**Molecular characterization of *Echinococcus granulosus sensu lato* genotypes in dromedary camels from extreme Sahara of Algeria based on analysis of *nad*2 and *nad*5 genetic markers**

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**Highlights**

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Genotyping of [Echinococcus granulosus](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/echinococcus-granulosus" \o "Learn more about Echinococcus granulosus from ScienceDirect's AI-generated Topic Pages)*sensu lato* in [dromedary camels](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/dromedary" \o "Learn more about dromedary camels from ScienceDirect's AI-generated Topic Pages) based on analysis of recently developed mitochondrial genetic markers (*nad*2 and *nad*5).

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Occurrence of *E. granulosus sensu stricto* (G1 and G3) and *E. granulosus s.l.* G6.

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Eleven different haplotypes were detected based on phylogenetic network analysis of sequence data.

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The present study reports valuable molecular data (genotyping and haplotypic variability) on CE in [dromedary camels](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/dromedary" \o "Learn more about dromedary camels from ScienceDirect's AI-generated Topic Pages) from extreme Sahara of Algeria.

**Abstract**

Cystic echinococcosis is parasitic disease caused by the metacestodes belonging to the *Echinococcus granulosus sensu lato (s.l.)* species complex. Cystic echinococcosis is of considerable economic and public health importance. It is endemic in both livestock and humans in North African countries, including Algeria. The present study aimed to characterize *E. granulosus s.l.* genotypes in dromedary camels (*Camelus dromedarius*) from the extreme Sahara of Algeria, using recently developed mitochondrial genetic markers (NADH dehydrogenase subunit 2 and NADH dehydrogenase subunit 5) for reliable identification of different genotypes. A total of 75 *Echinococcus* cysts were collected from 49 dromedary camels, including 65 and 10 cysts from 45 and four camels originating from two slaughterhouses of Tindouf and Illizi provinces, respectively. *E. granulosus sensu stricto* (*s.s*.) G1 and G3 were identified in camels from both areas based on *nad5* (649 bp) gene sequences, whereas *E. granulosus s.l*. G6 was identified in camels from Tindouf region based on concatenated *nad5* and *nad2* gene sequences (total 1336 bp). Identified samples clustered into 11 different haplotypes (ALG1-ALG11), including four haplotypes (ALG8-ALG11) for *E. granulosus s.s*. G1, one haplotype (ALG7) for *E. granulosus s.s*. G3, and six haplotypes (ALG1-ALG6) for *E. granulosus s.l*. G6. The present study provides valuable molecular data, including genotyping and haplotypic variability, on *E. granulosus s.l*. in dromedary camels from two regions in the extreme Sahara of Algeria. Future characterization of the G1, G3, and G6 samples based on sequencing of complete mitochondrial genomes would be of considerable significance for a more comprehensive understanding of molecular epidemiology of CE in Algeria.

**Introduction**

Cystic echinococcosis (CE) is a neglected parasitic disease caused by the metacestodes belonging to the *Echinococcus granulosus sensu lato* (*s.l*.) (genus *Echinococcus*, Rudolphi 1801) that are of both economic and public health significance worldwide. Two types of hosts are involved in the life cycle of these parasites, including canids (mainly dogs) as definitive hosts for the development of the adult forms in the intestine and various herbivorous and omnivorous mammal species as intermediate hosts for the larval (metacestode) development in the viscera (Romig et al., 2017). Currently, at least nine species are recognized within the genus *Echinococcus*, in which *E. granulosus s.l.* represents a complex of multiple species (Thompson, 2020; Vuitton et al., 2020).

Using two mitochondrial gene fragments, namely cytochrome c oxidase subunit I (*cox*1; 366 bp) and NADH dehydrogenase subunit I (*nad*1; 471 bp), 10 genotypes (G1-G10) were initially distinguished within *E. granulosus s.l.* (formerly strains of *E. granulosus*; Bowles et al., 1992, 1994; Scott et al., 1997; Lavikainen et al., 2003). However, genotypes G2 and G9 were subsequently shown to be microvariants of G3 and G7, respectively (Kedra et al., 1999; Thompson, 2008; Kinkar et al., 2017). Data on distinct differences in their epidemiology, including life cycle patterns and host ranges, as well as morphological data later warranted the classification of these genotypes into several species within *E. granulosus s.l*.: *E. granulosus sensu stricto* (*s.s*.; G1, G3), *E. equinus* (G4), *E. ortleppi* (G5), and the *E. canadensis* cluster (G6-G8, G10) (Thompson and McManus, 2002; Nakao et al., 2007, 2013a, 2013b; Romig et al., 2015; Kinkar et al., 2017). However, the taxonomic status of the aforementioned *E. canadensis* cluster is still under debate and no clear consensus has been reached. Some authors have suggested that the *E. canadensis* cluster is one species (Nakao et al., 2007, 2013a, 2013b, 2015; Yanagida et al., 2017), whereas others have suggested a split into two species (*E. intermedius* G6/G7, *E. canadensis* G8/G10; Thompson, 2017, 2020; Laurimäe et al., 2018a), and some into three - *E. intermedius* G6/G7, *E. borealis* G8 and *E. canadensis* G10 (Lymbery et al., 2015a, 2015b). Given that the taxonomic issue of this cluster has so far not been resolved, we will henceforth use the terminology "*E. granulosus s.l*. genotypes G6/G7″ when referring to these variants.

