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ORIGINAL PAPER

Molecular Study of *Echinococcus granulosus* Cestodes in Ukraine and the First Genetic Identification of *Echinococcus granulosus Sensu Stricto* (G1 Genotype) in the Country

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Abstract

Introduction Cystic echinococcosis is a globally distributed zoonotic disease of great medical and veterinary importance, which is caused by the cestode *Echinococcus granulosus sensu lato*. In Ukraine, two areas of the prominent circulation of the parasite are established, the southern steppe zone with sheep as the main transmitter, and the northern forest-steppe zone and Polissia, where pigs are mainly responsible for maintaining the *E. granulosus* transmission.

Methods Given that only a few studies have so far addressed the genetic diversity of the parasite in Ukraine, we have sequenced partial mitochondrial genes of cytochrome *c* oxidase 1 (789 bp), NADH dehydrogenase 1 (602 bp) and 12S rRNA (333–334 bp) in pig metacestodes from the Sumy region (farms close to Sumy, northeastern Ukraine) and the Kyiv region (a farm in Bila Tserkva, central Ukraine).

Results Four isolates from four pigs in the Sumy region were identified as *E. canadensis* (G7 genotype), the major *E. granulosus s.l.* species circulating in Eastern Europe, including the three microvariants (G7A, G7B, G7C). Three isolates from the two pigs in the Kyiv region were classified as *E. granulosus s.s.* (G1 genotype), including one microvariant (G1A).

Conclusion To our knowledge, this is the first genetic record of *E. granulosus s.s.* with the presumed highest infectivity and virulence among the *E. granulosus s.l.* species in Ukraine. The finding has implications for public health as local control programmes should take into consideration different development rate of this parasite in dogs and the greater risk of the species for human infection.

Keywords *Echinococcus granulosus* · *Echinococcus canadensis* · Ukraine · Haplotype · Pig

Introduction

The larval stages of the tapeworm *Echinococcus granulosus* (Batsch [1]) *sensu lato* (*s.l.*) are the causative agents of cystic echinococcosis (CE), a neglected zoonotic disease of global significance and an important cause of human morbidity, especially in regions where there is a close association between human and livestock. In 2014, a joint FAO/WHO panel ranked CE as the third most important foodborne parasitic diseases at the global level [2], which affects

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more than 1 million people currently suffering from the disease globally [3]. The annual financial burden associated with cystic echinococcosis is estimated to be up to US$ 3 billion for treating medical cases and losses for the livestock industry [4, 5].

In the current state of ongoing taxonomic debates, *E. granulosus s.l.* cluster is divided into five species, *E. granulosus* (Batsch [1]) *sensu stricto* (*s.s.*), *E. canadensis* (Webster and Cameron [6]), *E. ortleppi* Lopez-Neyra and Soler Planas [7], *E. felidis* Ortlepp [8], and *E. equinus* (Williams and Sweatman [9]) [10, 11]. *E. granulosus s.s.* (genotypes G1, G3) with the most cosmopolitan distribution are responsible for the vast majority of human CE (approximately 89%), and are often associated with transmission via sheep as intermediate hosts [12]. Sheep farming has a strong impact on the distribution of *E. granulosus s.s.* in Europe, with the Mediterranean basin and parts of Great Britain being the most affected [13].

The second species within *E. granulosus s.l.* infecting humans is *E. canadensis* cluster (thus assigned sensu Vuitton et al. [11], conventionally involving genotypes (or strains) of G6–G10, of which G6–G7 are globally responsible for about 11% of human infections [12]. Of these, the G7 genotype of *E. canadensis* ('pig strain') is well-adapted to pigs as the main intermediate host and has been reported in Europe, South/Central America, and to a lesser extent in West Asia and Africa [9–16]. In the eastern European countries such as Poland, Slovakia, and Lithuania, *E. canadensis* G7 is being predominantly recorded [(e.g., [12–20]). Currently available data on genetically classified *E. granulosus s.l.* circulating in Ukraine are fragmentary and restricted to a limited area. *E. canadensis* G7 was so far detected in pigs and wild boar from the Lebedyn district in the Sumy region in eastern Ukraine [17, 21], and in pigs from the Sumy district in the same region [22].

