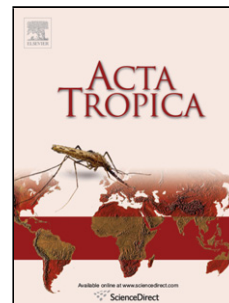# Accepted Manuscript

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Echinococcus granulosus **infection dynamics in livestock of Greece**

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Highlights

▯ the occurrence of *Echinococcus* hydatid cysts in livestock was determined in Greece ▯ Infection ranged from 30.2% sheep to 0% deer and cyst fertility from 7.9% to 0% ▯ Infection rates remain about the same to past surveys in spite applied regulations ▯ Genotyping, based on

cox1 and *nad*, showed predominance of *E. granulosus s.s.* (G1) ▯ Presence of a single genotype within hosts verifies a dog-sheep transmission cycle

Abstract

An epidemiological and molecular survey on the occurrence of *Echinococcus* hydatid cysts in livestock was conducted in Greece. In total 898 sheep, 483 goats, 38 buffaloes, 273 wild boars and 15 deer were examined and 30.2% (6.45% cyst fertility), 7.86% (3.2% cyst fertility), 42% (7.9% cyst fertility), 1.1% (0% cyst fertility), 0% of them were found infected, respectively. Infection rate in different geographical regions varied between 26.14%-53.8% (cyst fertility 2.04%-34.6%) in sheep, 7.33%-13.3% (cyst fertility 0%-3.2%) in goats. Genotyping, based on *cox1* and *nad1* analyses, demonstrated the predominance of *E. granulosus s.s.* (G1 genotype). The presence of one single genotype-complex within a relatively large spectrum of intermediate host species in Greece indicates the presence of a dominant transmission dog-sheep cycle involving additional host species which may act as disease reservoir for human infections.

Key words

Echinococcus granulosus, cystic echinococcosis, livestock

1. Introduction

Cystic echinococcosis (CE) is a zoonotic disease caused by the larval stage of the tapeworm *Echinococcus granulosus*. The parasite has a wide geographical distribution, and despite significant progress achieved in the field of control it still remains a

considerable problem for animal health and livestock economy (Eckert et al., 2000). Its worldwide distribution is mostly on account of the adaptability of the larval stage (hydatid cyst) to several domestic and wild mammalian intermediate hosts, including humans (Craig et al., 2007).

E. granulosus is characterized by high genetic diversity; ten variants/genotypes (G1-G10) have been reported (Eckert and Thompson 1997). The taxonomy of the genus *Echinococcus* has been recently revised based on the complete mitochondrial genome (Nakao et al., 2007) and on nuclear data (Saarma et al., 2009). Thus today, *E. granulosus* is considered as complex consisting of four species*: E. granulosus* sensu stricto (s.s.) (G1-G3), *E. equinus* (G4), *E. ortleppi* (G5), and *E. canadensis* (G6-G10) (Moks et al., 2008, Nakao et al., 2013, 2010, 2007; Saarma et al., 2009).

The phylogenetic relations within *E. canadensis* (G6-G10) remain under controversial discussion. It was suggested that the *E. canadensis* cluster might be divided into two sub-species *E. canadensis* (G8/G10) and *E. intermedius* (G6/G7) (Thompson et al., 2006, Thompson 2008). At least seven of nine *E. granulosus* "genotypes" are infective to humans, four of which exist in Europe. Globally, most human cases of cystic echinococcosis (CE) are caused *E. granulosus* s.s. (G1) which predominantly has a dog-sheep life-transmission cycle (Eckert and Deplazes, 2004).

The Mediterranean/Balkan area is endemic or highly endemic, and in most of the countries involved, including Greece, high prevalences have been reported in dogs and livestock (Breyer et al., 2004; Dakkak, 2010; Seimenis and Battelli, 2003 Simsek et al., 2010; Sotiraki and Chaligiannis, 2010; Utuk et al., 2008; Xhaxhiu et al., 2011).

