



Cystic echinococcosis in sheep and goats of Lebanon

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Research Article

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Abstract

Cystic echinococcosis (CE), a zoonotic disease caused by the species complex of *Echinococcus granulosus* sensu lato (s.l.), is endemic in Middle East and the Mediterranean basin, where pastoral activity is widespread. Despite the chronic endemicity of the disease in Lebanon and neighbouring countries, recent data are scant. The objectives of this survey were to evaluate the current epidemiology of CE in Lebanon, investigate the prevalence and determine the infecting genotypes in locally raised sheep and goats. A multidimensional approach combining post-mortem inspection of slaughtered animals and molecular diagnosis of the parasite was conducted to this end. From 2018 to 2020, 62.9% of the sheep and 20.9% of the goats were found positive for CE. The presence of hydatids varied between organs, showing higher prevalence in the liver of sheep vs the lungs of goats, however, a higher fertility rate of hydatid cyst was observed in lungs for both animals. Molecular diagnosis based on partial mitochondrial *cox1* gene (795 bp) showed that the majority of isolates were identified as *E. granulosus* sensu stricto (98.7%) and only one isolate from goat was identified as *Echinococcus canadensis* (genotype G7; 1.3%). *Echinococcus granulosus* s.s. population among the sheep and goats was represented by 22 haplotypes having very little genetic differentiation and relatively moderate haplotype diversity. Population demographics explored through neutrality indices suggested expanding population within the intermediate hosts. These results document the high prevalence of CE in the livestock of Lebanon and reveal for the first time the presence of three different genotypes G1, G3 and G7.

Introduction

Cystic echinococcosis (CE) is ranked second in the list of food-borne parasites worldwide, listed among the most severe parasitic diseases in humans (World Health Organization and Food and Agriculture Organization of the United Nations, 2014) and prioritized by the World Health Organization as one of the 17 neglected tropical diseases (WHO, 2015). It is also known to be the most important parasitic zoonosis in the Mediterranean region (Dakkak, 2010; Ismail *et al.*, 2018; Borhani *et al.*, 2020), with stable endemicity in the Middle East due to the high diversity of intermediate hosts, mainly sheep and goats, followed by camels and cattle (Al Kitani *et al.*, 2015). CE is creating a serious public health problem and is a burden to national economy for most countries in this region (Grosso *et al.*, 2012; Thys *et al.*, 2019).

CE occurs as a result of infection at the larval stage of the tapeworms belonging to the species complex *Echinococcus granulosus* sensu lato (s.l.). To complete the lifecycle, the cestode needs as intermediate hosts a wide range of herbivorous and omnivorous mammals and as definitive hosts dogs and wild canids (Thompson *et al.*, 2017; Kinkar *et al.*, 2018a). The adult worm develops in the small intestine of the definitive host while the larval stage grows into a hydatid cyst in intermediate hosts, causing CE (Kinkar *et al.*, 2018a; Thompson, 2020). Transmission of the infection occurs through egg ingestion either by direct contact with definitive hosts or indirectly by drinking water or consuming contaminated fruits and vegetables (Possenti *et al.*, 2016). However, the infection in definitive hosts does not cause morbidity, while it is considered severe in intermediate hosts (including humans) (WHO, 2020).

The taxonomy of *E. granulosus* s.l. has been a challenging issue for decades. Studies have identified a number of genotypes/species within *E. granulosus* s.l. (Knapp *et al.*, 2011; Kinkar *et al.*, 2018b) on the basis of mitochondrial DNA. At least 10 strains (G1–10) of *E. granulosus* s.l. have been described, forming four major clades (G1–G3, G4, G5 and G6–G10) (Nakao *et al.*, 2013). Some of these genotypes are now considered as distinct species: *E. granulosus* sensu stricto (s.s.; comprising genotypes G1 and G3) (Kinkar *et al.*, 2018b), *Echinococcus equinus* (G4) and *Echinococcus ortleppi* (G5) (Thompson and McManus, 2002), whereas the status of genotypes G6–G10 is under dispute (Lybery *et al.*, 2015; Nakao *et al.*, 2015; Laurimäe *et al.*, 2018). Some authors have proposed the consideration of G6–G10 provisionally as single

species, *Echinococcus canadensis* awaiting further data from the nuclear genome (Nakao *et al.*, 2013; Addy *et al.*, 2017), while others consider them as two distinct species: G6/G7 as *Echinococcus intermedius* and G8/G10 as *E. canadensis* (Thompson, 2008; Saarma *et al.*, 2009; Laurimäe *et al.*, 2018) or even as three species: G6/G7 as *E. intermedius*, G8 as *Echinococcus borealis* and G10 as *E. canadensis* (Lybery *et al.*, 2015).