In the country, the host involvement in the spread of echinococcosis varies substantially according to the geographical zone. In the midwestern and northern regions of Ukraine, echinococcosis is diagnosed mainly in pigs, while in cattle it is less common. In the southern regions, echinococcosis is reported in domestic ungulates such as sheep, cattle, goats, pigs, with predominant infections in sheep and cattle [23]. Human infections are more frequently registered in the southern areas associated with sheep-raising, with almost half of the cases reported in the Odessa region giving an annual incidence of 2.9 cases per 100,000 people [24]. The only human CE case that has so far been genetically analysed in Ukraine (in a 21-year-old woman from the Volyn region in the northwestern part of the country) resulted as *E. canadensis* G7 [22].

The study was undertaken to broaden the knowledge about the genotype and species spectrum of *E. granulosus s.l.* in Ukraine in pigs, which is one the main transmitters of the parasite in the country, by molecular analysis of the metacestodes they harboured in two areas.

Materials and Methods

The *E. granulosus s.l.* isolates examined in this study were obtained from two regions of Ukraine, the Kyiv region (located in the north of central Ukraine) and the Sumy region (northeastern Ukraine). In the Kyiv region, two pigs were found to be infected on a private farm in the city of Bila Tserkva. In one animal, the spleen was infected with metacestodes, while in the other pig individual, hydatid cysts were found in both the liver and lungs. In the Sumy region, livers with hydatid cysts were found during routine veterinary inspections at the slaughterhouse of the Sumy city in four pigs coming from three villages (Kosivshchyna, Stepanivka, Mykolaivka) near Sumy. The metacestode content of one cyst (considered as one isolate) was analysed from one affected anatomical organ, and thus seven isolates from six pigs were molecularly examined. The list of geographical origins and cyst localizations in the pig isolates is given in Table 1. Sampling sites in the territory of Ukraine are depicted in Fig. 1.

The infected organs were stored at −20 °C prior to transportation to the research laboratories. The contents of cysts were examined under a light microscope for the presence of protoscoleces, rinsed in physiological saline and fixed in 70% ethanol before molecular analyses. Active fertile cysts with viable protoscoleces were observed in all isolates.

Total genomic DNA was extracted from the protoscoleces or germinal layer using the DNeasy tissue kit (QIAGEN, Germany) according to the manufacturer's instructions. Fragments of three mitochondrial genes, cytochrome *c* oxidase 1 (*cox1*, 789 bp), NADH dehydrogenase 1 (*nad1*, 602 bp) and 12S rRNA (333–334 bp), were examined. DNA was amplified using specific primers described by Xiao et al. [25] for *cox1* (F: 5′´–TTGAATTTGCCACGTTTG AATGC3′, R: 5′´–GAACCTAACGACATAACATAATGA –3′´), Hüttner et al. [26] for *nad1* (primers assigned to the second PCR in the article; F′´–TATTAAAAATATTGAGTT TGCGTC–3′, R: 5′´–TCTTGAAGTTAACAGCATCACGAT –3′), and Dinkel et al. [27] for 12S rRNA (F: 5′´–TTAAGA TATATGTGGTACAGGATTAGATACCC–3′, R: 5′´–AAC CGAGGGTGACGGGCGGTGTGTACC–3′´).

For the PCR amplification of the two partial genes, cycling conditions were as follows: the initial denaturation at 95 °C for 5 min, 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and final extension at 72 °C for 5 min. PCR products were visualised after electrophoresis on 1.5% (w/v) agarose gels and purified using the Nucleospin Extract II kit (Macherey Nagel, Germany). Amplicons were sequenced in both directions using a dye terminator cycle

Table 1 Characteristics of *Echinococcus granulosus sensu lato* isolates examined in the study and final genotypes recorded

Isolate	Host	Geographical origin	Organ	Observed (analysed) cysts/organ	GB number (*cox1*)	GB number (*nad1*)	GB number (12S)	Genotype
UKR-BT1	Pig	Bila Tserkva (Kyiv region)	Spleen	6 (1)	MT380912	MW542591	MT396430	G1
UKR-BT2-1	Pig	Bila Tserkva (Kyiv region)	Liver	> 30 (1)	MT380850	MW542592	MT396429	G1
UKR-BT2-2	Pig	Bila Tserkva (Kyiv region)	Lung	> 30 (1)	MT380908	MW542953	MT396431	G1A
UKR-S1	Pig	Kosivshchyna (Sumy region)	Liver	5 (1)	MT383992	MW542587	MT396434	G7B
UKR-S2	Pig	Stepanivka (Sumy region)	Liver	3 (1)	MT383995	MW542588	MT396433	G7A
UKR-S3	Pig	Stepanivka (Sumy region)	Liver	4 (1)	MT383994	MW542589	MT396435	G7
UKR-S4	Pig	Mykolaivka (Sumy region)	Liver	8 (1)	MT383990	MW542590	MT396432	G7C

Fig. 1 Geographical locations of the origin of the pig isolates in Ukraine examined in the study, with assigning the genotypes detected

sequencing kit (DYEnamic ET terminator, Amersham Biosciences) and analysed with an ABI PRISM 377 automated sequencer (Applied Biosystems).