According to previous surveys, the common sheep strain (G1 genotype) is predominant in this area (Breyer et al., 2004; Simsek et al., 2010; Utuk et al., 2008; Varcasia et al., 2007; Xhahiu et al., 2011).

As far as Greece is concerned, during the last decades several epidemiological studies focused mainly on sheep and goats, reporting high prevalences, and also the presence of the G1, G3 and G7 strains (Christodoulopoulos et al., 2008; Kantzoura et al., 2013; Sotiraki et al., 2003; Varcasia et al., 2007), thus indirectly documenting that cystic echinococcosis is still an ongoing problem. Unfortunately, these studies were spatially and temporally restricted; therefore the information provided is not sufficient to draw country-wide conclusions.

Hence, the aim of our study was to contribute to the assessment of the current epidemiological situation of cystic echinococcosis in Greece by defining a) the incidence of *Echinococcus* infection in as many animal species as possible in a strategically selected area b) the intensity of the infection and the cyst fertility in the different animal species and c) genotypes and/or species as far as possible.

2. Materials and methods

2.1 Study design

The study was carried out in Northern and Central Greece in a total of 9 abattoirs scattered in 4 different regions. The study area was selected because of a) a significant livestock population being present in the area, and b) its neighbouring characteristic with other Balkan countries. The abattoirs were selected on the basis of their capacity (number

of animals slaughtered per month) and the variety of animals slaughtered. More precisely, the abattoirs were situated in the following locations: Komotini (Thrace); Kozani and Kastoria (Western Macedonia); Serres, Galatista, Lagkadas, Chalastra and Korinos (Central Macedonia) and Karditsa (Thessaly). The study lasted over a period of 12 months, and in total 898 sheep, 483 goats, 273 wild boars, 38 water buffaloes and 15 deer were inspected.

2.2 Sample collection

In each abattoir the slaughtered animals were inspected by some of the authors during regular visits throughout the study. More precisely the viscera of each carcass were examined for the presence and location of hydatid cysts by visual inspection, palpation and systematic incision of each organ. All infected organs were recorded, removed and separately transported in individual bags as soon as possible to the laboratory.

In each organ the number and the location of the cysts were recorded. Hydatid cysts were ranked as fertile, sterile or calcified/caseous cysts. Fertility was determined after dissection of the cyst, and after aseptical collection of the germinal layer and the protoscoleces if present. Protoscoleces were examined by light microscopy to determine viabilities (flame cell activity, peristaltic motility together with staining with a 0.2% aqueous eosin solution).

2.3 Molecular study

A total of 59 *E. granulosus* cysts were collected from different intermediate hosts (sheep

n = 24, buffalos n = 27, goats n = 5, wild boars n = 3) and from different body locations (liver and lungs). All cysts examined were fertile except those from wild boars.

The 59 *E. granulosus* genomic DNA (gDNA) samples were extracted from protoscoleces and/or germinal layer using the DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). The gDNAs were subsequently used as a template for the amplification of two mitochondrial gene markers COX1 (cytochrome *c* oxidase subunit 1) and NAD1 (NADH dehydrogenase 1) as described previously (Bart at al., 2006).The PCR was performed in a 25-μl volume containing 2 μl of gDNA, 2.5 μl 10X Buffer, 25 nmol dNTP Mix (Promega corporation, Fitchburg, WI, USA), 20 pmol each of primers (Invitrogen, Carlsbad, CA, USA) and 0.5 U GoTaq DNA Polymerase (Promega corporation, Fitchburg, WI, USA). The characteristics of the two primer pairs (EgCOI1/EgCOI2 and EgNDI 1/EgNDI 2 (Bart et al., 2006) are shown in Table 1. The amplification conditions were as follows: an initial step of denaturation (3 min at 94°C) followed by 40 cycles [94°C–30 s, 60°C (*cox1*) / 45°C (*nad1*) –30 s, 72°C–75 s (*cox1*) / 30 s (*nad1*)] and a final elongation step of 7 min at 72°C. The specificity of amplications and the size of products were then assessed by electrophoresis in 1.5% (w/v) Tris–acetate/EDTA (TAE) agarose gels. Then, PCR products were purified using the High Pure PCR Product Purification Kit (Roche Applied Science) according to the manufacturer's instructions.