In the Middle East, the most dominant species responsible for CE infections in both wild and domestic animals is *E. granulosus* s.s. (G1–G3) (Kim *et al.*, 2020) followed by genotype G6 (Rostami *et al.*, 2015; Khademvatan *et al.*, 2019). Information on strains and species from the Middle East, especially Iraq, Lebanon, Palestine, Syria and the Persian Gulf countries, is currently scant (Thompson, 2017). Regarding Lebanon, in 1930 the prevalence of 41.5% CE in cattle was reported in Beirut (Goodale and Krischner, 1930; Matossian *et al.*, 1977). In 1936, the epidemiological surveys conducted in Beirut revealed a prevalence of 22.1% in sheep and 45.1% in cattle (Turner *et al.*, 1936a), and 20–25% in dogs (Turner *et al.*, 1936b). Another study demonstrated prevalence rates of 11.6% in sheep, 67.4% in camels, 47% in cattle and 11.75% in dogs (Pipkin *et al.*, 1951). Another survey undertaken in 1963 reported that almost one-third of mature swine, one-third of cattle and one-fourth of sheep and goats were infected with CE in Beirut (Luttermoser and Koussa, 1963). In 1964, the prevalence rates of echinococcosis were 100% in camels and 42% in donkeys in Lebanon and Syria, whereas an infection rate of 28% was documented in dogs in Lebanon (Dailey *et al.*, 1966). These historical reports concerning CE in Lebanon suggest the endemic nature of CE in the country (Frayha, 1970).

CE has been an important public health problem in Lebanon; however, no recent information is available regarding the prevalence and impact of the different genotypes of *E. granulosus* s.l. in the country. Moreover, Lebanon has a high number of free-ranging dogs, illegal slaughtering and other cultural and socio-economic conditions that can contribute to the transmission and perpetuation of CE, particularly in sheep and goats. Therefore, the aim of this study was to investigate the prevalence of CE in sheep and goats of Lebanon and determine genotypes of isolated metacestodes to get a better understanding of *E. granulosus* s.l. species and genotypes that are represented in this part of the Mediterranean region.

Materials and methods

It was decided to proceed through a multidimensional approach combining: (i) exhaustive sampling and post-mortem inspection of slaughtered animals, (ii) morphological characterization of hydatid cyst, (iii) statistical analysis of the gathered data and (iv) molecular identification of the circulating genotypes.

Sample collection

According to the Raosoft sample size calculator (http://www.raosoft.com/sample_size.htm), the sample size number was determined in a population of 400 000 sheep and 400 000 goats, which supposed prevalence for CE of 60% and confidence level of 95%.

During three consecutive years (2018, 2019 and 2020), 369 sheep and 335 goats have been examined in various abattoirs from five different Lebanese regions (Mount Lebanon, Beirut, Bekaa, North and South of Lebanon). The animals' ages were estimated by examining teeth, and only animals above 2 years old were enrolled in this study (Varcasia *et al.*, 2007).

Liver and lungs were examined for cysts by post-mortem visual inspection and palpation. When hydatid cysts were found, these

were counted and examined to verify exact location, fertility and morphological characteristics.

Hydatid cysts were classified as fertile, sterile, calcified or caseous on the basis of morphological examination. Fertility was determined by microscopic observation, without staining protoscolices, at 10× to assess morphologic characteristics along with protoscolices' flame cell movements (Varcasia *et al.*, 2006). Protoscolices and germinal layers were then removed and preserved in ethanol (95%) before being further analysed.

Statistical analysis

Data were recorded on a spreadsheet (Excel® Microsoft Corp., Redmond, WA, USA) and prevalence values were calculated according to the type of hydatid cyst (fertile, sterile, caseous and calcified cysts). Differences in prevalence were statistically tested using the *chi*-square test for independence (Epi-Info® 7.0, CDC/WHO, Atlanta, GA, USA).