The obtained sequences were manually edited and sequence similarity searches compared to the GenBank reference sequences were performed using the BLAST

algorithm (https://blast.ncbi.nlm.nih.gov). Nucleotide sequences were aligned with the Clustal Omega multiple sequence alignment programme [28] for *cox1*, *nad1* and 12S rRNA partial genes. Phylograms were constructed using a concatenated dataset with the MEGA7 software [29] using the neighbour-joining (NJ) method with 1000 bootstrap pseudoreplicates. The concatenated sequences covering the same gene region of *Taenia solium* (NC_004022) was used as an outgroup.

A TN93 substitution model with gamma-distributed rate heterogeneity was selected based on the maximum likelihood evaluation of possible substitution models using the Modeltest in MEGA [30]. Statistical parsimony networks using TCS implemented in the PopART software [31] were performed to analyse the haplotype genealogy in *cox1, nad1* and 12S rRNA concatenated datasets. The networks were constructed with a 95% probability limit. Nucleotide sequence translation was carried out using the EMBOSS Transeq programme (http://www.ebi.ac.uk/emboss/transeq) to distinguish between synonymous and non-synonymous mutations.

Results

Using the sequences of the partial mitochondrial genes of *cox1* (789 bp), *nad1* (602 bp), 12S rRNA (333–334 bp), three isolates obtained from two pig individuals from Bila Tserkva (Kyiv region) were identified as *E. granulosus s.s.* (G1 genotype), including one microvariant (G1A) detected in one isolate. By contrast, four isolates collected from four pigs from three villages in the Sumy region were classified as *E. canadensis* (G7 genotype), including the three microvariants (G7A–C) detected in three isolates (Table 1).

Based on the concatenated sequences of the three partial genes, the samples in the clade belonging to *E. granulosus s.s.* grouped together (bootstrap value of 99% supporting the node), including the three pig isolates examined, with G1-type samples being clearly distinguished from the closely related G3 genotype by good bootstrap support of 98% (the NJ phylogram is shown in Fig. 2). A network of haplotypes for related samples of *E. granulosus s.s.* and the genetic subdivision for Ukrainian samples is depicted in Fig. 3.

Two pig isolates from the Kyiv region (designated as UKR-BT1 and UKR-BT2-1) had a pattern characteristic of the most common G1 haplotype. Interestingly, the third isolate UKR-BT2-2 (lung allocation) derived from the same pig individual as the latter pig isolate (liver allocation) differed in three nucleotide substitutions (one in *nad1* and two in *cox1*) from these samples (here, therefore, referred to as the G1A haplotype). As a result, UKR-BT2-2 was placed in a separated cluster (bootstrap value of 53% lower due to slight nucleotide variation) together with isolates from Italy, Turkey and China (Figs. 2 and 3). Among the polymorphic sites, 117C/T synonymous mutation for UKR-BT2-2 was detected in *nad1*. In GenBank we found two isolates from both Turkey (KU925402, MG672198) and China (MN269988, AB786664), and single isolates from Italy (MG672278) and Morocco (EF367302) corresponding to the above *nad1* pattern. Thus, the samples in this branch were not firmly grouped according to geographic origin. In *cox1*, nucleotide substitutions of a 343C/T (synonymous) and a 476 T/C, generating a non-synonymous exchange in the corresponding amino acid sequence (alanine/valine) compared to the remaining Ukrainian G1 samples, were detected in UKR-BT2-2. This *cox1* pattern was previously recorded in the Iranian human (MF004308) and in the Iraqi goat (MF004308). The former sample represented the most distinct haplotype among the 12 haplotypes detected in Iran by Yanagida et al. [32] and was four mutational steps away from the most common ancestral haplotype.

Four porcine isolates from three localities in the Sumy region were identified as *E. canadensis* G7, and showed four different haplotypes, allocated to the ancestral G7 genotype and its three microvariants, referred to herein as G7A, G7B, G7C (Table 1). The G7 clade is located in the second branch of the resulting phylogram (Fig. 2), receiving a good bootstrap value (99%). A network of haplotypes depicting mutational relationships between closely related G7 haplotypes is given in Fig. 4.

