and E.g.cs1rev. (5′ CTA AAT AAT ATC ATA TTA CAA C 3′) primers to verify the presence of *E. ortleppi*/*E. canadensis* species. The positive samples of this primer set were confirmed by semi-nested PCR using the E.g.camel.for. (5′ ATG GTC CAC CTA TTA TTT CA 3′) and E.g.cs1rev. (5′ CTA AAT AAT ATC ATA TTA CAA C 3′) primers for *E. canadensis* species and the E.g.cattle.for. (5′ ATG GTC CAC CTA TTA TTT TG 3′) and E.g.cs1rev. (5′ CTA AAT AAT ATC ATA TTA CAA C 3′) primers for *E. ortleppi* species.

Five microliter of each PCR product was applied to a 1.5 % ethidium bromide stained agarose gel, as well as the 100 bp DNA ladder (AppliChem, Germany). DNA was separated using TBE buffer (89 mM Tris-HCl, 89 mM Boric Acid and 2 mM EDTA.Na_2, pH 8.0) at an electric current of 90 V for forty-five minutes. The produced bands

were visualized on a UV transilluminator (Bioblock Scientific, France).

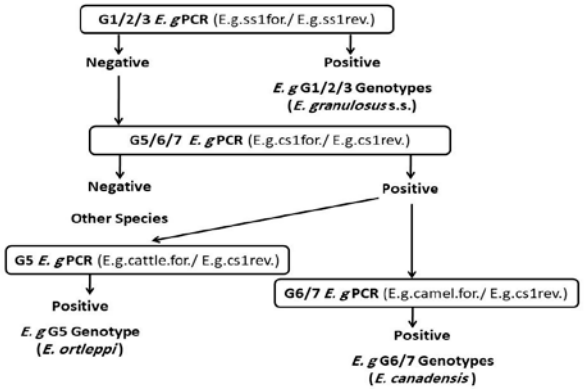

Figure 1. *E. granulosus* s.s., *E. ortleppi* and *E. canadensis* species identification protocol using PCR/semi-nested PCR system (Modified from Dinkel *et al.*, 2004).

2.4. Mitochondrial COX1 Partial Gene Sequencing

Sequencing of a 444 bp segment of the mt *COX1* gene was done for twelve selected samples (1L.9, 3g.3, 11L.2, 13L.3, 14g.6, 16L.3, 18L.4, 19L.3, 22g.3, 23g.4, 26g.2 and 29L.7). The PCR reaction was performed in a 50µL volume containing 1µL DNA template, 1X colorless Taq reaction buffer (Bio-Basic, Canada), 0.4mM of each dNTPs (Promega, USA), 1.5 units Taq DNA Polymerase (Bio-Basic, Canada), 4 mM $MgCl_2$ and 0.2 µM of JB3 (5′ TTT TTT GGG CAT CCT GAG GTT TAT 3′) and JB4.5 (5′ TAA AGA AAG AAC ATA ATG AAA ATG 3′) primers. The amplification was carried out after the initial denaturation step, four minutes at 94 ºC, for thirty-five cycles as follows: denaturation step for thirty seconds at 94 ºC, annealing step for forty seconds at 55 ºC, elongation step for forty seconds at 72 ºC, and a final elongation step for five minutes at 72 ºC (Bowles *et al.*, 1992).

The *COX1* partial gene sense and anti-sense strands were sequenced by ABI 3730XL DNA Analyzer (Applied Biosystems, USA) using BigDye® Terminator V3.1 Sequencing Kit. The sequencing results were analyzed using the BLAST programs and databases of the National Center for Biotechnology Information (NCBI).

