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Cystic Echinococcosis in Lebanese animals and humans

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Preface

The following thesis is written in a 'thesis by publication' format and includes one published article in an international journal and one paper submitted for publication.

This thesis entitled "Cystic Echinococcosis in Lebanese animals and humans" is divided in two main chapters:

Chapter 1. Includes a research article entitled "Cystic Echinococcosis in sheep and goats of Lebanon" published in Parasitology Journal; focusing on epidemiological study and molecular characterization of metacestodes *E. granulosus* s.l. species involved in echinococcosis from sheep and goats in Lebanon.

Chapter 2. Consists of "Human cystic echinococcosis in Lebanon: a retrospective study and molecular epidemiology". This chapter discusses the human epidemiology concerning temporal and spatial distribution of cystic echinococcosis in the patients who underwent surgical removal of hydatids in the hospitals of all different districts in Lebanon, across the years 2005-2018.

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General Introduction

Cystic echinococcosis (CE), also known as hydatid disease, is a neglected zoonotic parasitic disease of livestock and humans, caused by metacestodes (larval stage) of the tapeworm *Echinococcus granulosus sensu lato (E. granulosus s.l.)* (Tamarozzi *et al.,* 2019). This disease has known since ancient times, as described in Greece by Hippocrates and Aristotle (Sotiraki *et al.,* 2003); and is one of the epidemic diseases that are dangerous to humans in terms of health and economy in most countries of the world (Al-Khalidi *et al.,* 2020).

The parasite *E. granulosus s.l.* is transmitted from carnivores (dogs, foxes, leopards, lions, and hyenas), which act as definitive hosts, to herbivores (sheep, goats, camels, cows, buffaloes, horses, donkeys, wild ungulates, rabbits) as intermediate hosts, and humans (aberrant hosts) as accidental intermediate host, via oral-fecal cycle (Craig *et al.,* 2017; Al-khalidi *et al.,* 2020).

The infection of definitive host starts when the viable protoscoleces of infected domestic intermediate hosts are ingested by purposeful feeding of contaminated offal after home slaughter, improper management of abattoirs and slaughterhouses, or by stray dogs scavenging on livestock carcasses left on the pasture (Otero-Abad and Torgenson, 2013). In the small intestine of the definitive host, they develop into adult worms and start shedding eggs in the environment with faeces (Otero-Abad and Torgenson, 2013, Romig et al., 2017).

When the eggs are ingested by the intermediate hosts or humans (accidental host), they hatch in the small intestine and release six-hooked oncospheres (embryo) that penetrate the intestinal wall and migrate through the circulatory system into various organs, especially the liver and lungs (Eckert and Thompson, 2017). In the organs, the oncosphere develops into unilocular hydatid cyst that enlarges gradually and produces daughter cysts or protoscoleces that fill the cyst interior (Al-khalidi *et al.,* 2020). If cysts rupture, the protoscoleces are liberated and may create secondary cysts in other sites within the body (WHO, 2020).

Both humans and animals can be accidentally infected by ingesting *E. granulosus s.l.* eggs through consuming contaminated food and water or through close contact with infected soil or objects (with subsequent ingestion of eggs), including dogs' mouths and fur; in addition to several ecological factors, human behavior and hygiene habits that are

biologically plausible potential risk factors (Chaâbane-Banaoues *et al.,* 2015; Alvarez Rojas *et al.,* 2018; Cerda *et al.,* 2018).

The disease found in most people involved in raising sheep (acted as an intermediate host of the parasite) and in the presence of dogs that are allowed to eat the offal of infected sheep (Al-khalidi *et al.,* 2020).

Human infection with *E. granulosus s.l.* leads to the development of one or more hydatid cysts located most often in the liver and lungs, and less frequently in the bones, kidneys, spleen, muscles and central nervous system (Bhutani and Kajal, 2018; Yaghoobi *et al.,* 2019). The symptoms in humans depend on the hydatid cyst number, location, size and pressure exerted on the surrounding tissue (Sarkar *et al.,* 2016). Clinical signs and manifestations may range from asymptomatic infection to fatal disease in humans and takes few days to months after infection (Thys *et al.,* 2019; WHO, 2020). Hepatic and pulmonary signs/symptoms are the most common clinical manifestations, as these are the most common sites for cysts to develop (Ito and Budke, 2017).

The majority of human cases of echinococcosis are diagnosed through ultrasonography and/or other imaging techniques supported by positive serologic tests (ELISA or immunoblotting) (Fadel *et al.,* 2018).

Despite advances in diagnosis and therapeutic techniques in hydatid disease, recurrence remains one of the major problems in the management of hydatidosis (Velasco-Tirado *et al.,* 2017). The management and treatment of cystic echinococcosis may vary from endoscopic, surgical intervention to minimally invasive treatments (percutaneous drainage) or medical therapies (as chemotherapy), watch and wait (Fadel *et al.,* 2018; Velasco-Tirado *et al.,* 2018). The choice of the treatment depends on the stage of the cyst, the complications, and the locally available resources (Brunetti *et al.,* 2010).

In intermediate hosts CE is usually asymptomatic and diagnosis is usually performed post mortem through the detection of the cysts particularly in the liver and lungs (WHO, 2020). Cysts can be incidentally discovered by radiographic methods, especially portable ultrasound scanners, which are more practical and reliable approach for the potential premortem diagnosis of CE in small ruminants (Craig *et al.,* 2015).

In definitive hosts, the diagnosis is difficult to be performed by microscope technique since eggs of *Taenia* species and *Echinococcus* are morphologically identical (Eckert and Deplazes, 2004). Traditionally, the detection of canine echinococcosis was diagnosed ante

mortem by purging with arecoline hydrobromate (Eckert *et al.,* 2001). Later, arecoline testing was replaced by enzyme-linked immune-adsorbent assay (ELISA) and recently several PCR protocols have been used for the identification of *E. granulosus* DNA from eggs or from adult parasites (Varcasia *et al.,* 2004).

Based on phenotypic characters and gene sequences, *E. granulosus s.l.* complex has been delimited into 5 species including: *E. granulosus sensu stricto* (*s.s.*; former sheep strain, G1 and G3), *Echinococcus felidis* (former lion strain), *Echinococcus equinus* (former horse strain, G4), *Echinococcus ortleppi* (former cattle strain, G5), and *Echinococcus canadensis* (former camel, pig and cervid strains, including G6/G7 and G8/G10) (Romig *et al.,* 2015; Thompson, 2020). However, studies have revealed that genotypes G2 and G9 are no longer consider valid, since G2 is a micro variant of G3 and G9 a microvariant of G7 (Kinkar *et al.,* 2018; Laurimäe *et al.,* 2018). In contrast, the species *E. equinus* (G4), *E. orteleppi* (G5) and *E. felidis* has remained undisputed (Kinkar *et al.,* 2018). The situation of *E. canadensis* (G6-G10) genotypes is controversial and it suggested to be divided into two distinct species, one comprising *Echinococcus intermedius,* (G6/G7) of camel/pig genotypes, and the other species consist of *E. canadensis* (G8 and G10), cervid genotypes G8/G10 (Yanagida *et al.,* 2017; Laurimäe *et al.,* 2018).

Genotypes of *E. granulosus s.l.* have a variable geographic distribution expanding in multiple locations across the world (Eckert and Thompson, 2017), extending from China to the Middle East and from Mediterranean countries to the sub-Saharan Africa and South America (Romig *et al.,* 2011; Cardona and Carmena, 2013). Furthermore, it is considered endemic in areas of South America, the Mediterranean, Central-Eastern Europe, especially Romania and Bulgaria, north and east Africa, the Middle East, central Asia, all countries of the Indian subcontinent, western and north-eastern China, and Australia (Deplazes *et al.,* 2017). Among the species of *E. granulosus s.l.*, genotypes G1 and G3 (associated with sheep) are the most commonly reported at present and world widely distributed (Sharma *et al.,* 2013; Ito and Budke, 2017), and especially, the G1 genotype of *E. granulosus s.l.* which is the main responsible for the vast majority (88%) of human cases in the world (Alvarez Rojas *et al.,* 2014).