Within *E. granulosus s.l., E. granulosus s.s*. is the most frequent species in both livestock and canids. It is widely distributed in the world and has the highest global impact on public health (Alvarez Rojas et al., 2014). *E. granulosus s.l.* genotypes G6/G7 are the second most important agents of CE regarding their worldwide distribution and public health importance (Alvarez Rojas et al., 2014; Deplazes et al., 2017; Romig et al., 2017).

Cystic echinococcosis is endemic, or even hyperendemic in both animals and humans in North African countries, including Algeria (Sadjjadi, 2006; Dakkak, 2010; Deplazes et al., 2017). Main available data from Algeria consist of prevalence figures of unspecified CE in livestock. Infection rates up to 78% in sheep, 91% in cattle, and 26% in dromedary camels have been reported (Laatamna et al., 2019). Additionally, there is considerable lack of data on the epidemiology of *Echinococcus* spp. in dogs from both urban and rural areas. Infection rates ranging from 16% to 51% have been reported in dogs from eastern Algeria (Benchikh-Elfegoun et al., 2008; Bentounsi et al., 2009). Furthermore, available epidemiological data on human CE consisted of cases declared either in hospitals or by public health authorities (Zait et al., 2014; Laatamna et al., 2021). Genotyping and genetic diversity of causative CE agents have been reported in a few studies from Algeria. *E. granulosus s.s.* (predominance of G1 as compared to G3) has been characterized in both livestock (sheep, cattle, goats, and dromedary camels) and humans (Bardonnet et al., 2003; Bart et al., 2004; Maillard et al., 2007; Zait et al., 2016; Laatamna et al., 2019; Moussa et al., 2021). Maillard et al. (2007) have also reported the occurrence of G2 (microvariant of G3) in sheep, dromedary camels, and humans. Additionally, G6 has been identified in dromedary camels (Bardonnet et al., 2003; Bart et al., 2004; Maillard et al., 2007; Zait et al., 2016) and in one old Touareg women from the area of Tamanrasset, extreme south of the Algerian Sahara (Zait et al., 2016). Similar data have been recorded from other North African countries (Tunisia, Morocco, and Libya) where *E. granulosus s.s*. (mainly G1) is the most common species in both livestock and humans, and G6 is the predominant type in dromedary camels (reviewed in Deplazes et al., 2017).

Most of the molecular studies from Algeria as well as from other North African countries, which were conducted to determine *E. granulosus s.l.* genotypes either in livestock or humans, have mainly been based on analysis of shorter fragments of single mitochondrial genes including *cox*1(366 bp) and *nad*1(471 bp). Very few studies have used the full *cox*1 gene (1608 bp) to provide a clearer picture on genotyping of *E. granulosus s.l.* in Algeria (Laatamna et al., 2019). Recent molecular analyses of a large dataset of near-complete or complete mitogenomes have showed that in some cases, the genotyping of *E. granulosus* s.s. (G1/G3) and *E. granulosus s.l*. G6/G7 isolates based on either fragments or even the full *cox*1and *nad*1 genes remains ambiguous, with a number of isolates clustering in between the two closely related intraspecific genotypic groups (Romig et al., 2015; Kinkar et al., 2018a). Whereas for G6 and G7, it was demonstrated that a number of G7 isolates would have been incorrectly classified as G6 or closer to G6 than to G7 based on the widely used *cox*1 (366 bp) fragment as well as on the complete *cox*1 (1608 bp) gene (Laurimäe et al., 2018b). Nevertheless, since the cost of sequencing of complete mitogenomes for a larger number of samples remains rather high and a cumbersome task, a more cost-effective method was suggested that would be suitable for reliable intraspecific genotype discrimination by targeting two suitable genetic mitochondrial markers (Kinkar et al., 2018a; Laurimäe et al., 2019a). Sequencing of *nad5* gene (680 bp) was suggested for optimal consistent identification and discrimination of G1 and G3 (Kinkar et al., 2018a), and concatenated *nad2* (714 bp) and *nad5* (680 bp) gene sequences were suggested for discriminating G6/G7 (Laurimäe et al., 2019a). Precise determination of *E. granulosus s.l.* species and intraspecific genotypes, specifically for G1/G3 and G6/G7 that are the most important agents of CE in humans, is of considerable epidemiological and clinical significance.

For this purpose, the present study was conducted to perform molecular characterization of *E. granulosus s.l.* cyst samples collected from dromedary camels in two slaughterhouses in the extreme Sahara of Algeria, using the mtDNA markers *nad*2 and *nad*5 for reliable determination of the genotypes.