3. Results

3.1. All tested samples were E. granulosus s.s. species

Cytb gene target was used to demonstrate the presence of intact DNA within the amplified 139 PSC and GL isolates of hydatid cyst samples. All PCR results were positive for Cytb gene amplification (the 612 bp upper band, Figure 2) except for one sample (sample 15g.1, Fig. 2, lane 16). Moreover, PCR result using mt 12S rRNA gene of the first set of primers (E.g.ss1for./ E.g.ss1rev.) was positive for all samples, which indicates that all the isolated samples were identified as *E. granulosus* s.s. (254 bp, Figure 2). A PSC isolate from camel cysts collected from Iraq in 2003 was used as positive control for *E. canadensis*. DNA from this isolate was negative for the first primer set (absence of 254 bp band, Figure 2, lane 18), but showed positive PCR result for the *E. ortleppi/ E.canadensis* primers (254 bp band, Figure 2, lane 19). Further semi-nested PCR showed that this isolate was positive for the *E. canadensis* set primers (171 bp band, Figure 2, lane 20). None of the isolates from Jordan tested here was positive using the second set of primers (E.g.cs1for./ E.g.cs1rev.).

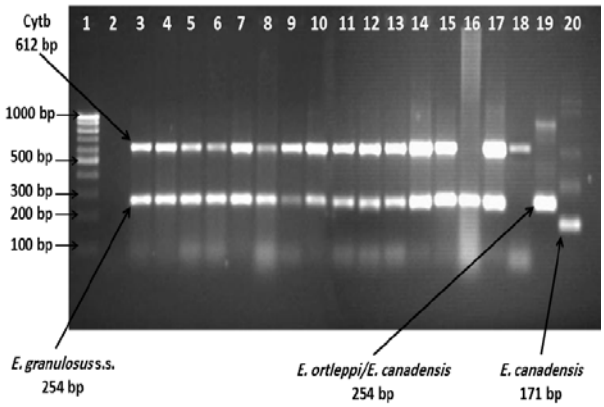

Figure 2. PCR analysis of DNA samples extracted from protoscolices and laminated membranes of selected hydatid cysts from sheep, goat, cattle and camels from Jordan. DNA was amplified using primers specific for 12S rRNA (254 bp lower band) and Cyt b (612 bp upper band) genes. Lane 1: 100 bp DNA marker, lane 2: PCR -ve control, lanes 3 to 17 represent samples from sheep, goat, cattle and camel obtained from liver or lung hydatid cysts, lane 18: 12S rRNA *E. granulosus* s.s. primers -ve control, lane 19: 12S rRNA *E. ortleppi*/*E. canadensis* primers +ve control, and lane 20: 12S rRNA *E. canadensis* primers +ve control.

3.2. Mitochondrial COX1 partial gene sequencing matched the already published sequences in the GenBank

The selected samples representing twelve cysts originated from the lungs and livers of twelve animals were sequenced for the *COX1* partial gene sense and antisense strands. Figure 3 shows the PCR amplification product of the 444 bp of *COX1* partial gene. One sample (13L.3, lane 11) showed a smaller band size. Further analysis of this sample and another one from the same animal (13L.9) revealed the presence of the 444 bp band and an extra band of about 400 bp (Figure 4). All the twelve samples were sequenced including the 400 bp bands of the 13L.3 and 13L.9 samples. Using the BLAST programs and databases of the NCBI, the 444 bp amplification product sequences were compared with *E. granulosus COX1* partial gene sequence published from Jordan sheep/G1 genotype deposited in the EMBL GenBank databases as AB688598.1 (Yanagida *et al.*, 2012). Among the twelve hydatid cysts *COX1* partial gene sequences, four polymorphisms with single-base pair substitution occurred in five DNA samples. Three of them were C to T and one was T to C nucleotide substitution. The other seven DNA samples were completely identical to the reference sequence.

Samples 13L.3 and 13L.9 400 bp band sequence showed higher variations when compared to the original *E. granulosus* s.s. *COX1* partial gene sequence (AB688598.1). The sample sequence showed two main deletions of 14 bp and 32 bp sizes (Figure 5). The sequence shows forty mismatched nucleotides with a max identity of 78 % (311/397 bp) compared to *E. granulosus* sheep/G1 genotype sequence, while twenty-seven mismatched nucleotides were counted compared to other

published *COX1* partial gene sequences, *E. vogeli* (accession number JX315616.1) with a higher identity (81 %, 323/397 bp). The two main deletions were found at the same sites in both reference *E. granulosus* and *E. vogeli* sequences. However, when both *E. granulosus* and *E. vogeli* sequences were aligned, 91 % max identity occurred with thirty-five mismatched nucleotides (Figure 5).