Only the UKR-S3 isolate (obtained from the Stepanivka village) possessed sequences that matched the most common G7 profile based on the concatenated sequences of the three gene fragments. In UKR-S2 (also coming from Stepanivka), a pattern designated as belonging to the G7A genotype was identified. Two synonymous nucleotide substitutions 72A/G, 96G/A observed in *cox1* were responsible for the location of UKR-S2 in a different subgroup. These polymorphisms were previously documented in two isolates from Ukraine for the given concatenated sequences (MH201021, MH201022).

In UKR-S4 (obtained from the Mykolaivka village), a common G7 profile was detected in *cox1* and 12S, while in *nad1* a single nucleotide substitution (synonymous) 225 T/C was found (profile designated as genotype G7B). This polymorphism (reflected in bootstrap support of 61%, Fig. 2) had previously been reported in four countries—Poland (MH301004), Romania (MH300982), Spain (MH300985) and Mexico (MH300972) in a study by Laurimäe et al. [33], with several findings in Mexico (six GenBank entries) and Poland (two GenBank entries) (Fig. 4).

Isolate UKR-S1 (derived from the Kosivshchyna village and bearing the haplotype G7B) also exhibited a 225 T/C mutation (shared with UKR-S-4), and one additional synonymous substitution (420G/A) in *cox1*. This sequence type was previously found only in two pigs in Argentina (MH300963,