For each sample, 1 μl of purified amplicon was applied in a PCR-Sequencing mix containing 0.5 μl of 5X Sequencing Buffer, 0.5 μl of BigDye (BigDye Direct Cycle Sequencing Kit, Applied Biosystems) and 0.4 μl sequencing primer (final concentration of 10 pmol/ μl). Final PCR-Sequencing reaction was adjusted to 6μl distilled, sterile

water.. The amplification conditions were as follows: an initial step of denaturation (1 min at 96°C) followed by 25 cycles [96°C–10 s, 55°C–5 s, 60°C–2 min]. Subsequently, 2 μl EDTA 125 mM and 25 μl 100% ethanol were added to each PCR-Sequencing tube. After an incubation step of 15 min at room temperature, the PCR-Sequencing tube was centrifuged 30 min at 3000×g and the supernatant was removed and the pellet was dried at room temperature during 15 min. Then, the pellet was resuspended in 10 μl of ABI HiDi Formamid (Applied Biosystem, Perkin-Elmer, Waltham, MA, USA), was added to each and the for 30 min at room temperature. The products were finally sequenced using an automated DNA sequencer (Applied Biosystems, ABI 3130× I Genetic Analyzer Sequencer).

Resulted sequences for *cox1* and *nad1* were edited and aligned separately using BioEdit software. The Basic Local Alignment Search Tool (BLAST) of NCBI (National Center for Biotechnology Information, Bethesda, MD, USA) was used to identify and confirm the identity of each sequence in relation to the GenBank nucleotide database. For each of the 59 samples, *cox1* and *nad1* DNA sequences were concatenated (http://users-birc.au.dk/biopv/php/fabox/alignment_joiner.php).

The dataset of the concatenated *cox1* + *nad1* sequences was compiled, together with key reference sequences (comprising concatenated *cox1* + *nad1* sequences representing, *E. granulosus* s.s. (G1) (AF297617), *E. granulosus* s.s. (G3) (KJ559023), *E. equinus* (G4) (AF346403), *E. ortleppi* (G5) (AB235846), *E. canadensis* (G6) (AB208063), *E. canadensis* (G7) (AB235847), *E. canadensis* (G8) (AB235848), *E. canadensis* (G10) (AB745463), *E. multilocularis* (AB018440), *E. felidis* (AB732958), *E. shiquicus*

(AB208064), *E. vogeli* (AB208546), *E. oligarthrus* (AB208545) . *Taenia solium* (AB086256) was employed as outgroup). Concatenated sequences were aligned using BioEdit version 7.0.9.0. The conversion of the alignment format to PHYLIP was also done with BioEdit.

All *cox1* and *nad1* sequence data from this study as well as reference sequences listed above were then used to generate dendogram using MEGA software (Molecular Evolutionary Genetics Analysis 3.1, (http://www.megasoftware.net). For reconstructing of the phylogenetic tree, we used the neighbor-joining (NJ) approach which is related to the cluster method but does not require the data to be ultrametric (Saitou and Nei, 1987). The principle of NJ takes into account (i) the minimum evolution (the minimum number of variation that occurs between the analysed sequences) and (ii) the potential differences in the speeds of occurrence of those variations. In this study, we used NJ method with the Kimura 2-parameter correction model (Kimura 1980) which assumes equal base frequencies and it distinguishes between the rate of transitions (purine (Adenine and guanine) -pyrimidine (Thymine and Cytosine) pair is substituted by the other purine-pyrimidine pair) and transversions (substitution of a purine (Adenine and Guanine) for a pyrimidine (Thymine and Cytosine) or conversely). The robustness of individual branches, a bootstrap (BS) value was assessed by bootstrap resampling (BP) (Felsenstein, 1985) (1000 random repetitions). Thus a gathering of genotypes was considered relevant when its robustness was more than 70%.

