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A molecular survey of *Echinococcus granulosus* sensu lato in central-eastern Europe

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Abstract: Central-eastern Europe is an endemic region for cystic echinococcosis where multiple species of intermediate hosts are commonly infected with *Echinococcus granulosus* sensu lato tapeworms of major medical and veterinary importance. Investigations of the genetic variation of 25 *Echinococcus* isolates from five countries (Slovakia, Romania, Ukraine, Hungary, Poland) were undertaken using three mitochondrial DNA markers. The 18 isolates from pigs derived from Slovakia and Ukraine and the four human isolates from Slovakia, Poland and Ukraine were identified as *E. canadensis* G7, whereas the three human isolates from Romania and Hungary were classified as *E. granulosus* sensu stricto G1. This study reports the first confirmed human case of *E. granulosus* s.s. in Hungary. The haplotype G7A with two polymorphic sites relative to the most common regional variant of *E. canadensis* G7 was recorded in both pigs from Ukraine and in a single pig isolate from Slovakia. The results of this study support the circumstantial evidence that *E. canadensis* G7 with low infectivity for humans is highly prevalent in the northern parts of the region (Poland, Slovakia, forest-steppe zone of Ukraine), while infections with *E. granulosus* s.s. which are highly infectious for humans are more commonly encountered in Romania and Hungary.

Keywords: *Echinococcus granulosus*, genotype, DNA sequences, human, pig, central-eastern Europe

1 Introduction

The larval stages of the tapeworm *Echinococcus granulosus* sensu lato (s.l.) are the causative agents of cystic echinococcosis (CE), one of the most important cestode infections causing morbidity and mortality in humans and significant economic losses in livestock. Around one million or more people are currently suffering from CE globally and the financial burden of the disease on the livestock industry is substantial, with up to two billion dollars lost annually [1]. Recent molecular phylogenetic analyses have revealed that *E. granulosus* is a complex of spesies/genotypes with at least five different species: *E. granulosus* sensu stricto (s.s.) (genotypes G1-G3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), *Echinococcus canadensis* (G6-G10), and *Echinococcus felidis* („lion strain“) [2-4].

CE remains one of the most important helminth zoonoses in many regions of Europe, especially the Mediterranean littoral (Spain, Italy, Greece and Turkey) and southeastern countries such as Bulgaria and Romania. The disease seems to be less prevalent in the UK, Central Europe, the Baltic States and the Scandinavian countries [5,6]. Surveys that addressed the incidence of CE over the last decade from central-eastern Europe has reported a relatively greater incidence in Romania (average incidence annual rate was 0.29 per 100,000 population) and Ukraine (0.30), whereas in Hungary (0.07), Poland (0.08) and Slovakia (0.10) lower numbers of human cases were documented [7]. This also is due to the fact that *E. granulosus* s.s., which is responsible for the majority

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of the global burden of CE infections, predominantly circulates in Romania and the southern steppe zone of Ukraine associated with sheep-raising regions; whereas *E. canadensis* G7, which prevails in the three remaining countries, is of minor importance for human health due to low infectivity for humans [8,9]. Particularly in Bulgaria and Romania, socio-economic changes in the 1990s accompanied by a breakdown of veterinary control efforts and administrative irregularities led to a re-emergence of CE [10,11].

To extend knowledge of the genotype spectrum responsible for CE with fragmentary data available for central-eastern Europe, the study was conducted to evaluate the genetic variation of *Echinococcus granulosus* in pigs and humans from five neighbouring countries of the region.

2 Material and Methods

Echinococcus isolates examined in this study were derived from 18 pigs and seven humans originating from countries of central-eastern Europe, i.e. Slovakia, Ukraine, Romania, Hungary and Poland (Table 1, Figure 1). Pig hydatid cysts were collected at abattoirs during routine meat inspections and stored at -20°C before transfer to the laboratories of cooperating institutes in the above countries. Human hydatid cysts collected at surgery in Slovakia, Romania and Poland were isolated from livers, hydatid cysts from Hungarian patient were recovered from the liver and lung. Cyst contents were examined under light microscopy for the presence of protoscoleces, which were thereafter rinsed several times in phosphate buffered

Table 1. Characteristics of *Echinococcus granulosus* sensu lato isolates used in this study