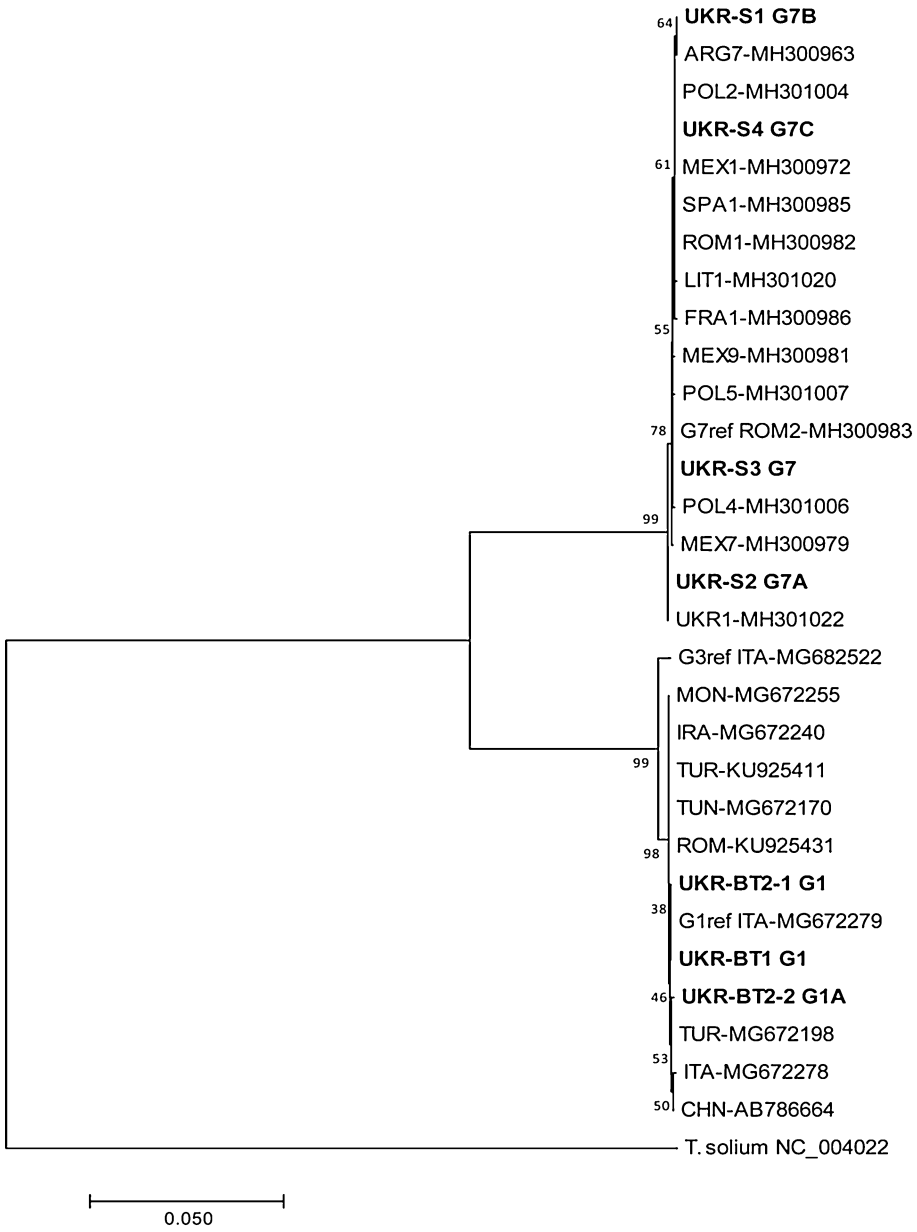

Fig. 2 Neighbour-joining phylogram generated from concatenated sequences of partial genes of cytochrome *c* oxidase 1 (789 bp), NADH dehydrogenase 1 (602 bp) and 12S rRNA (333–334 bp) showing relationships between the examined Ukrainian isolates and GenBank-retrieved related sequences of *Echinococcus granulosus sensu stricto* (G1 genotype), *Echinococcus granulosus sensu stricto* (genotype G3) and *Echinococcus canadensis* (G7 genotype). The concatenated sequences *Taenia solium* originating from China was used as the outgroup. The scale bar refers to a phylogenetic distance of 0.050 nucleotide substitutions per site. Numbers next to the branches indicate the bootstrap value calculated from 1,000 pseudoreplicates. Geographical origins of referenced isolates: UKR, Ukraine; POL, Poland; LIT, Lithuania; ROM, Romania; ITA, Italy; FRA, France; SPA, Spain; TUR, Turkey; CHN, China; MON, Mongolia; IRA, Iran; TUN, Tunisia; MEX, Mexico; ARG, Argentina

MH300962) by Laurimäe et al. [33] and a corresponding cluster was differentiated with a bootstrap value of 64% due to low genetic variation (Fig. 2).

The gene sequences obtained were deposited in GenBank® under Accession Numbers MT380850, MT380908, MT380912, MT383990, MT383992, MT383994, MT383995 for *cox1* target, MW542587-MW542593 for *nad1* target, and MT396429-MT396435 for 12S rRNA target.

Discussion

The data presented provide, to the best of our knowledge, the first molecular evidence of the presence of an autochthonous infection with *E. granulosus s.s.* in Ukraine, which was found in three isolates from two pigs from the central part of the country. In four pigs from northeastern

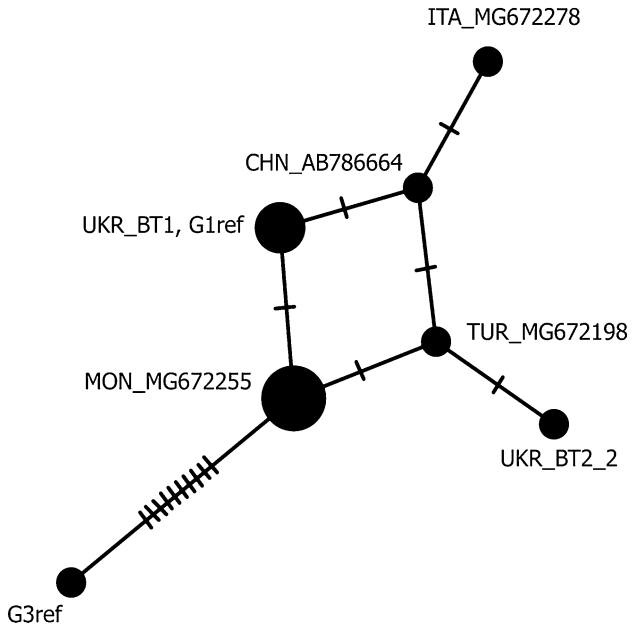

Fig. 3 Maximum parsimony haplotype network generated from concatenated sequences of *cox1* (789 bp), *nad1* (602 bp), 12S rRNA (333 bp) and illustrating genealogical relationships between the examined Ukrainian isolates and the most related haplotypes of *Echinococcus granulosus sensu stricto* G1 and *Echinococcus granulosus sensu stricto* G3. Labelled circles represent distinct haplotypes, transversal bars at branches represent point mutations, circle size correlates with haplotype frequency