Various studies related to the epidemiology in the Middle East (such as Turkey, Cyprus, Israel, Palestine, Lebanon, Syria, Jordan, Iran and Egypt), have indicated that the hydatid disease is mainly caused by *E. granulosus s.s.* (G1-G3) followed by *E. canadensis* (G6/7) (Lopez-Bernus *et al.,* 2015; Deplazes *et al.,* 2017; Matini *et al.,* 2018); and commonly

found in sheep and goats as principle intermediate hosts, followed by camels and cattle (Al kitani *et al.,* 2015). These countries are highly exposed to several risk factor for *E. granulosus s.l.* infection, associated to cultural practices in sheep breeding and home slaughtering, dogs keeping freely near slaughterhouse, and poor hygienic conditions in slaughterhouse, in addition to the rural/nomadic lifestyle accompanied by use of sheep dogs on farms and the consumption of raw vegetables (Otero-Abad and Torgerson, 2013; Thys *et al.,* 2019). However, some other factors work as opposing sources against the CE transmission, such as the arid/semi-arid climatic conditions that prevent the survival of *Echinococcus* eggs, religious traditions that do not allow pig breeding and the habit of avoiding dogs in Muslim communities (Chaâbane-Banaoues *et al.,* 2015).

The infection caused by CE appears to be increasing annually with >200,000 new cases/year and causing annual economic losses related to human infection (Cerda *et al.,* 2018), as well as animal production losses due to death of infected animals, condemnation of internal organs of slaughtered livestock, reduction in carcass weight, decrease in milk production, fecundity and other production-based losses (Borji *et al.,* 2012; Valieva *et al.,* 2014; Kere *et al.,* 2019).

According to WHO, 1 to 3 million people around the world are suffering from CE with an estimated burden of 184.000 DAILYs (disability-adjusted life years) every year (Torgerson *et al.,* 2015), with an estimated loss of US$ 3 billion in terms of medical expenses yearly and livestock industry losses (Agudelo Higuita *et al.,* 2016; WHO, 2020).

These economic losses can be reduced by several preventive measures, as primarily targeted to better availability of surveillance tools, optimal application of livestock vaccination, management and ecology of dog and wildlife host populations and health education about hygiene (Craig *et al.,* 2017).

Lebanon, a small country in the Middle East could be a good epidemiological model to study CE for: (i) it's geographic location in the Mediterranean region where the disease is widespread, (ii) the presence of all the important factors that contribute to the transmission of CE like the extensive livestock farming, presence of stray dogs, illegal slaughtering, and improper disposal of carcass, (iii) the lack of recent data regarding the epidemiology and the molecular characterization of *E. granulosus* in livestock (sheep and goats) and humans of Lebanon.

Historical reports concerning CE in Lebanon suggest the endemic nature of hydatidosis in the country, but recent data is unfortunately not available (Araj and Mourad, 2014).

The present epidemiological study was carried out in different Lebanese regions in order to do a survey about the current situation of cystic echinococcosis caused in livestock and humans of Lebanon.

The first chapter was (i) to determine the current situation of hydatid cyst infection in Lebanese sheep and goats, (ii) to classify cysts morphologically as fertile, sterile, calcified or caseous focusing on the prevalence of hosts harboring viable protoscoleces, that represents the greatest risk of spreading the infection to both humans and animals, and (iii) to characterize using molecular techniques, the present genotypes/species of *E. granulosus* in sheep and goats of Lebanon.

The second chapter was (i) to assess the incidence of human CE in Lebanon by conducting a survey for the period 2005-2018 using hospital's medical records for patients that underwent surgical removal of hydatid cysts, (ii) to check the distribution of the disease in the country, (iii) to characterize using molecular techniques, the present genotypes/species of *E. granulosus* in humans of Lebanon.

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Abstract

This doctoral thesis aims to provide original data on the epidemiology and transmission of cystic echinococcosis disease in order to improve the current knowledge and the ongoing control strategies. The thesis is presented in form of "thesis by publication" and is composed by two chapters as submitted articles.

An epidemiological survey was investigated the diffusion of cystic echinococcosis (CE) in small ruminants and Hunan in Lebanon. The objectives of this survey were to evaluate the current situation of CE in Lebanon, investigate the prevalence and determine the infecting genotypes in locally raised sheep, goats and Human.

During 2 consecutive years, a survey was carried out on 369 and 335 slaughtered sheep and goats respectively, 62.9% of the sheep and 20.9% of the goats were found positive for CE.

From our results, we identified a high prevalence of CE in livestock of Lebanon and reveal for the first time the presence of three different genotype.

In addition, epidemiologic features of human CE surgical cases were assessed from 2005 to 2018 in the five main regions of Lebanon. A total of 894 surgically confirmed cases of hydatidosis were recorded from five anatomy and pathology laboratories, with a mean annual surgical incidence of 1.23/100,000 inhabitants. Additionally, predominant involvement of *Echinococcus granulosus* sensu stricto was recorded. The lack of epidemiological data and control measures has resulted in higher incidence of human CE.

The current study is a step forward to fill the gap of knowledge for the hydatidosis in Lebanon, where prevention and control programs should be implemented to reduce the risk of hydatidosis in all over the country.

Chapter 1.

Research article

Cystic Echinococcosis in sheep and goats of Lebanon

Adapted from:

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1. 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 neighboring 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, of 369 and 335 slaughtered sheep and goats respectively, 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 liver of sheep versus lungs of goats, however, 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 *E. canadensis* (genotype G7; 1.3%). *E. 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 high prevalence of CE in livestock of Lebanon and reveal for the first time the presence of three different genotypes G1, G3 and G7.

Key words: Cystic echinococcosis, sheep, goats, Lebanon, haplotypes, *E. granulosus s.s.*, *E. canadensis*

Key Findings

1. This is the largest epidemiological survey carried out in Lebanon in the last 30 years revealing high CE prevalence among sheep (62.9%) and goats (20.9%).
2. Cysts in sheep were predominantly located in the liver (58.2%), whereas in goats, lung was the most affected organ (61.3%), however, the probability of finding fertile cysts in lungs was higher than in liver in both animals.
3. Two species, *E. granulosus* s.s. (genotypes G1, G3) and *E. canadensis* (genotype G7) were found to be circulating among sheep and goats out of which G1 was highly prevalent.
4. Twenty-two (22) haplotypes were recorded for *E. granulosus* s.s. with two predominant haplotypes identified from other Mediterranean countries.
5. Among the 22 haplotypes arranged in a star like configuration, 18 haplotypes belonged to G1 genotype whereas 4 haplotypes were of G3 genotype mainly identified from sheep.

2. Introduction

Cystic echinococcosis (CE) is ranked second in the list of food-borne parasites worldwide, listed amongst the most severe parasitic diseases in humans (WHO, 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.* 2018*a*). 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.* 2018*a*; 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.* 2018*b*) on the basis of mitochondrial DNA (mtDNA). At least 10 strains (G1–10) of *E. granulosus* s.l. have been described, forming 4 major clades (G1–G3, G4, G5, 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.* 2018*b*), *Echinococcus equinus* (G4) and *Echinococcus ortleppi* (G5) (Thompson and McManus 2002), whereas the status of genotypes G6–G10 is under dispute (Lymbery *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* (Lymbery *et al.* 2015).

In the Middle East, the most dominant species responsible for CE infections in both wild and domestic animals is *E. granulosus* sensu stricto (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, are currently scant (Thompson, 2017). Regarding Lebanon, in 1930 the prevalence of 41.5% CE in cattle was reported in Beirut (Goodale *et al.* 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.* 1936*a*), and 20-25% in dogs (Turner *et al.* 1936*b*). 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 socioeconomic 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.

3. 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 characterisation of hydatid cyst, (iii) statistical analysis of the gathered data, and (iv) molecular identification of the circulating genotypes.

3.1.Sample collection

According to the Raosoft sample size calculator (http://www.raosoft.com/samplesize.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 5 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 cyst were found, these were counted and examined to verify exact location, fertility and morphological characteristics.

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

3.2.Statistical analysis

Data was 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).

3.3.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 ^{o}C till digestion of the membrane. Following digestion, absolute ethanol was added to each sample and the sample was transferred to Nucleospin tissue column and centrifuged at 12000 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 12000 g centrifugation. PCR was done using primer pairs F/COI (TTGAATTTGCCACGTTTGAATGC) and R/COI GAACCTAACGACATAACATAATGA) for the amplification of partial mitochondrial *cox1* gene (Nakao *et al,* 2000). 29 isolates were further screened through *nad5* mitochondrial gene

employing the primers EGnd5F1 (GTTGTTGAAGTTGATTGTTTTGTTTG) and EGnd5R1 (GGAACACCGGACAAACCAAGAA) for correct identification of genotypes G1 and G3 (Kinkar *et al.* 2018*b*). A G7 isolate was also amplified using G7for (GTGTTGTGTTGTTGATAGATTG) and G7rev (GTAAAAATAATCACCACCCAAC) primers for *nad2* gene (Laurimae *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.* (2018*c*) 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 Fs) and genetic differentiation (Fst), the data were analyzed on Arlequin package 3.5 (Excoffier and Lischer, 2010). Haplotype network formation was executed on PopArt software using a median joining network (Leigh and Bryant, 2015). Estimation of pairwise divergence was done on MEGA X software (Kumar *et al.* 2018).