Molecular studies

DNA was extracted from 77 samples of hydatid material obtained from sheep (65) and goats (12) using NucleoSpin Tissue (Macherey-Nagel GmbH & Co. KG, Düren, North Rhine-Westphalia, Germany). Germinal layers of the hydatid cysts were lysed using proteinase K and the samples were incubated at 56°C until digestion of the membrane. Following digestion, absolute ethanol was added to each sample and the sample was transferred to the NucleoSpin Tissue column and centrifuged at 12 000 g for 1 min. Samples were subsequently washed with buffers provided in the kit and finally the DNA was eluted in a pre-labelled Eppendorf tube with BE buffer at 12 000 g centrifugation. Polymerase chain reaction (PCR) was conducted using primer pairs F/COI (TTGAATTTGCCACGTTTGAATGC) and R/COI (GAACCTAACGACATAACATAATGA) for the amplification of partial mitochondrial *cox1* gene (Nakao *et al.*, 2000). Twenty-nine isolates were further screened through *nad5* mitochondrial gene employing the primers EGnd5F1 (GTTGTTGAAGTTGATTGTTTTGTTT) and EGnd5R1 (GGAACACCGGACAAACCAAGAA) for the correct identification of genotypes G1 and G3 (Kinkar *et al.*, 2018b). A G7 isolate was also amplified using G7for (GTGTTGTGTTGTTGATAGATTG) and G7rev (GTAAAATAATCACCACCCAAC) primers for *nad2* gene (Laurimäe *et al.*, 2019).

PCR products were purified using a NucleoSpin Gel and PCR cleanup (Macherey-Nagel GmbH & Co. KG, Düren, North Rhine-Westphalia, Germany) and sent to an external sequencing service (Eurofins Genomics, Ebersberg, Germany) for bidirectional sequencing.

The base-calling errors on sequenced chromatograms were checked on Finch TV viewer (Geospiza Inc., Seattle, WA, USA). Reference sequence (GenBank number: MG672237) from Kinkar *et al.* (2018c) was used for multiple alignment of all *E. granulosus* s.s. sequences, whereas *E. canadensis* sequence was aligned with a reference sequence (GenBank number: MH301020) from Laurimäe *et al.* (2018). *Cox1* dataset (795 bp) for *E. granulosus* s.s. was exported to DnaSP (Rozas *et al.*, 2017) for the analysis of basic genetic variability indices (polymorphism, number of mutations, singleton variable and parsimony informative sites). For computation of haplotype and nucleotide diversities, neutrality indices (Tajima's *D* and Fu's *F_s*) and genetic differentiation (*F_{st}*), the data were analysed using the package Arlequin 3.5 (Excoffier and Lischer, 2010). Haplotype network formation was executed on PopArt software using a median joining network (Leigh and Bryant, 2015). The

Table 1. Percentage of types of hydatid cysts in infected Lebanese sheep and goats

	Sheep (no.)	Prevalence (%)	Goats (no.)	Prevalence (%)
Examined no.	369	100	335	100
Infected with cysts	232	62.9	70	20.9
Infected with fertile cysts	79	21.4	13	3.9
Infected with caseous cysts	43	11.6	11	3.3
Infected with calcified cysts	201	54.5	42	12.5
Infected with sterile cysts	91	24.7	4	1.2

Note: A significant number of sheep and goats show more than one type of hydatid cyst. Only a restrained number of observed targets are limited to one type of hydatid cyst.

estimation of pairwise divergence was performed on MEGA X software (Kumar *et al.*, 2018).

Results

Among 369 examined sheep, 232 sheep were found positive (62.9%) with a total number of 2707 hydatid cyst. Parasitological examination showed a fertility rate of 21.4%: 79 out of the 369 examined animals harboured at least one fertile hydatid cyst. The prevalence of sheep infected with caseous cysts, calcified cysts and sterile cysts was 11.6, 54.5 and 24.7%, respectively. The overall prevalence of hydatid cyst infection and the prevalence of the different cyst categories are summarized in Table 1.

The presence of hydatids in sheep varied between the main target organs, showing a prevalence of 44.4% (164/369) in lungs and higher (48.5%; 179/369) in the liver ($\chi^2 = 1.2256$; $P = 0.2682$). Hydatid cysts were found in both liver and lungs in 30.1% of the examined animals (111/369). Cyst abundance (number of cysts/examined animal) was 7.3 (range: 0–129), while cyst intensity (number of hydatids/positive animal) was 11.6. Of the infected animals, 32% had 1–5 cysts, while 21.9% of animals had massive infection harbouring 10–129 cysts. The fertility rate of hydatid cyst was higher in lungs (17.1%) compared with the liver (14.6%) but no statistical differences were observed ($\chi^2 = 0.8227$; $P = 0.3643$).