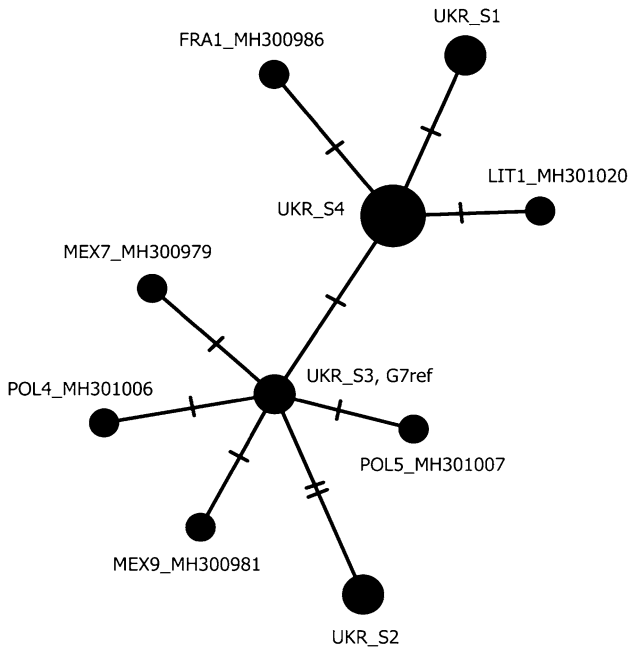

Fig. 4 Maximum parsimony haplotype network generated from concatenated sequences of *cox1* (789 bp), *nad1* (602 bp), 12S rRNA (334 bp) and illustrating genealogical relationships between the examined Ukrainian isolates and the most related haplotypes of Echinococcus canadensis G7. Labelled circles represent distinct haplotypes, transversal bars at branches represent point mutations, circle size correlates with haplotype frequency

Ukraine, we have also recorded *E. canadensis* G7, the major species circulating in Eastern Europe, which was identified in Ukraine also in previous genetic studies [17, 21, 22].

In Eastern Europe northward from Romania and Hungary, *E. canadensis* G7 is prevailing (except for the European part of Russia), while *E. granulosus s.s.* is being detected only sporadically. Specifically, human infections with the latter species were recently recorded in two cases in Slovakia [34], and in one case in a part of Russia belonging to Europe, in the Republic of Bashkiria [35]. Among animal hosts, *E. granulosus s.s.* was documented in one sheep in southern Poland [36] and in four dogs in Estonia [37]. In the European part of Russia, the species was recorded in a domestic cat from Saint Petersburg [38] and in four sheep originated from the Permskiy Krai [35].

The fact that different strains (now considered as different species) circulate in Ukraine in the pig/dog and sheep/dog cycles has previously been indicated by differences in their prepatent periods (53–57 days for the "pig strain", 45–50 days for the "sheep strain"), and by the low motility of "pig" proglottids (more than 90% are inactive, staying in the faeces of the dogs) [39, 40]. In addition, morphological differences between the strains were significant in lengths of large hooks: 22.98 ± 0.5 for sheep protoscoleces, 25.97 ± 0.51 for pig protoscoleces and in hook numbers: 35.83 ± 0.69 (34–38) for sheep protoscoleces, 34.5 ± 0.16 (34–36) for pig protoscoleces [41].

E. granulosus s.s. (genotypes G1, G3) is the most widespread species of the *E. granulosus s.l.* complex. Cysts are often fertile in humans, and numerous observations suggest that the high number of cases may be due to increased infectivity (and pathogenicity) of *E. granulosus s.s.* compared to other *Echinococcus* species [10]. Rural livestock-raising areas are particularly affected and some of the main factors contributing to the CE persistence include frequent illegal and home slaughtering of animals for food, feeding of raw offal to dogs, low public awareness of the disease, large populations of stray dogs and poor hygiene conditions [42, 43]. In private farms of central Ukraine with the current findings of *E. granulosus s.s.* G1, several animal species (including sheep and pigs) are often bred together, which may contribute to facilitating the species transmission of the parasite by dogs.

In Ukraine, 2,153 cases of CE in humans were reported between 2000 and 2013, accounting for an annual incidence of 0.36 per 100,000 inhabitants [24, 44]. Since 2009, there has been a decrease in the incidence in the country, which correlated with the animal prevalence (average prevalence of 3.21% in the years 2004–2008 in sheep, pigs and cattle