4. 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 harbored 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 (χ^2 = 1.2256; P = 0.2682). Hydatid cyst were found in both liver and lungs in 30.1% of 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 infected animals, 32% had 1-5 cysts, while 21.9% of animals had massive infection harboring 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 (χ^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%, 7.4% were found to be fertile, sterile and caseous respectively. Looking at all the 2707 recovered hydatid cyst 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 in lungs (11.9%; 40/335) than in the liver (9.9%; 30/335), but no statistical differences were observed (χ^2 = 1.5952; P = 0.2065). Hydatid cyst 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 (χ^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%, 9.3% were found to be caseous, fertile and sterile, respectively. The presence of hydatids in goats varied when considering 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.* 2018*b*) 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 genes sequence reflected similarity with 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.

The analyzed *cox1* region (795 bp) contained 24 point mutations at 24 segregating loci of which 17 (70.83%) were singleton sites and 7 (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 (Figure 1). G1 genotype was represented by 18 haplotypes (81.81%) whereas, G3 genotype was represented by 4 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 as 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 in 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 higher number of haplotypes (n = 6) in 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, Fs = -19.891) in the overall population of *E. granulosus* s.s. identified on the basis of *cox1* dataset. Furthermore, estimation of gene flow among the two intermediate hosts was calculated using pairwise Fst. Due to occurrence of common haplotypes and less divergence between the *E. granulosus* s.s. haplotypes, Fst value was very low (-0.01956, $p>0.05$).

5. Discussion

To our knowledge, the present article 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 present survey, the prevalence of CE in sheep and goats has increased to 62.9% and 20.9% respectively. Current results show 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 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 done in

several 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 done 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, 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 high infection rate was seen in sheep with the liver being the most affected organ (Varcasia *et al*. 2006; Grosso *et al.* 2012; Almalki *et al.* 2017; Brik *et al.* 2018; Toulah and Albalawi, 2019; Varcasia *et al.* 2020).

Different situation has been observed in epidemiological studies done in Iran and Pakistan, where sheep had highest infection in lungs and liver was most infected organ in goats (Lotfi *et al.* 2010; Mehmood *et al.* 2020*a*).

When considering cyst fertility, the probability of finding fertile cysts in lungs was higher than 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). Increased presence of fertile cysts in lungs can be explained by the variation in tissue resistance between organs (Fikire *et al.* 2012).

Determination of 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 analyzed 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.* 2020*b*). 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 like China (Nakao *et al.* 2010), Iran (Yanagida *et al.* 2012), United Kingdom (Boufana *et al.* 2015), Italy (Mehmood *et al.* unpublished data). 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 like 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, indication of hypothetical haplotypes in the haplotype network implied that high genetic variability exists among *E. granulosus* s.s. population of Lebanon which required molecular surveillance encompassing more isolates.

E. granulosus s.s. has shown to have undergone rapid radiation globally (Kinkar et al. 2018*a*; Kinkar *et al.* 2018*b*). Current study estimated moderate genetic variability (Hd: 0.7614±0.0391) among the *E. granulosus s.s.* specimens isolated from sheep and goats. Even though sheep harbored 20 haplotypes, the haplotype diversity was lower for sheep in comparison to 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 (Fst) for the goat and sheep population yielded a very low value (-0.01956) suggesting 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 has largely been influenced by humans (Kinkar et al., 2018*c*).

E. 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 (680bp) and *nad2* (714 bp) genes, it was identified that this sequence belonged to G7b haplogroup of *E canadensis* which was also recognized from other Mediterranean countries 'France and Italy' (Laurimäe *et al.* 2019). 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 harbor 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 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 reasonable request.

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Author Contribution

Conceptualization, A.V. and G.J.; investigation, G.J., G.D., C.T., F.N.; data curation, M.N., C.T.; writing original draft preparation, G. J., A.V.; writing review and editing, C.T., U.S., A.S., M.N., C.H.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare there are no conflicts of interest.

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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List of Figures and Tables

Figure 1. Haplotypic structure of *E. 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.

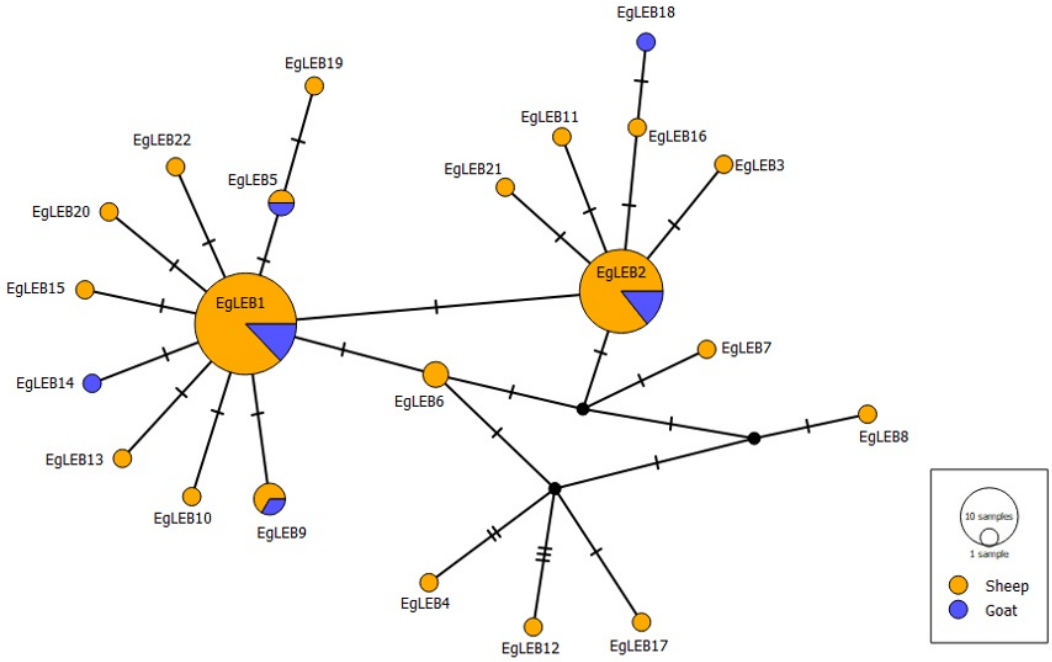

Table 1: Percentage of types of hydatid cysts in infected Lebanese sheep and goats

	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.

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

Table 3: Number, prevalence and geographic localization of haplotypes (n = 22) of *E. 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	Hn	Diversity indices		Neutrality indices	
		Hd±SD	Nd±SD	Tajima's *D*	Fu's Fs
Sheep	20	0.7567±0.0423	0.00189±0.00129	-2.1265 *	-17.097*
Goat	6	0.8364±0.0887	0.00184±0.00137	-1.2181	-2.5079*
Overall	23	0.7614±0.0391	0.00188±0.00026	-2.1627*	-19.891*

Hn: haplotype number, Hd: haplotype diversity, Nd: nucleotide diversity

* significant at $p<0.05$

Chapter 2.

Original article

Human cystic echinococcosis in Lebanon: a retrospective study and molecular epidemiology

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1. Abstract

Human cystic echinococcosis (CE) is a zoonotic parasitic disease that constitutes a public health challenge and a socioeconomic burden in endemic areas worldwide. No specific surveillance system of CE infections in humans exists in Lebanon. The incidence and trends over time have not been documented. The current study aimed to assess the demographic and epidemiologic features of human CE surgical cases over a 14-year period in the five main regions of Lebanon. From 2005 to 2018, a total of 894 surgically confirmed cases of hydatidosis were recorded from five anatomy and pathology laboratories, with a mean annual surgical incidence of 1.23/100,000 inhabitants. Over the span of these years, the incidence increased from 0.53 to 1.94 cases/100,000 inhabitants in 2005 and 2018, respectively). CE is present in Lebanon with an uneven distribution from one region to the other with higher prevalence in Bekaa (29.0%), a rural area where sheep raising is widespread. Human CE cases were more common in females (60.1%) than in males (39.9%) and a high burden of infection was reported for the age group of 30-39 years. Besides, 66.7% of the cases expressed only liver complications whereas 20.5% only related to the lungs. The 7.8% of cases presented cysts in other organs, and 1.3% showed multiple localizations. Additionally, predominant involvement of *Echinococcus granulosus sensu stricto* was recorded. The current study is a step forward to fill the gap of knowledge for the hydatidosis in Lebanon, where lack of epidemiological data and control measures have resulted in higher incidence of human CE.

Key words

Human cystic echinococcosis, Lebanon, Zoonosis, *E. granulosus s.s.*, Haplotypes.