In relation to the morphology of the recovered hydatid cyst, 49.5% of the hydatids were calcified, while 23.9, 19.2 and 7.4% were found to be fertile, sterile and caseous, respectively. Looking at all the 2707 recovered hydatid cysts in the 369 concerned sheep, it was underlined that 41.8% (1132/2707) were prevalent in lungs and 58.2% (1575/2707) in the liver. Conversely, fertile cysts were found more often in lungs (13.4%) compared with the liver (10.5%) (Table 2).

Among 335 investigated goats, 70 goats were CE positive, with an overall prevalence of 20.9% and a total number of 204 hydatid cyst. Parasitological examination showed a fertility rate of 3.9% (13/335) in the examined animals. The prevalence of sheep infected with caseous cysts, calcified cysts and sterile cysts was 3.3, 12.5 and 1.2%, respectively (Table 1). Contrary to what was found in sheep, the prevalence of hydatids in goats was found to be higher

Table 2. Number and percentage of the different categories of hydatid cysts and site of infections in Lebanese sheep and goats

Type of cyst	Sheep		Goat	
	Cyst no.	Prevalence (%)	Cyst no.	Prevalence (%)
Lung				
Fertile	364	13.4	15	7.4
Caseous	138	5.1	18	8.8
Calcified	386	14.3	82	40.2
Sterile	244	9	10	4.9
Total	1132	41.8	125	61.3
Liver				
Fertile	284	10.5	9	4.4
Caseous	62	2.3	11	5.4
Calcified	953	35.2	50	24.5
Sterile	276	10.2	9	4.4
Total	1575	58.2	79	38.7
Lung + Liver				
Total	2707	100	204	100

in lungs (11.9%; 40/335) than in the liver (9.9%; 30/335), but no statistical differences were observed ($\chi^2 = 1.5952$; $P = 0.2065$). Hydatid cysts were found in both liver and lungs only in 0.9% of examined animals (3/335). None of the examined animals showed massive infection and hydatid cyst abundance.

The abundance was 0.6 (range: 0–9), while the mean intensity was 2.9. Fertility rate was higher in lungs (3.0%; 10/335) compared to the liver (1.8%; 6/335) having no statistical difference ($\chi^2 = 1.0245$; $P = 0.3114$). In relation to the morphology of the recovered hydatid cyst, 64.7% of the hydatids were calcified, while 14.2, 11.8 and 9.3% were found to be caseous, fertile and sterile, respectively. The presence of hydatids in goats varied when considering the examined organs, showing that 61.3% (125/204) of hydatid cyst were found in lungs and 38.7% (79/204) in the liver. Fertile cysts were found more often in lungs (7.4%) compared with the liver (4.4%) (Table 2).

In this study, 77 hydatid cyst isolates originating from sheep ($n = 65$) and goats ($n = 12$) from five different regions of Lebanon were subjected to genetic analysis. Molecular characterization of these isolates was based on partial mitochondrial *cox1* gene (795 bp) and yielded sufficient polymorphism for further population genetics analysis. It was identified that the majority of isolates ($n = 76$; 98.7%) belonged to *E. granulosus* s.s., of which G1 genotype was more prevalent ($n = 72$; 94.73%); G3 was identified from only four isolates (5.27%) belonging to sheep. Genotypic assessment was further confirmed through *nad5* gene (680 bp; Kinkar *et al.*, 2018b) sequencing of 29 isolates and the presence of similar genotypes was reaffirmed. One isolate (1.3%) originating from goat was identified as G7 genotype and was found 100% similar to G7 sequence (MH301020) for the partial *cox1* region, whereas *nad5* and *nad2* mitochondrial gene sequences reflected similarity to G7b at five fixed positions for this haplogroup [804 and 1060 bp (*nad5*); 6491, 6524 and 6620 bp (*nad2*); Laurimäe *et al.*, 2018]. Because of only single sequence of *E. canadensis* (G7 genotype), it was subsequently excluded from the analysis covering *E. granulosus* s.s. population among the intermediate hosts.