Results

In total 898 sheep, 483 goats, 38 buffaloes, 273 wild boars and 15 deer were examined

and 30.2% (6.4% cyst fertility), 7.86% (3.2% cyst fertility), 42% (7.9% cyst fertility), 1.1% (0% cyst fertility), 0% of them were found infected, respectively. Infection rate in different regions varied between 26.1% - 53.8% (cyst fertility 2% - 34.6%) in sheep, 7.3% - 13.3% (cyst fertility 0% - 3.2%) in goats. Hydatid cysts were found in both lungs and liver in sheep and goats whereas only in lungs in buffaloes. Cyst fertility was constantly higher in lungs than liver in both sheep and goats (4% and 2.7%; 2.4% and 0.45% respectively). The detailed results are shown in Table 2.

Out of 639 infected animals, 59 *E. granulosus* cyst isolates were assessed by molecular identification. Two independent (*nad1* and *cox1*-PCR-sequencing) investigations were carried out on these isolates, and all samples showed fragments of approximately 530 bp and 443 bp, respectively. The *cox1* and *nad1* regions of the 59 isolates fully corresponded with the *E. granulosus* s.s. (G1/G2/G3) cluster (Figure 1). The sequencing of *cox1* (443bp) and *nad1* (530 bp) shown 4 and 5 segregating sites, resulting in 6 (*cox1*_1 to *cox1*_6) and 7 (*nad1*_1 to *nad1*_7) genetic variants, respectively, among the 59 analyzed isolates (Table 3 and 4). Except of *nad1*_3, *nad1*_7 and *cox1*_4 (accession number KT152150, KT152151, KT152149 respectively), which were identified for the first time in the present study, all others haplotypes had been previously reported (Table 3 and 4). The most frequent haplotypes in the studied population were cox1_6 (45 samples) and *nad1*_5 (35 samples).

For the concatenated sequence (cox1 + nad1), analysis revealed the presence of 14 haplotypes and all of them clustered with *E. granulosus* s.s. (G1 and G3) (Figure 1). Exclusively, haplotypes H4 and H9 were found in Buffalo (2 Buffalos from Sidirokastro)

and in sheep (2 sheep from Komotini), respectively. Six haplotypes were isolated each from single animal (H3 (1sheep), H6 (1 goat), H7 (1sheep), H8 (1sheep), H10 (1Buffalo) and H12 (1sheep)).The haplotype H2 was the most abundant and it was present in the 4 intermediate hosts; sheep, goat, Buffalo and wild boar.

Thus, despite the large spectrum of intermediate host species (sheep, buffalos, goats, wild boars), only *E. granulosus* s.s. (G1/G2/G3) was involved.

Discussion

CE is considered an emerging disease in humans and a serious animal production problem and thus economic concern in many areas of the world. Great progress has been achieved in the last years in understanding the geographical distribution and genetic diversity of *E. granulosus*, but still there is a clear need to complete the epidemiological, molecular, and economic data sets for many endemic areas in order to implement standardized recording and reporting systems for livestock CE (Eckert et al., 2000; Jenkins et al., 2005).

Despite the fact that *E. granulosus* is considered endemic in Greece, and has been repeatedly reported in both humans and animals, relatively little information is available on the characterization of the circulating strains of this parasite (Cardona and Carmena, 2013; Sotiraki and Chaligiannis, 2010). Till now, studies on the genetic variability of *E. granulosus* in Greece were only based on analysing partial sequences of the parasite mitochondrial genome such as cox1, nad1 (Varcasia et al., 2007). Coding and non-coding

regions from the nuclear genomes might be suitable/useful for analyzing the genetic diversity within the *Echinococcus* genus (intra- and inter-species) (Boubaker et al., 2014, Haag et al., 1999, Saarma et al., 2009). Thus, further molecular investigations with both nuclear and mitochondrial targets are required to clarify/confirm the genetic variability status of Greek *E. granulosus* isolates.