2. Introduction

Cystic echinococcosis (CE), or hydatidosis, is a parasitological disease carrying a vast history, with its first records dating all the way back to ancient Greece (Dakkak, 2010; Eckert & Thompson, 2017). However, despite being long identified and the considerable scientific advancements, CE still represents a persistent zoonosis with significant socio-economic impacts (Moro & Schantz, 2009; Dakkak, 2010; Eckert & Thompson, 2017). Overall economic losses due to this disease are estimated at two billion US$ annually and CE is believed to affect more than one million people worldwide (FAO/WHO, 2014). Furthermore, besides being considered among the most severe parasitic diseases in humans (ranking the second most concerning food-borne parasites globally, FAO/WHO, 2014), CE is also one of the most important parasitic diseases in the Mediterranean region and the Middle East (Dakkak, 2010; Varcasia et al., 2011; Al-Kitani et al., 2015). In general, CE is most common in pastoral regions where sheep, cattle and camelids are prominent and is present worldwide with endemic foci on every inhabited continent (Dakkak, 2010).

CE is caused by the tapeworms belonging to the *Echinococcus granulosus sensu lato* (*E. granulosus s.l.*) species complex comprised of 10 separate genotypes (G1-10) and *E. felidis*, each with specific geographical distribution and host affinities (Nakao et al., 2013; Romig et al., 2015). The tapeworms exhibit an indirect lifecycle involving two hosts; various herbivorous and omnivorous mammals act as intermediate hosts while the domestic and wild canids act as the definitive hosts (Moro & Shantz, 2009; Eckert & Thompson, 2017). Whereas canids harbor the adult tapeworms in their small intestine, larvae grow into hydatid cyst in the intermediate host's tissues, causing CE. The latter acquire the infection by oral ingestion of infectious eggs shed through the faeces of the definitive host. Consumption of cyst bearing tissue by canids closes the cycle up (Thompson, 2017).

E. granulosus s.l. is also capable of infecting humans who, as such, work as a dead-end (aberrant) host (Al-Kitani et al., 2015; Pakala et al., 2016). Just like the other intermediate hosts, human infection results from oral ingestion of infectious eggs. People can accidentally ingest eggs through intimate contact with infected dogs (Buishi et al., 2005). Alternatively, indirect transmission can occur by ingestion of contaminated food, water or soil carrying the eggs of the parasite (Moro & Schantz, 2009; Brehm & Koziol, 2017; Torgerson et al., 2020). The infection starts when oncospheres released from ingested eggs penetrate the intestinal wall, then the larvae migrate through the portal venous system reaching the liver and possibly various other internal organs developing into hydatid cysts (metacestode). As these cysts grow slowly, this first phase of CE occurs asymptomatically (Moro & Schantz, 2009; Pakala et al., 2016; Brehm & Koziol, 2017). Only later, when cysts reach a considerable size, which can be responsible of organ dysfunction, could symptoms associated with involved organs arise. Additionally, the risk of rupture of cysts could possibly lead to anaphylactic shock and death, or to the dispersion of oncospheres resulting in multiple secondary echinococcosis disease (Moro & Schantz, 2009; Pakala et al., 2016). Human infections have largely been attributed to *E. granulosus sensu stricto (s.s.)* (G1 genotype, sheep strain) due to its cosmopolitan distribution and maintenance through sheep-dog cycle (Alvarez Rojas et al., 2014); genotype G3 is also implicated in human CE (Muqaddas et al., 2020). Other taxa having significant contribution in human CE are genotypes G6 and G7 which usually transmit through camels, goats and pigs respectively in areas where these genotypes have predominant occurrence in animals (Alvarez Rojas et al., 2014).

Globally, occurrence of CE is closely related with specific socio-economic conditions, including extensive livestock farming and pasture sharing, unsupervised slaughtering practices, improper disposal of carcasses, uninformed public and the widespread presence of numerous stray and shepherd dogs. One particularly critical aspect of endemic CE is the

feeding of raw offal to dogs (Scala et al., 2006; Scala & Mazzette, 2009; Dakkak, 2010; Varcasia et al., 2011).

The presence of *E. granulosus s.l.* tapeworms as well as the prevalence of CE in both animals and people has been extensively recorded around the Mediterranean basin, including France, Spain, Italy, Greece, Turkey, Cyprus, Syria, Israel, Egypt, Libya, Tunisia, Algeria and Morocco (Dakkak, 2010). Scarce recent information regarding the epidemiology and impact of the different *E. granulosus s.l.* species and genotypes is available for Lebanon (Araj & Mourad, 2014) despite CE has been known to be an important public health problem in the country (Frayha, 1970). Furthermore, all the socio-economic conditions contributing to disease perpetuation are prominently present in Lebanon and account for the endemic nature of hydatidosis in this country.

For these reasons, an epidemiological survey was carried out in order to investigate the distribution of CE in Lebanon. Additionally, metacestodes isolated from patients diagnosed with CE in Lebanese hospitals were genetically characterized as to get a better understanding of what *E. granulosus s.l.* species and genotypes are represented in this region of the Mediterranean.

3. Materials and Methods

3.1. Survey and data collection

In total, data for 894 human CE cases were obtained from five main pathology laboratories, each located in one of the five principal Lebanese regions: Beirut, Bekaa, Mount Lebanon, North Lebanon and South Lebanon (Figure 1). Each of those five pathology laboratories complements the area hospitals laboratories in each of the five mentioned areas. Most hospitals laboratories lack the pathology specialty and subcontract those services to external

specialized units. This puts our selected laboratories as significant representatives of the area dynamics.

Retrospective epidemiological and clinical data from the laboratories included in the investigation were obtained for all patients who underwent surgical treatment after confirmation of CE diagnosis between January 2005 and December 2018. Only data regarding patients who underwent surgical removal of a hydatid cyst was included in this research.

The collected data consisted of hospital discharge records (HDRs) containing patients' personal and medical information. Only a selected number of parameters from the HDRs were used during the analyses: gender, age, location of the excised cyst(s) and year of treatment. Finally, the gathered information from each patient was linked to the respective hospital of treatment together with its corresponding region and centralized in a spreadsheet using the software Microsoft Office Excel®.

Furthermore, human CE data was also collected from the register of the Ministry of Health in Lebanon (MOH, available online) in order to compare that with the data collected from the hospitals.

3.2. Molecular analysis

In order to identify the etiological agents of the human CE in Lebanon, four freshly excised cysts were collected from pathology departments of different hospitals under the supervision of a specialist doctor. Fresh cysts located in lungs and liver were collected from different patients; the germinal layer and the cystic liquid of the hydatid were excised and preserved in ethanol (95%) before being analyzed by the Department of Veterinary Medicine (University of Sassari, Italy). In addition, ten paraffin-embedded fixed samples from surgically confirmed CE cases were collected from the pathology departments of two hospitals located in the Bekaa region.

Thin sections of paraffin-embedded samples were soaked in xylol and then in ethanol 100% in order to remove the paraffin prior the DNA extraction. DNA from fresh (n = 4) and paraffin-embedded samples (n = 4) was extracted using NucleoSpin Tissue (Macherey-Nagel GmbH % Co.KG, Düren, North Rhine-Westphalia, Germany). A fragment within the cytochrome c subunit 1 (*cox1*) was amplified from DNA of both fresh and paraffin-embedded samples using two different set of primers and protocols. In particular, the primers COIF (5′-TTGAATTTGCCACGTTTGAATGC-3′) and COIR (5′-GAACCTAACGACATAACATAATGA 3′) were used to amplify a fragment of approximately 800 bp from DNA of fresh samples, as previously described (Nakao et al., 2000). Additionally, a fragment of 396 bp was amplified using the primers JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and JB4.5 (5′-TAAAGAAAGAACATAATGAAAATG-3′) from the DNA of the paraffin-embedded samples according to Bowles et al. (1992), given the DNA fragmentation due to the fixation process of those samples. DNA of the fresh cyst material was also amplified for *nad5* gene (680 bp) for proper discrimination of *E. granulosus s.s.* genotypes (Kinkar et al., 2018a).

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. Obtained electropherograms for partial *cox1* regions (800bp and 396 bp) were aligned with their respective reference sequences (Bowles et al., 1992; Nakao et al., 2000) for determination of genotypes.