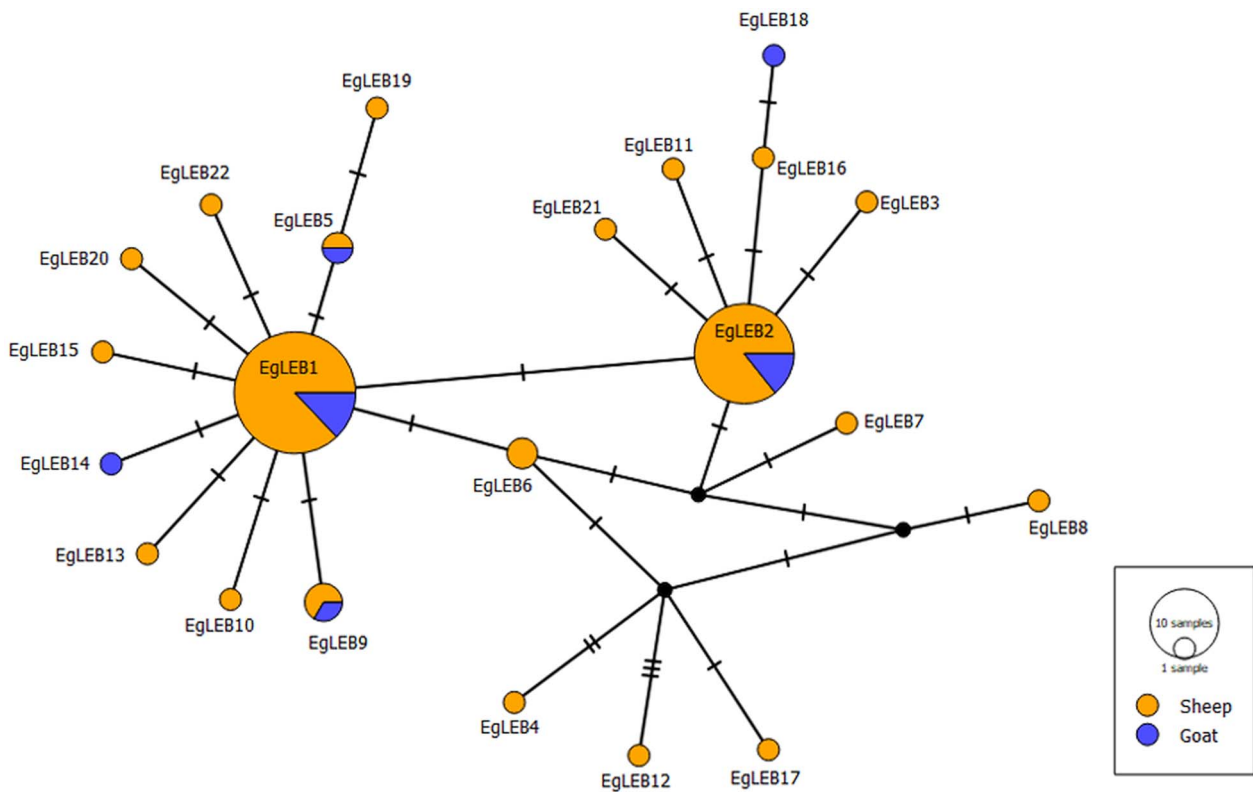


Fig. 1. Haplotypic structure of *Echinococcus granulosus* s.s. among sheep and goats of Lebanon. Hatch marks correspond to the number of mutations between haplotypes and size of the circle indicates frequency of each haplotype (see also Table 4). Black dots represent hypothetical haplotypes in the population.

The analysed *cox1* region (795 bp) contained 24 point mutations at 24 segregating loci of which 17 (70.83%) were singleton sites and seven (29.17%) were parsimony informative. Parsimony informative sites contain at least two nucleotide variants which appear twice or more within the population for a given gene sequence. No insertions or deletions were observed in the sequences. Overall, there were more transitions ($n = 23$, 95.83%) than the transversions ($n = 1$, 4.17%) in the variable sites. The polymorphic loci had 10 non-synonymous (41.66%) and 14 synonymous substitutions (58.33%). In total, 22 haplotypes were found in 76 isolates for *E. granulosus* s.s. Discerning genealogical relationships between the haplotypes, it was identified that two haplotypes, EgLEB1 and EgLEB2, predominated the population appearing in 31 (40.78%) and 21 (27.63%) isolates, respectively (Fig. 1). G1 genotype was represented by 18 haplotypes (81.81%), whereas G3 genotype was represented by four haplotypes (18.19%) which were present as singleton variants. The number of mutational differences between the most common G1 haplotype and others ranged from one to five. Ten haplotypes had more than one nucleotide variation and seven of these microvariants shared similar mutation to that of the second most common haplotype, LEB2. Majority of the haplotypes (77.27%) occurred as singleton variants appearing only once in the population. Haplotypes identified on the basis of partial *cox1* gene were also submitted to the NCBI database under the accession numbers: MW428227–MW428248 (Table 3).