All *E. granulosus* isolates analysed in this work showed high sequence identity levels with *E. granulosus* s.s. (G1/G2/G3), and this both upon *cox1* and *nad1* markers. Thus *E. granulosus* s.s. (G1/G2/G3) is the major causative agent of cystic echinococcosis in livestock animals in Greece. Our results are consistent with previous epidemiological data reported from Greece that have also shown that *E. granulosus s.s*. (G1/G2/G3) was a dominant species in the Balkan and Mediterranean countries (Bulgaria, Italy, Romania, Turkey) (Breyer et al., 2004; Casulli et al., 2012). As already reported in other parts of Europe (Cardona and Carmena, 2013; Eckert et al., 1993; Scott et al.,1997; Snabel et al., 2000), the *E. granulosus* s.s. (G1/G2/G3) genotype complex has always been considered as the major source of human contamination (Eckert et al., 2000 and Thompson et al., 1995), therefore we anticipate to perform future analyses with parasite material of human origin to confirm this assumption for Greece.

From 2000 onwards, the surveys based on meat inspection carried out in different Greek regions, reported infection rates in livestock ranging from 30.4 to 39.32% for sheep and 10.3 to 14.7 % for goats with a respective cyst fertility ranging from 8 to 16.2 for sheep and 0 to 7.4 for goats. As regards other animal the prevalences found were 0.6 % in pigs, and 0% in cattle (Christodoulopoulos et al., 2008; Sotiraki et al., 2003; Varcasia et al.,

2007). Taking those into account and comparing with the data presented in the current study we can confirm that the parasite is still circulating among livestock in Greece and in spite all efforts taken and applied regulations the infection rates remain roughly the same. Livestock-rearing communities with subsistence-farming practices, such as Greece, are high-risk areas for cystic echinococcosis (Otero-Abad & Torgerson, 2013). Failure of hydatid control programmes is not such an infrequent situation and the failure is not only connected to the nature of the programme but to various other reasons such as the general social/scientific relaxation attitude against the endemic neglected zoonoses, which when combined with an unfavourable economic situation may result for example to the withdrawal of government/regional funding and to an inadequate management of stray dogs (Craig et al., 2007). Future control plans should follow an integrative approach implementing initiatives such as the "One Health approach" and encourage the involvement of government officials, relevant veterinary and medical professionals and farmers in order to make the most of the existing resources and share the benefits.

In line with previous studies (Bart et al., 2006; Mwambete et al., 2004; Varcasia et al., 2007), we found that *E. granulosus* s.s. (G1/G2/G3) infection in ovine is associated with cyst fertility rates.

Although infections due to *E. canadensis* (G7)) have also been documented in the past (Varcasia et al., 2007) we did not identified any respective haplotypes in the present study. However, this strain appears to circulate in sheep and primarily in goats from

Hungary, Spain and Turkey (Casulli et al., 2012; Mwambete et al., 2004;) possibly less frequently.

The abundance of the parasite differs quite a lot between different regions in Greece. An interesting observation of this study is the high infection rates found in the region of Thessaly (53.8%), comparing to rates of 39.3% and 85.9% that were documented respectively during a meat inspection control (Christodoulopoulos et al., 2008) and from a study of CE seroprevalence study (Kantzoura et al., 2013). These findings support the theory of infection "hot spots" found in different locations all over the country, which may be linked to husbandry practices applied in the region and/or low educational status of the farmers. Precisely, as documented in similar studies, although control programs are still in action, still in the majority of the farms home-slaughtering is still happening and viscera are either used as dog meal raw or not discarded properly to avoid infection. Moreover farmers have usually more than the required number of dogs which are not regularly dewormed (Christodoulopoulos et al., 2008; Kachani & Heath 2014; Varcasia et al., 2011).