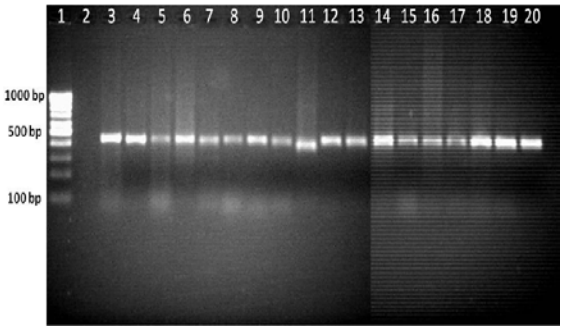

Figure 3. PCR amplification of 444 bp *COX1* for selected hydatid cyst samples from Jordan. Lane 1: 100 bp DNA marker, lane 2: PCR -ve control, lanes 3 to 20 represent samples: 1L.9, 3g.5, 4L.1, 7g.4, 9L.4, 10g.2, 11L.2, 12L.2, 13L.3, 14g.6, 16L.3, 18L.4, 19L.3, 22g.3, 23g.4, 24L.4, 26g.2, and 29L.7, respectively.

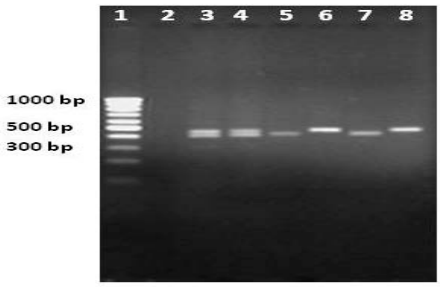

Figure 4. PCR amplification of *COX1* gene for two hydatid cyst samples. Lane 1: 100 bp DNA marker, lane 2: -ve control PCR product, lanes 3&4: *COX1* partial gene PCR amplification product for sample 13L.3 and 13L.9, respectively. Lane 5&7: the purified 400 bp band of the 13L.3 and 13L.9 samples, respectively. Lanes 6&8: the purified 444bp band of the 13L.3 and 13L.9 samples, respectively.

```
E.granulo 1-60      GTGTTGATTTTGCCTGGATTTGGTATAATTAGTCATATTTGTTTGAGTATTAGTGCTAAT
13L.3               GTGTTAATTTTGCCAGGGTTTGGCATAATTAGTCATATTATGTTTGAGAATTAGTGCTAAT
E. vogeli           GTGTTAATTTTGCCTGGGTTTGGTATAATTAGTCATATTATGTTTGAGGGTTAGTGCTAAT

E.granulo 61-120    TTTGATGCGTTTGGGTTCTATGGGTTGTTGTTTGCTATGTTTTCTATAGTGTGTTTGGGT
13L.3               TTGGATGCATTTGGGTTTTATGGGTTGTTATTTGCTATGTTTTCTATAGTAT-------A
E. vogeli           TTGGATGTGTTTGGGTTTTATGGGTTATTGTTTGCTATGTTTTCTATAGTATGTTTAGGA

E.granulo 121-180   AGCAGGGTTTGGGGTCATCATATGTTTACTGTTGGGTTGGATGTGAAGACGGCTGTTTTT
13L.3               -------GTTTGAGGGCATCATATGTTTACTGTTGGGTTAGATGTTAAGACGGGTGT-TTT
E. vogeli           AGTAGGGTTTGAGGGCATCATATGTTTACTGTTGGGTTGGATGTGAAGACGGCTGTKTTT

E.granulo 181-240   TTTAGCTCTGTTACCTATGATTATAGGGGTTCCTACTGGTATAAAGGTGTTTACTTGGTT
13L.3               TTTAATTCTGTTAGCTATGATTATAGGGTGTTCCTACGGGTATAAAGGTTTTAACTTGGTT
E. vogeli           TTTAGTTCTGTTACCTATGATTATAGGGGTTCCTACTGGTATAAAGGTGTTTACTTGGTT

E.granulo 241-300   ATATATGTTGTTGAATTCGAGTGTTAATGTTAGTGATCCGGTTTTGTCATGCGGTTGTTTC
13L.3               GTACATGCTGTTGAATTTGAGTGTGAATAAGAGTGATCCGGTTT-----------------
E. vogeli           GTATATGTTGTTGAATTCGAGTGTGAATAAGGGTGATCCGGTTTTATGGTGAGTGGTTTC

E.granulo 301-360   TTTTATAGTGTTGTTTACGTTTGGGGGAGTTACGGGTATAGTTTTGTCTGCTTGTGTGTT
13L.3               ----------------ACTTTTGGTGGTATGACTGGTATAGTTTTGTCTGCATGTGTATT
E. vogeli           TTTTATAGTGTTGTTTACGTTTGGTGGTGTTACTGGTRTAGTTTTGTCTGCTTGTGTATT

E.granulo 361-396   AGATAATATTTTGCATGATACTTGGTTTGTGGTGGCT
13L.3               GGATAATGTTTTGCATGATACTTGGTTTGTGGTGGCT
E. vogeli           GGATAATGTTTTGCATGATACTTGGTTTGTGGTAGCT
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Figure 5. DNA sequence alignment of the 400 bp segments of the 13L.3 *COX1* gene compared with *E. vogeli COX1* partial gene (accession number JX315616.1) and *E. granulosus COX1* partial gene (accession number AB688598.1) sequences. Gray blocks represent mismatched base pairs, while bold nucleotides represent three stop codons within the 13L.3 sample sequence according to the normal reading frame (without deletions).

4. Discussion