E. granulosus s.s. sequences for the human CE isolates (this study) and intermediate hosts from Lebanon (Joanny et al. unpublished data) and its neighboring countries (Iraq and Jordan) were compared to understand demographic and transmission patterns for this region. Available mitochondrial *cox1* nucleotide sequences for Iraq (Hassan et al., 2017) and Jordan

(Yanagida et al., 2012) were retrieved from GenBank database and in total, 130 sequences were analyzed from Lebanon (n = 80), Iraq (n = 38) and Jordan (n = 12). Dataset for the partial *cox1* gene was trimmed to equal lengths and computed in DnaSP (Rozas et al., 2017) for information on haplotypes and polymorphism. Furthermore, population diversity indices (haplotype and nucleotide diversities), neutrality indices (Tajima's *D* and Fu's Fs) and pairwise fixation index (F_{st}) were calculated using Arlequin (Excoffier & Lischer, 2010). A haplotype network was generated using TCS criteria through PopART software providing information on all haplotype linkages (Leigh and Bryant, 2015).

3.3. Data analysis and statistics

All data (i.e., cyst surgical removal site, age and gender of patients, and hospital location) were analyzed with a descriptive analysis, according to region, age, gender of patients and lesion site.

Mean annual incidence rates of surgery have been calculated on the basis of the total population in Lebanon from 2005 to 2018 obtained from the World Bank Data and WHO data (https://data.worldbank.org/country/LB).

Moreover, data were compared with the data retrieved from the MOH.

4. Results

The analysis of data provided the temporal and spatial distribution of CE in the patients who underwent the surgical removal of hydatid cysts in different hospitals of Lebanon. Based on HDRs, a total of 894 human CE cases occurred between 2005 and 2018 in five different Lebanese regions (Beirut, Bekaa, Mount Lebanon, North and South). Retrospective data analysis yielded that CE was present in all regions with the Bekaa region showing significant highest percentage (29.0%; 259/894) followed by Mount Lebanon (24.8%; 222/894), South

Lebanon (17.9%; 160/894), Beirut (14.8%; 132/894) and North Lebanon (13.53%; 121/894) (Figure 1).

The total number of registered surgical cases increased from 21 in 2005 to 133 in 2018 with a mean annual incidence rate of 1.23/100,000 inhabitants. Across the years, an increasing trend could be pointed from the year 2005 (0.53/100,000 inhabitants) to date (1.94/100,000 inhabitants) (Figure 2).

Age distribution of the CE cases in Lebanese population showed that patients belonged to multiple age groups ranging between 4 and 94 years (mean age 41). Highest prevalence was observed for the age cohort of 30-39 years (17.3%;155/894) (Figure 3). Furthermore, 60.1% (537/894) of recorded cases referred to females and 39.9% (357/894) referred to males.

Data regarding the cysts localization was available for 861 cases (33 cases were unspecified): 849 CE cases with single organ infection and 12 with multiple organ infections. The majority of patients were affected by liver echinococcosis (66.7%), followed by pulmonary CE (20.5%). The 7.8% of cysts were present in other sites like the abdomen, the spleen, bones, kidneys and ovaries, whereas 1.34% cases showed multiple cyst localization (Table 1, Figure 4).

Furthermore, a comparison between our survey and data obtained by the MOH was made in order to check the similarities in the number of cases of CE reported per year and gender distribution. Although our survey included only surgically confirmed CE cases from a limited number of Private, Public and University hospitals in Lebanon, we could note that the MOH presented a lack in case reporting and data registration (Figure 5). In regards to gender of patients, females were more predisposed to CE than males in both data sets collected directly from the hospitals and those recorded by the MOH.

Genotyping of the examined isolates revealed the involvement of *E. granulosus s.s.* in the human CE in Lebanon. Sheep strain (G1 genotype) was identified from seven isolates (87.5%) whereas, buffalo strain (G3 genotype) was characterized from a single cyst removed from liver of a female patient. The population structure analysis encompassing three regional populations (Lebanon, Iraq and Jordan) of the *E. granulosus s.s.* revealed the presence of 54 haplotypes with 49 point mutations. Predominant occurrence of two haplotypes, EG5 (27.7%) and EG17 (21.5%) resulted in the formation of a double clustered haplotype network (Figure 6).

Four haplotypes (EG4, EG5, EG8, EG17) were shared among the three countries whereas the haplotypes EG16 and EG23 were only present in Iraq and Lebanon. Shared haplotypes for Iraq-Jordan and Jordan-Lebanon populations were EG12 and EG34 respectively.

Molecular analysis of the *E. granulosus s.s.* sequences from Lebanon, Iraq and Jordan revealed the presence of 54 haplotypes with 49 point mutations. Overall, high haplotype diversity (0.8769±0.021) and low nucleotide diversity (0.00264±0.00021) were estimated for all countries (Table 2). Neutrality indices were highly negative (D = -2.39256, Fs = -80.185) and significant ($p < 0.01$) suggesting the presence of rare haplotypes and therefore, population expansion. The pairwise fixation index values were also very low ranging from -0.02153 (Iraq and Jordan) to 0.00449 (Lebanon and Jordan) indicating gene flow among these countries (Table 3).

5. Discussion

This is the first comprehensive retrospective study on human epidemiology of CE in Lebanon to date since a survey carried in 1961 (Schwabe & Abou Daoud, 1961). From previous studies it is well known that CE is highly endemic in Lebanon, with an incidence of 3.8/100,000 inhabitants (Matossian et al., 1977). However, the incidence seemed to decrease in recent years, although no evidence is currently available in the literature to support this statement (Araj & Mourad, 2014). As CE is an important health concern in both humans and animals and is paired with considerable economic burden due to the medical treatment costs, work impairment, morbidity and mortality (Piseddu et al., 2017), we decided to undertake this current study and to assess the status of human CE and the population structure of *E. granulosus s.s.* in Lebanon and its neighboring countries. Our aim is to better understand the transmission dynamics of this parasite in this region.

Estimation of regional prevalence revealed the highest frequency of human CE to be in the Bekaa region which is a fertile valley in eastern Lebanon and the most important farming region of the country. Highest prevalence of CE usually occurs in regions with extensive and traditional sheep farming (Otero-Abad & Torgerson, 2013) leading to human infections (Alvarez Rojas et al., 2014). Previously, higher CE prevalence has been reported from Beirut and Mount Lebanon among the dog owners (Araj & Mourad, 2014).

Mean annual incidence rate of surgically treated CE cases in the current study has been determined as 1.23/100,000 inhabitants which is comparable with the incidence in other middle eastern countries such as Israel (2.7 ± 1.2 /100,000 inhabitants) (Ben-Shimol et al., 2016) but higher than Iran (0.74/100,000 inhabitants; Shahriarirad et al., 2020). An incidence of 0.87 to 6.6 per 100,000 inhabitants has been reported for CE in Turkey (Altintas., 2003). Similarly, higher annual incidence rates were found in Jordan (2.3/100,000 inhabitants; Al-Qaoud et al., 2003) and Sardinia where the mean annual regional discharge rate for patients

hospitalized for symptoms correlated to CE was 9.3/100,000 inhabitants (Mastrandrea et al., 2012) and 6.62/100,000 inhabitants (Conchedda et al., 2010). Furthermore, the numbers of CE cases obtained in this study are higher than those reported by the Lebanese Ministry of Health showing that CE disease is under-reported by healthcare professionals and the general population. The disease is disseminating in Mediterranean countries, however, the exact incidence and prevalence of CE in humans and animals remains unknown (Dakkak, 2010).

Based on surgically confirmed CE cases in this study, the age group of 30-39 years remained most vulnerable to the infection showing highest prevalence among all age cohorts; people in this age group are probably the most active in livestock rearing (Mastrandrea et al., 2012). It was also observed that older age groups (40-49 and 50-59 years) had higher CE prevalence compared to younger ones which could indicate chronic but asymptomatic infections. Contrastingly, a study on human CE in Iraq (Abdulhameed et al., 2018) identified highest prevalence for 21-30 years age group. Analysis of gender-based data revealed that females were more prone to CE infection than males, a finding that has been previously observed in Lebanon (Frayha et al., 1989) and other Middle eastern countries like Iran (Chalechale et al., 2016; Moosazadeh et al., 2017) and Iraq (Abdulhameed et al., 2018). In contrast, higher male infection rates than female were reported for Sardinia (Conchedda et al., 2010). This can be explained by the result of the lifestyle of women, who are more likely to be in direct contact with a source of infection, since they tend to have main role in domestic activities, including food preparation and caring for the family dog (Rao et al., 2012; WHO, 2020).

Regarding cysts localization, the current results showed that the liver was the most commonly affected organ, followed by the lungs, in line with a previous Lebanese study (Araj & Mourad, 2014) and international literature (Salamone et al., 2016; Yaghoobi et al., 2019). Single cases in rare sites of occurrence of the hydatid cyst like in the adrenal gland, brain, breast, intramuscular, myocardium, ovaries, pancreas, peri-bladder, spine and subcutaneous

were also observed which could be explained by the dissemination of cysts through lymphatic channels (Wani et al., 2012).