Although the number of examined water buffaloes was low, the prevalence of CE infection was relatively high (42%). Cyst fertility in these animals was 7.9%, with all cysts being located in the lungs. Therefore, the lungs appear as predilection site for buffaloes, matching similar findings from surveys conducted in Italy (Capuano et al., 2006; Casulli et al., 2008; Rinaldi et al., 2008) and Turkey (Beyhan and Umur, 2011). It's worth to notice that water buffaloes in Greece are found only in limited wetlands in the

north, co-existing with high numbers of other ruminants and shepherd or stray dogs in the same area. The epidemiological value of such infection rates/cyst fertility found is even more significant since such wetlands are also popular recreational sites often visited by people. This situation seems to indicate that water buffaloes are potentially a suitable intermediate host for the maintenance of the *E. granulosus* s.s. life cycle in the area, although more extent epidemiological studies are needed before definitive conclusions can be drawn.

Red deer (*Cervus elaphus*) and wild boars (*Sus scrofa*) were included in this study in order to investigate their role in the parasite's epidemiology. These two animal species represent a new type of animal farming, and both are mostly reared under free ranging practices. The results, with regard to wild boar, showed low infection rates with the sheep strain (1.1% -with no fertile cysts). However, in similar surveys in France and Romania, wild boars where also found infected (although by *E. canadensis* (G7) as well) indicting a potential risk for human infection, especially for people involved in wild boar hunting or breeding (Onac et al., 2013; Umhang et al., 2014).

Pigs and cattle were not included in the current study because of the low infection rates found in the previous studies. Those animals are mostly reared intensively indoors in Greece and do not have close contacts to dogs. Furthermore, during all visits realised in the slaughterhouses during sample collection, no cyst was ever found in such animals by the authors.

The presence of one single genotype-complex within a relatively large spectrum of intermediate host species in Greece indicates the presence of a dominant transmission dog-sheep cycle involving additional host species which may act as disease reservoir for human infections. Based on these findings, it will be interesting to carry out additional studies on the prevalence in domesticated definitive hosts such as the dog and in the human population. Confirming the presence of a single genotype-complex also in humans will help to tackle the transmission and exposition risks in humans, and thus to elaborate appropriate measures of control and prevention in Greece.

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Primers 5′-3'		Product size (bp)	Tm (°C)	Ref.
cox1				Bart et al., 2006
EgCOI1	TTTTTTggCCATCCTgAggTTTAT	443	60 (75 sec.)	
EgCOI 2	TAACgACATAACATAATgAAAATg			
nad1				Bart et al., 2006
EgNDI1	AgTCTCgTAAgggCCCTAACA	530	45 (30 sec.)	

EgNDI 2   CCCgCTgACCAACTCTCTTTC

Table 1. Characteristics of the primers used for molecular study. Tm: annealing

temperature, sec.: seconds for the elongation time.

Geographic area	Animal species	Infection rate (%)	[a]No. liver cysts, [b] range [min-max]	[c]Cyst fertility rate (%)	[a]No. liver cysts, [b] range [min-max]	[c]Cyst fertility rate (%)
Thrace (n = 58)						
	sheep n = 57	33.3 (19/57)	65 [1-14]	5.3	42 [1-6]	5.3
	goat n = 1	0 (0/1)	0 [0-0]	0	0 [0-0]	
	total	**32.8 (19/58)**	**65**	**5.3**	**42**	
Western Macedonia (n = 487)						
	sheep n = 441	30.3(134/441)	363 [1-28]	0.4	371 [1-33]	1.6
	goat n = 46	13.3 (6/45)	11 [1-5]	0	2 [1]	0
	total	**28.7 (140/486)**	**374**		**373**	
Central Macedonia (n = 1110)						
	sheep n = 348	26.1 (91/348)	280 [1-16]	2.6	213 [1-10]	4.3
	goats n = 436	7.3 (32/436)	35 [1-9]	0.4	43 [1-6]	2.7
	buffaloes n = 38	42 (16/38)	25 [1-4]	0	13 [1-4]	7.9
	wild boars n = 273	1.1 (3/273)	1 [1]	0	3 [1-2]	0
	deer n = 15	0	0 [0-0]	0	0 [0-0]	0
	total	**12.8 (124/1110)**	**341**		**272**	
Thessaly (n = 52)						
	sheep n = 52	53.8 (28/52)	101 [1-19]	15.4	69 [1-10]	21.1
	total	**53.8 (28/52)**	**101**		**69**	
Total animal number N = 1707	TOTAL	18.2 (311/1707)	881	1.4 (24/1707)	756	3 (51/1707)