In the present study, semi-nested PCR system and mt*COX1* gene sequencing techniques were used to screen hydatid cysts collected from infected livestock in Jordan. The semi-nested PCR system used by Dinkel *et al.* (2014) to characterize the genotypes of *E. granulosus* with high specificity (100 %) and sensitivity (0.25 pg of DNA) was adopted successfully in this study. The system was comparable with other protocols of RFLP-PCR and sequencing of mt*COX1* and *NDI* genes (Dinkel *et al.*, 2004), and has been utilized by several research groups in the region for this purpose (Pour *et al.*, 2011; Elhag *et al.*, 2013; Hammad *et al.*, 2018). Using Dinkel's semi-nested PCR system, all the collected samples from Jordanian livestock in the present study proved to conform to *E. granulosus* (s.s.). All samples were distinctly different in their mt 12S rRNA gene profile from that of *E. canadensis* which characterized the camel control samples originating from Iraq (Figure 2).

However, *E. canadensis* species (camel G6 genotype) was isolated from sheep, goats, cattle, camels and humans in Sudan, Tunisia, Iraq, Iran, Egypt and Kenya (Dinkel *et al.*, 2004; Shahnazi *et al.*, 2011; Rajabloo *et al.*, 2012; Elhag *et al.*, 2013; Boufana *et al.*, 2014; Khalifa *et al.*, 2014; Pestechian *et al.*, 2014; Amer *et al.*, 2015; Hammad *et al.*, 2018). In the present study, none of the thirteen collected camel cysts were found to be infected with *E. canadensis*. This may be explained by the limited importation of exogenous camels in Jordan, the use of mixed livestock herds and the dominancy of the highly fertile *E. granulosus* sheep G1/dog cycle.

To verify the PCR results, twelve samples representing twelve infected intermediate hosts were selected for mt*COX1* partial gene sequencing. Sequencing of the mtDNA markers has more power than nuclear DNA in reconstructing the phylogenetic relationships among the closely-related species, and proved to be useful as an important genetic marker for the differentiation and studying of the population genetic structure of *E. granulosus* genotypes (Nakao *et al.*, 2013a; Sharma *et al.*, 2013). Mitochondrial *COX1* gene sequencing has been applied successfully in order to distinguish different *Echinococcus* species and genotypes (Bowles *et al.*, 1992; Abushhewa *et al.*, 2010; Casulli *et al.*, 2012; Nakao *et al.*, 2013a; Amer *et al.*, 2015; Abbas *et al.*, 2016; Debeljak *et al.*, 2016; Kandil *et al.*, 2016).

Although the identity of the 400 bp band sequence with *E. vogeli* was higher than that with *E. granulosus* (3 % difference, 12 bp), the life cycle and distribution of *E. vogeli* exclude the possibility of its existence in hosts indigenous to Jordan. *E. vogeli* typically uses the South American bush dog (*Speothus venaticus*) as a wild definitive host and pacas and agoutis as intermediate hosts (D'alessandro and Rausch, 2008). Taking into consideration the fact that both self and cross fertilization can occur in *Echinococcus* species (Haag *et al.*, 1999), the appearance of two bands in the 13L.3 and 13L.9 *COX1* partial gene PCR products may reveal the presence of two *COX1* gene alleles since the DNA source is genomic. Moreover, such finding may be explained by the presence of *pseudogenes*. *Pseudogenes* reported in mitochondrial and ITS genes of parasitic helminths, including