Population diversity and neutrality indices were also computed for *E. granulosus* s.s. population from the sheep and goats of Lebanon. Relatively moderate haplotype diversity (0.7614 ± 0.0391) was observed in the population along with low nucleotide diversity (0.00188 ± 0.00026). Harboring a higher number of haplotypes ($n = 6$) in a lower number of isolates ($n = 11$), higher haplotype diversity existed for *E. granulosus* s.s. in goats (0.8364 ± 0.0887) compared to sheep (0.7567 ± 0.0423). In total,

20 haplotypes were identified from 65 isolates originating from sheep (Table 4). Other demographic parameters computed *via* neutrality indices indicated a significantly negative bias ($D = -2.1627$, $F_s = -19.891$) in the overall population of *E. granulosus* s.s. identified on the basis of *cox1* dataset. Furthermore, the estimation of gene flow among the two intermediate hosts was calculated using pairwise F_{st} . Due to the occurrence of common haplotypes and less divergence between the *E. granulosus* s.s. haplotypes, F_{st} value was very low (-0.01956 , $P > 0.05$).

Discussion

To our knowledge, the current paper constitutes the first molecular characterization of *E. granulosus* s.l. isolates from sheep and goats of Lebanon, besides being the largest epidemiological survey carried out in this country in the last 30 years. In previous CE studies carried out in Lebanon between 1951 and 1989, the prevalence of CE in sheep and goat varied between 6.6 and 22.1% (Araj and Mourad, 2014). According to the current survey, the prevalence of CE in sheep and goats has increased to 62.9 and 20.9%, respectively. Current results show the persistence of the disease throughout the years; and the high prevalence can be justified with the age of the examined animals, as only adult animals were included in this study, the widespread home slaughtering and feeding dogs with infected offal. An increase in prevalence with age was also described in several studies (Scala *et al.*, 2006; El Berbri *et al.*, 2015; Varcasia *et al.*, 2020). It is well established that in the endemic areas of echinococcosis, the prevalence of hydatid cysts increases with the age of livestock, which become more susceptible to lower immunological resistance against infections (Torgerson and Heath, 2003; Fikire *et al.*, 2012).

Also, higher prevalence of hydatid disease was found among sheep compared to goats (62.9 vs 20.9%; $\chi^2 = 126.31$; $P < 0.0001$), which is in agreement with other studies carried out in several

Table 3. Number, prevalence and geographic localization of haplotypes ($n = 22$) of *Echinococcus granulosus* s.s. in the sheep and goats of Lebanon

Genotype	Haplotype name	Number in population	Prevalence (%)	Host animals	Geographic location	Accession number
G1	EgLEB1	31	40.78	Sheep, goat	South Lebanon, Bekaa, North Lebanon, Mount Lebanon, Beirut	MW428227
G1	EgLEB2	21	27.63	Sheep, goat	South Lebanon, Bekaa, Mount Lebanon	MW428228
G1	EgLEB3	1	1.31	Sheep	South Lebanon	MW428229
G3	EgLEB4	1	1.31	Sheep	South Lebanon	MW428230
G1	EgLEB5	2	2.63	Sheep, goat	Bekaa, North Lebanon	MW428231
G1	EgLEB6	2	2.63	Sheep	South Lebanon, Bekaa	MW428232
G1	EgLEB7	1	1.31	Sheep	Bekaa	MW428233
G3	EgLEB8	1	1.31	Sheep	Bekaa	MW428234
G1	EgLEB9	3	3.94	Sheep, goat	South Lebanon, Bekaa	MW428235
G1	EgLEB10	1	1.31	Sheep	Bekaa	MW428236
G1	EgLEB11	1	1.31	Sheep	Bekaa	MW428237
G3	EgLEB12	1	1.31	Sheep	Bekaa	MW428238
G1	EgLEB13	1	1.31	Sheep	Bekaa	MW428239
G1	EgLEB14	1	1.31	Goat	North Lebanon	MW428240
G1	EgLEB15	1	1.31	Sheep	Bekaa	MW428241
G1	EgLEB16	1	1.31	Sheep	Bekaa	MW428242
G3	EgLEB17	1	1.31	Sheep	Bekaa	MW428243
G1	EgLEB18	1	1.31	Goat	Mount Lebanon	MW428244
G1	EgLEB19	1	1.31	Sheep	Bekaa	MW428245
G1	EgLEB20	1	1.31	Sheep	South Lebanon	MW428246
G1	EgLEB21	1	1.31	Sheep	Bekaa	MW428247
G1	EgLEB22	1	1.31	Sheep	Bekaa	MW428248