compared to 2.54% prevalence in the years 2009–2013) [24], and in 2018 it reached a 0.14 incidence per 100,000 inhabitants [45]. Investigations of endemic foci in Ukraine in recent decades have revealed the highest average number of human cases in the southern steppe zone where sheep are the main CE transmitters, while pigs are mainly responsible for *Echinococcus* circulation in the northern forest-steppe zone and Polissia reporting the lower number of human infections [24, 40, 46]. In sheep-breeding areas in southern Ukraine, the CE cycle is maintained by close contact between livestock and shepherd dogs, resulting in a high rate of environmental contamination (grass, soil) by eggs due to relatively mobile intermediate hosts, which may increase the risk of human infection. Unlike this, the lack of active mobility of indoor pigs during breeding reduces the risk of contamination of dog fur and soil, thus decreasing the risk of infection of humans and animals in the central/northern part of the country [47]. It had been also presumed, on circumstantial grounds of epidemiological observations, that *E. canadensis* from pigs may have lower infectivity for humans [10]. Indeed, investigations of endemic foci in Ukraine demonstrated the common occurrence of *E. granulosus* infections in dogs and pigs, but less evidence of the disease in humans in the central and western parts of the country [24].

In 2000–2013, the highest prevalence of CE in sheep was reported in Crimea (21.3%), Odesa (12.2%) and Transcarpathian (8.9%) regions, all situated in southern Ukraine [48]. Sheep is a likely candidate for the introduction and maintenance of the *E. granulosus s.s.* and its transmission to pigs in the area of the Kyiv region (where the G1 genotype has now been detected in a private farm of Bila Tserkva), due to its high susceptibility to infection with this species exemplified by high cyst fertility rates reported from sheep (e.g., [49, 50]). In the Kyiv region, an average CE prevalence of 0.6% in sheep was found in the years 2000–2013 [48].

In cattle, CE is most common in the central and eastern parts of the country, with an average infection rate of 0.9% documented in 2003–2009 [51]. The highest prevalences were observed in the regions of Odesa (12.5%) and Dnipropetrovsk (4.7%). In general, a high fertility rate in cattle is commonly due to the *E. ortleppi* (bovine strain G5) and rarely to the sheep strains (G1–G3) of *E. granulosus s.s.*, which usually play little role in parasite transmission with cysts being mostly sterile in Europe [47–54]. Thus, the transmission of G1 from cattle to pigs in Bila Tserkva is unlikely given also the low CE prevalence (0.06%) in cattle in the Kyiv region [51].

The infection rate with CE in pigs in Ukraine remains relatively high, but significantly decreased to 3.2% in 2003–2013 compared to 6.2% documented in 1976–1986 [55]. According to this report, the main reason for a decline was a significant reduction in the number of reared pigs bred in Ukraine. In 1976, the number of reared pigs amounted to 57.9 million, while in 2013 the total amount decreased to 462,300 animals. In 2012, the area with the highest prevalence of CE in pigs stretched diagonally through the centre of the country from north to south, with the most affected regions of Chernihiv (3.8%), Khmelnytskyi (3.1%), Sumy (2.7%), Chernivtsi (2.4%), and Kyiv (2.1%) [54]. Our sampling was carried out in the areas of Sumy and Kyiv regions with high prevalences of pig CE. In the above report by Litvinenko [56], feed grain contaminated with *Echinococcus* eggs was indicated as the significant factor responsible for transmitting the infection to pigs. During the processing of such grain, cestode eggs largely retain their viability and infectivity. Specifically in the Sumy region, a survey in pig farms of the Trostyanets, Sumy and Romensky districts conducted in 2017 showed that the spread of CE was among biotic factors primarily caused by infected dogs (infection rate of 27.6%), with a significant number of stray dogs having the opportunity to feed infected pig carcasses [57].

The existence of genetic variants within *E. granulosus* strains is a commonly observed phenomenon using mitochondrial markers, the existence of which is facilitated by the great asexual reproduction potential and short generation time of the parasite [33, 58]. In the present study, we have identified four microvariants (G1A, G7A-C) in G1 and G7 genotypes among the seven examined isolates.

Noticeable geographical associations in the microvariant distribution were herein derived for the G7A haplotype (bearing two nucleotide substitutions 72A/G, 96G/A in *cox1* compared to the most common G7 structure). In Ukraine, the G7A variant was found in one of four *E. canadensis* G7 samples (UKR-S2) in this study, in one of three G7 samples in the report by Šnábel et al. [59], and in two of the three G7 samples in the consecutive report by Šnábel et al. [22]; thus, in total G7A was detected in up to 40% of samples examined from pigs and people in the country. Identical nucleotide exchanges by the analysis of the complete *cox1* gene (1,608 bp) were recorded also in three of four pig-derived isolates originated from Armenia and one of twelve pig-derived isolates from Serbia [60]. In that study of Addy et al., Armenian samples clustered with the European samples rather than samples from neighbouring Iran, indicating the livestock dispersal through common trade routes with Europe. Given the high frequencies of the G7A variant in pigs in both Armenia and Ukraine, this haplotype is assumed to be highly established in the region southeast of Russia extending from the Black Sea and since its colonisation primarily disseminated through the diffusion of stock raising. According to the official data, the agro-industrial complex of Ukraine in 1978 produced 23.6% of all meat production in the former Soviet Union and the pig industry was very pronounced in this segment [61]. A part of pig herds from Ukraine was redistributed to some other Soviet republics including Armenia

during those boom times for swine breeding [62], which may have markedly influenced the current sharing of the G7A genotype between the countries.