Molecular genotyping analysis of the partial *cox1* gene revealed the presence of the species *E. granulosus s.s.* (G1-G3 complex) with sheep strain having the predominant involvement in human CE in Lebanon, as identified in most Asiatic human populations (Matini et al., 2018; Yan et al., 2018; Yousefi et al., 2019). The *E. granulosus s.s.* nucleotide sequences obtained from humans in current study were similar to those of intermediate hosts in Lebanon (Joanny et al., unpublished data) reflecting genetic similarity between different intermediate host samples. Five nucleotide sequences identified from the cysts localized in lungs and livers of patients were similar to the second most common haplotype identified in Iran, Turkey and Iraq (Hassan et al., 2017). Haplotypic analysis of Lebanon and its neighboring countries yielded double clustered network topology with two central haplotypes. The haplotype EG5 identified in this study is considered to be a founder haplotype and is also reported from other regions like Iran and Jordan (Yanagida et al., 2012), Tunisia (Boufana et al., 2014) and Sardinia (Mehmood et al., unpublished data). The second most common haplotype (EG17) is reportedly present in Iran (Yanagida et al., 2012), Tunisia (Boufana et al., 2014) and Iraq (Hassan et al. 2017). Similarity in genetic structures among different geographical regions reflects common evolutionary scenario whereby the founder haplotype seemed to have spread across continents through anthropogenic movement of the intermediate hosts from the cradle (Middle east) of this parasite (Hassan et al., 2017).

Population genetics analysis further revealed absence of genetic differentiation and high degree of gene flow among the three countries as indicated by very low and non-significant F_{st} values. Moreover, high haplotype diversity, low nucleotide diversity and highly negative bias from neutrality suggested population expansion and occurrence of single nucleotide substitutions as identified from various parts of the world (Yanagida et al., 2012; Boufana et

al., 2014). Little genetic differentiation among the *E. granulosus s.s.* isolates originating from different intermediate hosts indicates presence of efficient transmission routes of this species to humans across different regions and among various intermediate hosts (Kinkar et al., 2018b). Likewise, presence of *E. canadensis* (G7) among goats in Lebanon was recorded (Joanny et al. unpublished data) and it is quite likely that this genotype could be involved in the human CE if more human isolates are characterized.

Retrospective studies have certain limitations. A selection bias is possible since the samples were recruited from selected anatomy and pathology laboratories. The machines used for analysis differed between hospitals, which might predispose us to information and validity biases. Only CE patients who underwent surgical removal of hydatid cysts were included. For this reason, the data presented are probably underestimating the incidence of CE in Lebanon. Data concerning morbidity and mortality associated with CE, mean hospital stay and follow-up time were not available. Future studies including factors associated with CE spread, including slaughterhouses without supervision, illegitimate slaughtering, little public and farmers' mindfulness of hydatid diseases, and the big number of homeless dogs, need to be conducted to correctly estimate the status of CE in Lebanon.

6. Conclusion

This study added information about the *status quo* of CE rate in Lebanon and its distribution across the different regions of the country. As a whole, this work clearly showed that CE represents a serious health problem in Lebanon and its extent is still underestimated due to under-reporting and weak maintenance of data by concerned officials and public institutions. Rise in incidence rates of surgery, however, suggested the perpetuation of the disease and involvement of multiple risk factors which are still to be defined for the Lebanese population. Limited knowledge on disease and poor surveillance may impede the efforts of disease

control as the exact burden of disease is unknown. In view of the important impact of such disease in terms of public health and economy, more control efforts need to be taken by the local authorities in order to establish more efficient control programs.

Declaration of Competing Interests

All authors hereby declare no conflict of interest.

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List of Figures and Tables

Figure 1. Human Cystic Echinococcosis cases distribution over 5 Lebanese regions between 2005 and 2018. According to Hospitals data during this period, it was registered 121 cases of hydatid cysts in North of Lebanon, 222 cases in Mount Lebanon, 132 in Beirut, 160 in South, and 259 in the Bekaa region.

Figure 2. Incidence rates of surgically removed cystic echinococcosis in Lebanon per 100,000 inhabitants during 2005 and 2018.

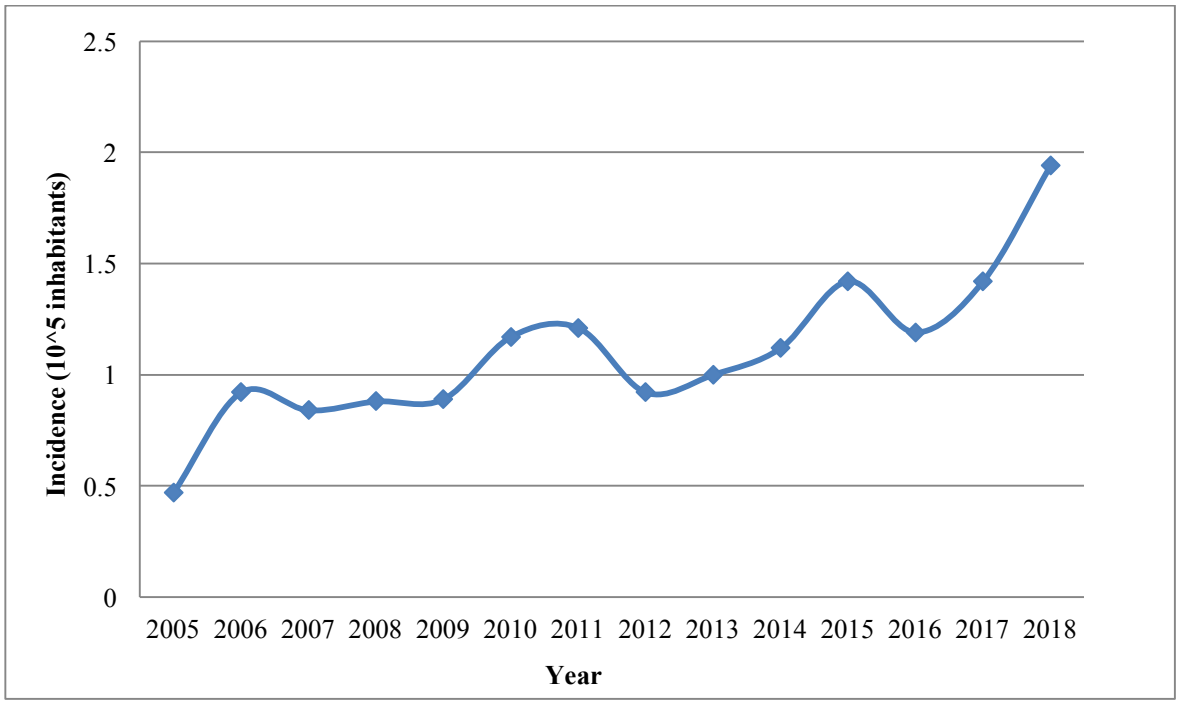

Figure 3. Age distribution of human cystic echinococcosis (CE) cases identified in Lebanon during 2005 and 2018.

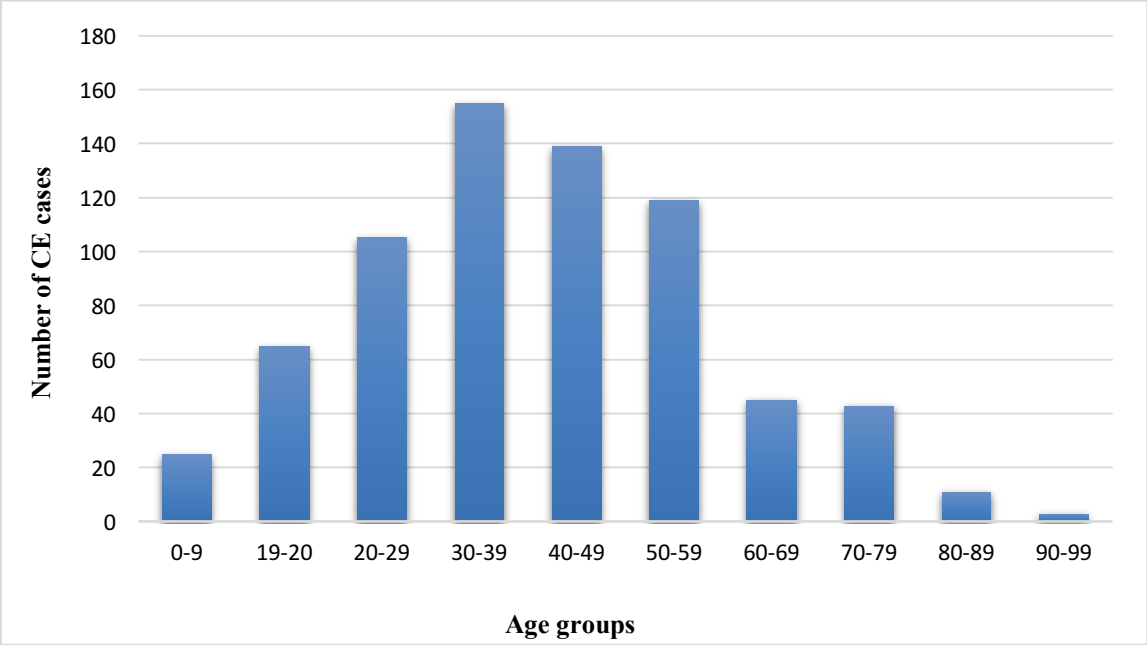

Figure 4. (a) Intraoperative Cystic echinococcosis found in human lungs in Lebanon. (b) Complete resection of a 4 cm lung hydatid cyst showing the pericyst and the inner germinal layer.