Table 4. Diversity and neutrality indices for *E. granulosus* s.s. in the sheep and goats of Lebanon

Host animals	H_n	Diversity indices		Neutrality indices	
		$H_d \pm s.d.$	$N_d \pm s.d.$	Tajima's D	Fu's F_s
Sheep	20	0.7567 \pm 0.0423	0.00189 \pm 0.00129	-2.1265*	-17.097*
Goat	6	0.8364 \pm 0.0887	0.00184 \pm 0.00137	-1.2181	-2.5079*
Overall	23	0.7614 \pm 0.0391	0.00188 \pm 0.00026	-2.1627*	-19.891*

H_n : haplotype number, H_d : haplotype diversity, N_d : nucleotide diversity.

*Significant at $P < 0.05$.

Arab countries (Ibrahim, 2010; Lotfi *et al.*, 2010; El Berbri *et al.*, 2015; Hassan *et al.*, 2016; Almalki *et al.*, 2017; Abdel-Baki *et al.*, 2018). Similarly, previous studies in other non-Arab Mediterranean regions reported higher prevalence in sheep (Seimenis *et al.*, 2006; Varcasia *et al.*, 2007; Manfredi *et al.*, 2011; Chaligiannis *et al.*, 2015), whereas a study conducted by Kamhawi (1995) in Jordan, reported that infection rates were similar for sheep and goats.

Current results provided evidence that cyst fertility rates were higher in sheep (21.4%) compared to goats (3.9%; $\chi^2 = 47.489$; $P < 0.0001$). Similar results have been reported in other surveys carried out in Jordan and Greece (Kamhawi *et al.*, 1995; Varcasia *et al.*, 2007; Chaligiannis *et al.*, 2015). The lowest rate seen in goats is likely due to the feeding habits of this animal, as they eat higher part of herbs (Otero-Abad and Torgerson,

2013); the latter represents difficulty for dogs to uphill to these areas for defecation and is exposed to sunlight, which decreases the viability of the *E. granulosus* eggs. Most importantly, high prevalence and cyst fertility rates in sheep correlate with the molecular results where majority of sheep and goat samples belonged to *E. granulosus* s.s., a species particularly well adapted to sheep as intermediate hosts (Deplazes *et al.*, 2017) whereas goats are considered to have more G6/G7 genotypes (Varcasia *et al.*, 2007). However, almost all other livestock species (goats, cattle, yak, camels, alpacas, pigs and donkeys) contribute to the transmission of CE and are usually known to develop fertile cysts of *E. granulosus* s.s., but considered less important for the life cycle due to lower prevalence, cyst fertility or availability to dogs (Deplazes *et al.*, 2017).

Cysts in sheep were predominantly located in the liver (58.2%), whereas in goats, lung was the most affected organ (61.3%). This has also been demonstrated in other Mediterranean Arab and non-Arab countries, where a high infection rate was seen in sheep with the liver being the most affected organ (Varcasia *et al.*, 2006, 2020; Grosso *et al.*, 2012; Almalki *et al.*, 2017; Brik *et al.*, 2018; Toulah and Albalawi, 2019). A different situation has been observed in epidemiological studies conducted in Iran and Pakistan, where sheep had highest infection in lungs and liver was the most infected organ in goats (Lotfi *et al.*, 2010; Mehmood *et al.*, 2020a).

When considering cyst fertility, the probability of finding fertile cysts in lungs was higher than that in liver in both animals. Similar results have been reported in other Mediterranean regions and the Middle East (Scala *et al.*, 2006; Daryani *et al.*, 2009; Conchedda *et al.*, 2012; Varcasia *et al.*, 2020). However, in some surveys the fertility rates were similar for liver and lungs (Khan *et al.*, 2001) or even higher in the liver (Dalimi *et al.*, 2002; Elham *et al.*, 2014). The increased presence of fertile cysts in lungs can be explained by the variation in tissue resistance between organs (Fikire *et al.*, 2012).

Determination of the epidemiological role of different *E. granulosus* s.l. species involved in echinococcosis is of paramount importance for disease control. Current investigation involving molecular characterization of metacestodes from sheep and goats in Lebanon revealed the presence of three genotypes, G1, G3 and G7 circulating among the analysed sheep and goats. Such information on the prevalence of CE in sympatric species has epidemiological implications and helps understanding the transmission dynamics of different genotypes (Nakao *et al.*, 2010; Mehmood *et al.*, 2020b). Of five different areas of Lebanon, it was revealed that a common haplotype EgLEB1 existed among the sheep and goats of these regions. Similar existence of a shared and predominant haplotype based on *cox1* mitochondrial marker has been identified in different countries such as China (Nakao *et al.*, 2010), Iran (Yanagida *et al.*, 2012), United Kingdom (Boufana *et al.*, 2015) and Italy (Mehmood *et al.*, unpublished data). The presence of a common haplotype among different populations suggests expansion from an ancestral haplotype (Yanagida *et al.*, 2012).