Table 2. Infection rates %, body localisation and fertility of cysts. [a]: number of liver cysts, [b] range of minimum and maximum number of cyst per animal, [c] The cyst fertility rate (number of fertile cyst /number of examined animal x 100)

Gene marker *cox1* Haplotype	I. Host	Locality	100% identity to	Country	I. Host	Species
cox1 _1 (n = 2)	sheep	Komotini	KJ782442	Moldova	_	*E. granulosus* s.s. (G3)
cox1_2 (n = 4)	sheep	Sidirokastro	KR070966	Bulgaria	*Homo sapiens*	*E. granulosus* s.s.
	buffalo	Karditsa Komotini				
cox1_3 (n = 1)	sheep	Karditsa	KR070967	Greece	sheep	*E. granulosus* s.s.
cox1_4 (*n = 1*)	goat	Korinos	KT152149	In this study		
cox1_5 (n = 5)	sheep	Sidirokastro	KP259871.1	Iran	cattle	*E. granulosus* s.s. (G1)
	buffalo	Komotini				
cox1_6 (n = 45)	sheep	Galatista	KR070964	Bulgaria	Homo sapiens	*E. granulosus* s.s.
	buffalo	Sidirokastro				
	goat	Xalastra				
	Wild boar	Korinos				
		Kozani				
		Komotini				
		Axioupoli				
		Kastoria				
cox1_7 (n = 1)	buffalo	Sidirokastro	KM100578.1	Turkey	sheep	*E. granulosus* s.s.
Total = 59						

Table 3. List of *cox1* haplotypes collected from different localities and host taxa in

Greece.

Gene marker *nad1* Haplotype	I. Host	Locality (Greece)	100% identity to	Country	I. Host	Species
nad1_1 (n = 2)	sheep	Komotini	EF125693	India	cattle	*E. granulosus* s.s.
nad1_2 (n = 10)	sheep	Sidirokastro	KR070990	Bulgaria	*Homo sapiens*	*E. granulosus* s.s. (G1)
	buffalo	Galatista Karditsa				
nad1_3 (n = 3)	sheep	Karditsa	KT152150	This study		
	buffalo	Sidirokastro				
nad1_4 (*n = 5*)	sheep	Sidirokastro	EF367331	Morocco	camel	*E. granulosus* s.s. (G1)
	buffalo	Xalastra				
nad1_5 (n = 35)	sheep	Komotini	KP245839	China	sheep	*E. granulosus* s.s. (G1)
	buffalo goat wild Boar	Sidirokastro Kastoria *Axioupoli Korinos Galatista Kozani*				
nad1_6 (n = 2)	sheep	Xalastra	KR070988	Bulgaria	Homo sapiens	*E. granulosus* s.s.
nad1_7 (n = 2)	buffalo	Sidirokastro	KT152151	This study		
Total = 59						

Table 4. List of *nad1* haplotypes collected from different localities and host taxa in Greece.

Figure 1. Dendogram drawn with the mitochondrial sequences identified in this study.

These genotypes were defined by combining the results obtained with *cox1* and *nad1*. The phylogenetic tree was drawn using the neighbour-joining method and the Kimura two parameter model of correction (MEGA software v. 3.1, http://www.megasoftware.net).

graphical-abstract .