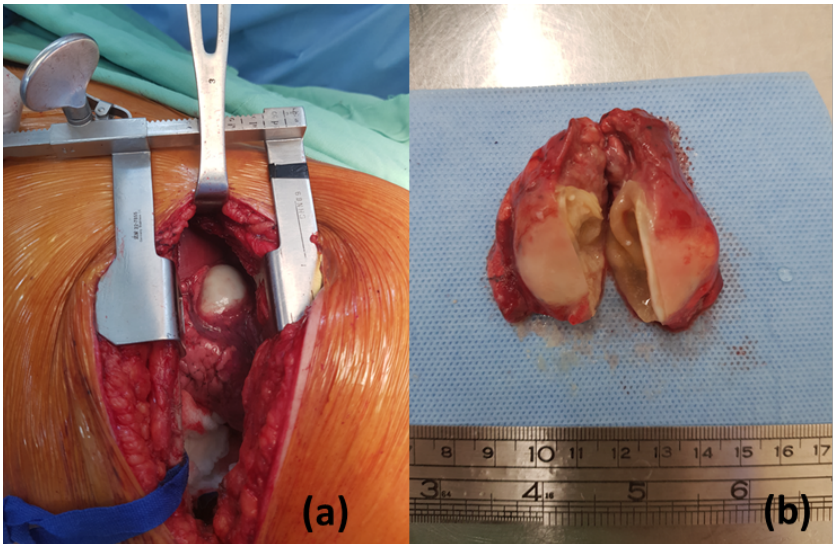

Figure 5. Comparison between cystic echinococcosis (CE) cases registered per year according to the Ministry of Health (MOH) and the collected data from the five anatomy and pathology hospital laboratories (HDR).

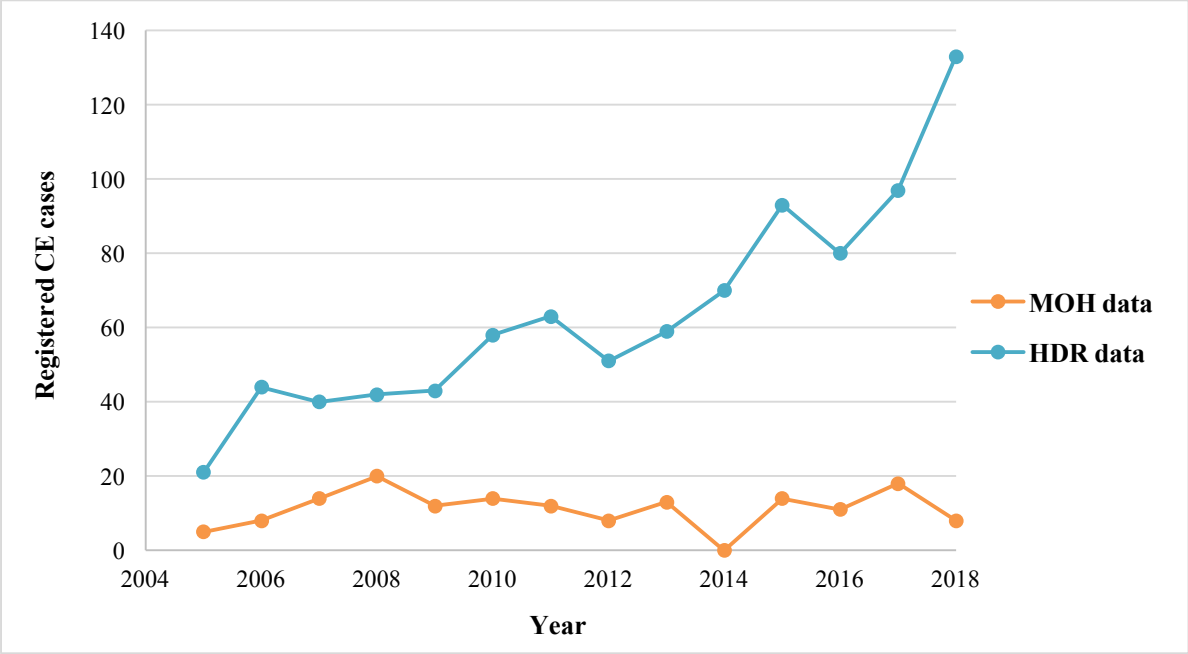

Figure 6. Haplotype network for *E. granulosus* s.s. sequences from Lebanon and neighboring countries. Hatchmarks represent number of mutations. Size of the circle indicates its prevalence in the studied populations.

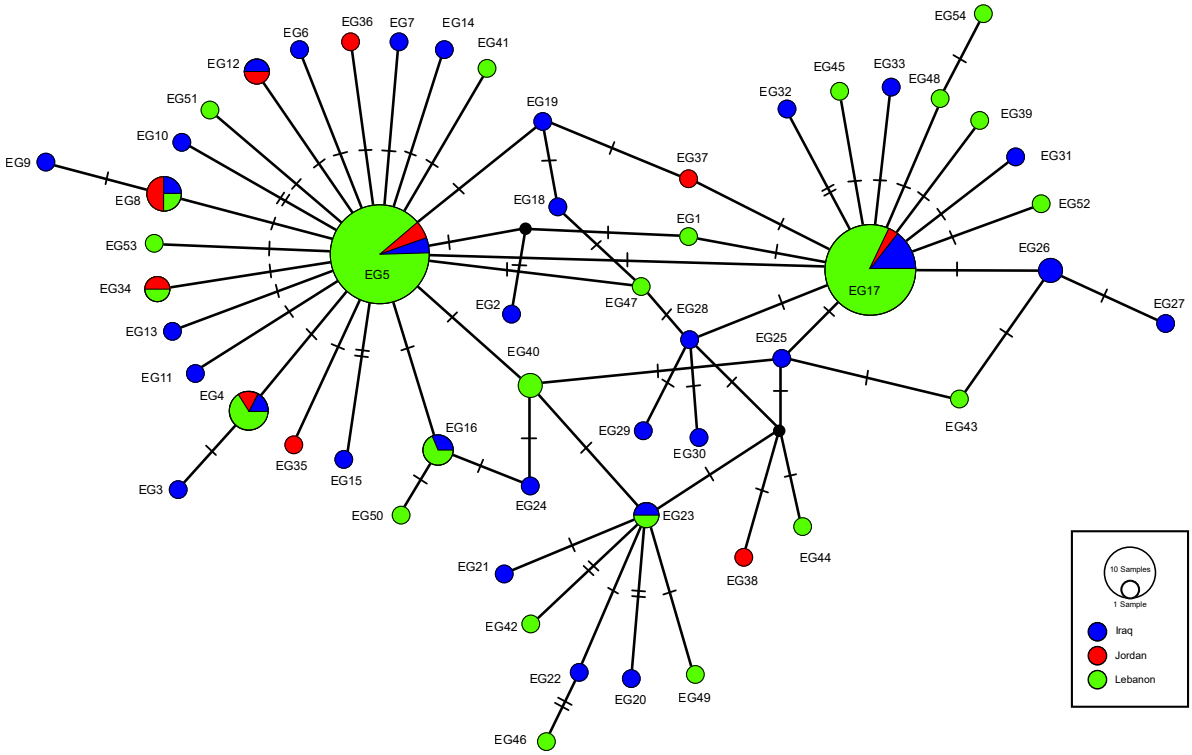

Table1. Distribution of CE cases based on their lesion site.