In a study by Laurimäe et al. [33] analysing a complete mitogenome (13,556 bp), two Ukrainian samples corresponding to the here defined G7A structure (based on *cox1*, 789 bp) formed a small haplogroup with the Romanian cattle isolate, and, more broadly, they clustered with G7 samples from Poland and Mexico. The G7A haplotype was also found in one of the 18 examined *E. canadensis* isolates in Slovakia in the eastern part of the country [22].

Notably, we have detected two different G1 haplotypes (differing by three nucleotide substitutions) in one pig individual in cysts located in the liver and lungs. The affected pig host came from a private farm in the city of Bila Tserkva in the Kyiv region. The presence of different haplotypes in one host individual can mainly be caused by multiple successive infection events of the host by infective eggs with different genetic profiles. This phenomenon could also be explained by a single infection with several eggs with different haplotypes of *E. granulosus s.s.* This might occur when the eggs are in spatial proximity to each other in the environment due to the simultaneous excretion of these eggs by a canid infected with adult worms with different profiles.

In intermediate hosts, multiple haplotype patterns of *E. granulosus s.s.* in one host individual using classical sequencing of mitochondrial genes (including different haplotypes in the liver and lungs) were identified in cattle in Hungary and Romania [63], in sheep in France [64], in sheep and cattle in Moldova [65], and in sheep and cattle in Chile [66]. The greater frequency of mixed infections is usually the result of high environmental contamination by eggs of *E. granulosus s.s.* and the presence of multiple haplotypes in the same animal could be thus useful for identifying highly endemic foci of the parasite transmission [65, 66]. One case of infection with multiple G1 haplotypes found in this study in one of the two pig hosts could therefore serve as an indication of higher egg contamination around a farm in the Kyiv region, but this should be corroborated by further evidence from multiple-cyst analyses in the area.

In the territory of the former Soviet Union, CE is common in those regions where livestock (mainly sheep) is bred—particularly in the North Caucasus, Transcaucasia, Kazakhstan, Kyrgyzstan, Uzbekistan, Moldova, Ukraine [49]. The increase of CE incidence has been linked to changes in farming practices following the collapse of the Soviet Union in 1991 towards private backyard slaughtering that promotes infection of dogs [67, 68]. In Ukraine, e.g. in the Odesa region, 99.8% of sheep, 91.4% of cattle and 41.9% of pigs were home-slaughtered in 2004–2017 in private facilities [69]. The approximately fivefold increase in clinical records between 2000 and 2013 (154 cases/year) compared to 1980–1986 (28 cases/year) in Ukraine is comparable to the figure recorded in Kyrgyzstan and Tadjikistan [40, 46, 70, 71].

The first molecular evidence of *E. granulosus s.s.* in Ukraine could be of importance regarding the dosing regimen employed in dogs that should be designed for different period of G1 and G7 developments. Further molecular and epidemiological studies are needed, especially in the southern part of Ukraine, where *E. granulosus s.s.* poses a greater risk for human health, with a focus on both clinical and sheep samples.

Conclusion

In summary, two species of the *Echinococcus granulosus s.l.* complex have been identified in pigs coming from two areas of northern Ukraine by DNA sequencing of mitochondrial genes. Specifically, *E. canadensis* (G7 genotype), which is the most common of *E. granulosus s.l.* species circulating in eastern Europe, and for the first time in the country by molecular methods, a virulent *E. granulosus s.s.* (G1 genotype) in the Kyiv region, which poses an increased health hazard.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors claim that all parasitological and genetic procedures contributing to this work are in accordance with the ethical standards of the relevant national and institutional guides. Samples were taken from slaughterhouses in Sumy and Bila Tserkva cities in Ukraine after the pigs were humanely slaughtered for meat according to the Law of Ukraine "on Veterinary Medicine" no. 3318 (as amended with changes from February 4, 2021).

Consent to participate Not applicable.

Consent for publication Not applicable.

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