Echinococcus (Van Herwerden *et al.*, 2000; Obwaller *et al.*, 2004). *Pseudogenes* have been defined as "mitochondrial-like sequences found in the nuclear genome" (Lavikainen *et al.*, 2008) or as "sequences containing internal stop codons and/or deletions associated with frame shifts (Obwaller *et al.*, 2004; Lee *et al.*, 2007). Both definitions conform to the present findings since the DNA was genomic and internal stop codons and deletions have occurred.

Moreover, variations among parasitic helminths mt*COX1* gene sequences have been documented in several studies. A study by Obwaller *et al.* (2004) reported on a single base deletion and an internal stop codon in the *COX1* gene sequences of four *Echinococcus* species with a variation level of 2.45%. Also, Lee *et al.* (2007) reported that differences in the *COX1* gene sequences ranged from 1-121 bp in trematodes and 0-28 bp in cestodes. The largest gap was 121 bp between *Heterophyes nocens* and *Plagiorchis muris* in trematodes, and 28 bp between *Diphyllobthrium latum* and *Taenia taeniaeformis* in cestodes. In addition, Lavikainen *et al.* (2008) reported on the high intraspecific nucleotide sequence variations in the *COX1* gene within both *T. polyacantha* and *T. taeniaeformis* species.

In conclusion, semi-nested PCR system and *COX1* partial gene sequencing are useful for the characterization of *Echinococcus* specimens. Moreover, molecular analysis of the cysts of sheep, goats, cattle, and camels collected from four different abattoirs in Jordan proved the dominance of *E. granulosus* s.s. (G1-G3 strains). This finding supports earlier studies in Jordan regarding the existence of the former genotype of *E. granulosus* s.s. G1 strain as the dominant species/genotype in Jordan (Abdel-Hafez and Kamhawi, 1997; Al-Qaoud *et al.*, 2003a; Yanagida *et al.*, 2012). As the cosmopolitan sheep/dog G1 strain of *E. granulosus* s. s. is responsible for 88.44 % of the human CE worldwide (Rojas *et al.*, 2014), the dominance of this species in Jordan as documented in the present study explains well the high endemicity of the disease in Jordan (Al-Qaoud *et al.*, 2003b). Accordingly, and as different strains or species of *E. granulosus* may exhibit variations in epidemiology, transmission patterns as well as sensitivity to chemotherapeutic agents and drugs (Siracusano *et al.*, 2012; Rojas *et al.*, 2014), any control program to be envisaged for CE in Jordan must take into consideration that *E. granulosus* s.s. is the dominant species or strain in the country.

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