Site	Number of cases	Percentage (%)
Liver	597	66.78
Lung	183	20.47
Abdominal cavity	15	1.68
Spleen	17	1.9
Bone	11	1.23
Kidneys	11	1.23
Liver & lung	8	0.89
Pelvis	3	0.34
Liver & spleen	2	0.22
Ovaries	2	0.22
Adrenal gland	1	0.11
Bladder	1	0.11
Brain	1	0.11
Breast	1	0.11
Heart	1	0.11
Inguinal	1	0.11
Intramuscular	1	0.11
Liver & abdomen	1	0.11
Liver, kidneys & diaphragm	1	0.11
Pancreas	1	0.11
Skin	1	0.11
Spine	1	0.11
Unspecific site	33	3.69

Table 2. Diversity and neutrality indices for *E. granulosus s.s.* populations from Lebanon and neighboring countries.

Country	Number of sequences	Diversity indices			Neutrality indices	
		Hn	Hd±SD	Nd±SD	Tajima's *D*	Fu's Fs
Lebanon	80	23	0.7614±0.0368	0.001877±0.001279	-2.13977*	-21.4702*
Iraq	38	33	0.9886±0.0106	0.004053±0.002389	-2.10706*	-26.2472*
Jordan	12	10	0.9697±0.0443	0.002873±0.001918	-1.63391*	-7.42411*
Overall	130	54	0.8769±0.0210	0.00264±0.00021	-2.39256*	-80.1850*

* significant at $p<0.05$

Table 3. Pairwise fixation index (Fst) for studied populations of *E. granulosus s.s.* from Lebanon and neighboring countries.

Fst	Lebanon	Iraq
Lebanon	-	
Iraq	0.00371	-
Jordan	0.00449	-0.02153

General discussion and conclusions

The present thesis is centered on the evaluation of the current situation of CE in Lebanon, providing key information about three aspects related to the parasitosis in the country: 1) the prevalence of CE in sheep and goats of Lebanon, 2) the epidemiology of human CE surgical cases in the five main regions of Lebanon, 3) the *E. granulosus s.l.* species and genotypes circulating in the region.

Chapter 1 focuses on the epidemiology and the molecular characterization of *E. granulosus* s.l. isolates from sheep and goats of Lebanon. The current study is the largest epidemiological survey carried out in this country in the last 30 years, and showed high CE prevalence in livestock. The high infection rates can be justified with the age of the examined animals, as only adult animals which are exposed to the disease (parasitic ova) over a long period, thus having higher chance of getting infection, were included in the sampling (Torgerson and Heath, 2003; Fikire et al. 2012). Moreover, Lebanon is characterized by a high concentration of free ranging dogs, illegal slaughtering and other cultural and socioeconomic conditions that can contribute to the transmission and perpetuation of CE, particularly in sheep and goats.

In addition, our results showed lower prevalence and fertility rates among goats compared to sheep; which correlate mainly 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), but also due to the feeding habits of goats, as they eat higher part of herbs, less exposed to defecation and more exposed to sunlight (Otero-Abad and Torgerson 2013).

Cysts in sheep were predominantly located in the liver, whereas in goats, the lung was the most affected organ; both organs have greater capillary fields, which allow them to efficiently filter the ingested onchospheres from the blood. However, the probability of finding fertile cysts in lungs was higher than in liver in both animals; this might be due to the softer consistency of lung tissue that allows easier development of the cyst, hence providing good environment for the fertility of hydatid cyst than other organs, the variation in tissue resistance between organs (Vaidya et al., 2018).

Our study is the first one to determine by molecular characterization, the infecting genotypes in locally raised sheep and goats. Among the analyzed samples, three genotypes of *E. granulosus s.l.* were described: G1, G3 and G7. Most importantly, and for the first time, G7b haplogroup of *E canadensis* was identified in Lebanese livestock, as was also recognized from other European Mediterranean countries (Laurimäe et al. 2019).

Considering the high prevalence of CE in livestock of Lebanon and the diversity of the circulating genotypes, prevention and control programs using genotype-specific approach for disease control should be implemented to reduce the risk of this zoonotic parasitic disease that presents a public health challenge and a socioeconomic burden on developing countries.

The Chapter 2 constitutes the largest retrospective survey carried out in all the Lebanese regions of this country. The aim of this study was to assess the demographic and epidemiologic features of human CE surgical cases over a 14-year period in the five main regions of Lebanon and to identify for the first time the genotypes responsible for the human hydatidosis in the area.

Over the span of these years, in parallel to the high prevalence of the disease seen in animals, an increasing trend of cystic echinococcosis incidence was recorded in humans. This increase is probably due to the lack of hydatid disease awareness program in our country. Also, a comparison between our survey and data obtained by the Lebanese Ministry of Health was made and showed that the numbers of CE cases obtained in this study are higher than those reported by the MOH demonstrating that CE disease is under-reported by healthcare professionals and the general population. The lack of epidemiological data and control measures, and weak maintenance of data by concerned officials and public institutions represent a serious health problem in Lebanon.

Highest prevalence of CE usually occurs in regions with extensive and traditional sheep farming (Otero-Abad & Torgerson, 2013) leading to human infections (Alvarez Rojas et al., 2014). Previously, higher CE prevalence has been reported from Beirut and Mount Lebanon among the dog owners (Araj & Mourad, 2014). In the present study, the highest frequency of human CE was determined in the Bekaa region, a rural area close to the Syrian border and where sheep raising is widespread.

Additionally, the most vulnerable to CE infection are those in the age group of 30-39 years, that are most probably the most active in livestock rearing (Mastrandrea et al., 2012). It has been also revealed that females were more prone to CE infection than males that has been previously observed in Lebanon (Frayha et al., 1989) and other Middle eastern countries (Chalechale et al., 2016; Moosazadeh et al., 2017; Abdulhameed et al., 2018). This can be explained by the result of women's lifestyle, who are more likely to be in direct contact with a source of infection, since they tend to have main role in domestic activities, including food preparation and caring for the family dog (Rao et al., 2012; WHO, 2020).

The results of the current study showed that the liver was the most commonly affected organ, followed by the lungs, in line with a previous Lebanese study (Araj & Mourad, 2014) and international literature (Salamone et al., 2016; Yaghoobi et al., 2019). Single cases in rare sites of occurrence of the hydatid cyst like in the adrenal gland, brain, breast, intramuscular, myocardium, ovaries, pancreas, peri-bladder, spine and subcutaneous were also observed which could be explained by the dissemination of cysts through lymphatic channels (Wani et al., 2012).

Finally, the molecular genotyping analysis of the examined isolates revealed the involvement of *E. granulosus s.s.* in the human CE in Lebanon, as identified in most Asiatic human populations (Matini et al., 2018; Yan et al., 2018; Yousefi et al., 2019). Moreover, the *E. granulosus s.s.* nucleotide sequences obtained from humans in current study were similar to those of intermediate hosts in Lebanon (Joanny et al., 2021) reflecting genetic similarity between different intermediate host samples.

The current research work showed that cystic echinococcosis/hydatidosis is an endemic zoonotic disease in the different regions of Lebanon, in different food animals and posing high risk to human population. Hence, this study has a perspective plan in building a prophylactic program involving strong cooperation among the agriculture, veterinary, medical, and health sectors and with neighboring countries in order to successfully eradicate the disease.

Control strategy should be implemented by starting with (i) planning phase which may include appointment of an appropriate authority supported by legislation, collection of baseline data, selection of appropriate control strategies, selection and training of staff and provision of sufficient fund for the program; (ii) attack phase, where control measures are

applied non-discriminately to the entire host population at risk; (ii) consolidation phase, risky areas or farms identified through surveillance and control measures and surveying involves the gathering of data about past infections and present status; and (iv) the maintenance of eradication phase, can be entered once the parasite has possibly been eliminated (Schurer *et al.,* 2015; WHO, 2020).

The control of cystic echinococcosis relies on interrupting the transmission between dogs and livestock and implementing measures in humans to avoid the ingestion of *E. granulosus s.l* eggs (Woolsey and Miller., 2021).

To interrupt the lifecycle in animals, a combined approach relying on long term interventions including dog population control and periodic deworming, vaccinating sheep with *E. granulosus* recombinant antigen EG95 vaccine and culling of aged sheep has proven to be effective in eliminating the disease (Craig *et al.,* 2017, Larrieu *et al.,* 2019).

To prevent human contamination, CE control campaigns for hygiene education are needed, such as washing hands with soap after gardening or touching the dog, avoid untreated water sources and washing vegetables that may have been contaminated by dog faeces (Tamarozzi *et al.,* 2019). Moreover, training courses targeting medical and paramedical personnel provide support on early diagnosis and clinical management of cystic echinococcosis in rural areas (WHO, 2020).

Finally, at the end of our study and based on the results and the prevalence of the disease in the country, a campaign can be undertaken to elaborate a prophylactic strategy including education of citizens, better availability of surveillance tools, effective measurements to control the stray dog population, superior conditions in abattoirs and good cooperation with regional countries to eradicate the disease.

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