A double-clustered haplotype network of *E. granulosus* s.s. having two predominant haplotypes, both belonging to G1 genotype (EgLEB1 and EgLEB2) was identified, signifying the importance of this genotype and the two haplotypes in this region. The second most common haplotype, EgLEB2, has been identified from other geographic areas of the world such as Iran (Yanagida *et al.*, 2012), Eastern Europe (Casulli *et al.*, 2012) and Iraq (Hassan *et al.*, 2017), implying that no significant phylogeographic segregation has occurred globally. This haplotype results in a non-synonymous substitution which may have led to its higher frequency and selection advantage and this is the same nucleotide position at which the point mutation led to initial description of G2 strain by Bowles *et al.* (1992). Assembled in a star-like configuration, haplotypes EgLEB3, EgLEB7, EgLEB8, EgLEB11, EgLEB16 and EgLEB18 seemed to have radiated from EgLEB2 which was separated by a single mutation from the common G1 haplotype. Genotype G3 was represented by four haplotypes having very low occurrence in the population. Furthermore, the indication of hypothetical haplotypes in the haplotype network implied that high genetic variability exists among the *E. granulosus* s.s. population of Lebanon which required molecular surveillance encompassing more isolates.

Echinococcus granulosus s.s. has shown to have undergone rapid radiation globally (Kinkar *et al.*, 2018a, 2018b). Current study estimated moderate genetic variability (H_d : 0.7614 ± 0.0391) among the *E. granulosus* s.s. specimens isolated from

sheep and goats. Even though sheep harboured 20 haplotypes, the haplotype diversity was lower for sheep in comparison with goats which had six *E. granulosus* s.s. haplotypes. An overall negative bias from neutrality was observed among the parasitic population which suggests population expansion after bottleneck event in the past. Genetic differentiation estimate (F_{st}) for the goat and sheep population yielded a very low value (-0.01956) suggesting the efficient transmission of *E. granulosus* s.s. among these hosts in this region. Transmission dynamics and spread of this parasitic species across different geographical regions have largely been influenced by humans (Kinkar *et al.*, 2018c).

Echinococcus canadensis G7 genotype was isolated from a goat specimen sampled from Beirut and is the first report on molecular identification of this genotype in Lebanon. Based on mitochondrial *nad5* gene (680 bp) and *nad2* gene (714 bp), it was identified that this sequence belonged to the G7b haplogroup of *E. canadensis* which was also recognized from other Mediterranean countries such as France and Italy (Laurimäe *et al.*, 2019). A similar occurrence of pig strain in goats was reported earlier from Greece (Varcasia *et al.*, 2007) and Iran (Fadakar *et al.*, 2015) indicating that goats could harbour this genotype despite low levels of infection. Goats are naturally more prone to *E. granulosus* s.s. infection, most probably due to the cosmopolitan distribution of sheep strain, however susceptibility of goats to other genotypes cannot be disregarded. Different genotypes of *E. granulosus* s.l. occur sympatrically across various geographical areas among different intermediate hosts (Maillard *et al.*, 2007), however, intrinsic barriers to gene exchange between sympatric species maintain genetic identity among the strains despite overlap in the host spectrum.

The unofficial slaughterhouses and inadequate tracking of animals are serious issues sustaining the CE in endemic countries that could have contributed to the high prevalence recorded in sheep and goats. Furthermore, the socioeconomic and cultural conditions of Lebanon could have led to reduction in the prophylactic approaches to a bare minimum contributing to the maintenance of the parasite lifecycle. In conclusion, high rates of infection in sheep and goats represent a zoonotic risk with particular relevance to urban areas. This was brought to light through a reliable assessment of the epidemiological situation that revealed the true burden of disease. Developing guidelines and an implementation plan to reduce the public health costs in endemic settings using genotype-specific approach for disease control is deemed necessary.

Data. The datasets used and/or analysed during the current study are available from the corresponding author on a reasonable request.

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Ethical standards. All the operations carried out on live animals were performed by the vet as part of the routine clinical visit and the study was carried out following the recommendations of European Council Directive (86/609/EEC) on the protection of animals.

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