Code	Host	Geographical origin
SK1	pig	eastern Slovakia (Michalovce district)
SK2	pig	eastern Slovakia (Trebišov district)
SK3	pig	eastern Slovakia (Trebišov district)
SK4	pig	eastern Slovakia (Trebišov district)
SK5	pig	eastern Slovakia (Trebišov district)
SK6	pig	eastern Slovakia (Košice district)
SK7	pig	eastern Slovakia (Rožňava district)
SK8	pig	eastern Slovakia (Rožňava district)
SK9	pig	central Slovakia (Revúca dsitrict)
SK10	pig	central Slovakia (Revúca district)
SK11	pig	central Slovakia (Lučenec district)
SK12	pig	central Slovakia (Rimavská Sobota district)
SK13	pig	western Slovakia (Komárno district)
SK14	pig	western Slovakia (Dunajská Streda district)
SK15	pig	western Slovakia (Dunajská Streda district)
SK16	pig	western Slovakia (Dunajská Streda district)
SK17	human	eastern Slovakia (Michalovce district)
SK18	human	eastern Slovakia (Stará Ľubovňa district)
UKR1	pig	north-eastern Ukraine (Sumy, Sumy oblast)
UKR2	pig	north-eastern Ukraine (Sumy, Sumy oblast)
UKR3	human	north-western Ukraine (Lutsk, Volyn oblast)
POL1	human	south-eastern Poland (Lublin, Lublin voivodeship)
HUN1	human	south-eastern Hungary (Szeghalom, Békés county)
ROM1	human	north-eastern Romania (Românești, Iasi county)
ROM2	human	eastern Romania (Tutova, Vaslui county)

Figure 1. Geographical origin of *Echinococcus granulosus* sensu lato isolates examined in this study. Full circles indicate pig isolates, empty circles human isolates. SK, abbreviation for Slovakia.

saline (PBS; pH 7.4) and fixed in 70% ethanol for further analyses.

Total genomic DNA was extracted from fertile hydatid cysts using a DNeasy tissue kit (Qiagen, Hilden, Germany) following the animal tissue isolation protocol. Fragments of three mitochondrial genes were targeted for PCR amplifications, namely cytochrome *c* oxidase 1 (*cox1*, 789 bp), ATP synthase subunit 6 (*atp6*, 513 bp), and NADH dehydrogenase 1 (*nad1*, 471 bp). DNA was amplified using specific primers: 5´-TTGAATTTGCCACGTTTGAATGC-3´/5´-GAACCTAACGACATAACATAATGA-3´ for *cox1* [12], 5´-GCATCAATTTGAAGAGTTGGGGATAAC-3´/5´-CCAAATAATCTATCAACTACACAACAC-3´ for *atp6* [13], and 5´-AGATTCGTAAGGGGCCTAATA-3´/5´-ACCACTAACTAATTCACTTTC-3´ for *nad1* [14]. PCR reactions were performed using following cycling conditions: initial denaturation step 94°C for 5 min followed by 35 cycles, 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. Amplified products were visualized after electrophoresis on 1.5% (w/v) agarose gels and purified using a Nucleospin Extract II kit (Macherey Nagel, Düren, Germany). Amplicons were directly sequenced using a dye terminator cycle sequencing kit (DYEnamic ET terminator; Amersham Biosciences, UK) and analysed with an ABI PRISM 377 automated sequencer (Applied Biosystems, USA).

Nucleotide sequences were aligned using ClustalX2 [15] and compared to those stored in GenBank using BLAST program. The branching pattern was generated by MEGA 6 software [16] using the neighbor-joining (N-J) method. The evolutionary distances were computed using the Tamura-Nei method [17], following the run with Modeltest to infer the best evolutionary model. The same model was used for all gene regions investigated, and the concatenated analysis was performed using all genetic markers. Bootstrap analyses were conducted by using 1000 replicates. Reference DNA sequences were taken from a study of Nakao *et al.* [18] who first screened the complete mitochondrial genome for *Echinococcus* species and genotypes. DNA sequences of these isolates under this study were deposited into the GenBank database: ROM1 isolate in *cox1* gene (Accession No. JF520817); ROM2 isolate in *cox1* gene (JF520818); HUN1 isolate in *cox1* gene (JF690976); ROM1 isolate in *atp6* gene (JF708944); ROM2 isolate in *atp6* gene (JF708943); HUN1 isolate in *atp6* gene (JF690977). Obtained sequences were analyzed for haplotype inference and genetic variation using DNAsp [19].

3 Results

Of the 25 isolates from central-eastern Europe, 22 from Slovakia, Poland and Ukraine were indentified as *E. canadensis* G7. The three isolates from Romania and Hungary were typed as *E. granulosus* s.s. G1. In total, twelve haplotypes were identified in the three analyzed gene portions.

In screening *cox1* fragment (789 bp), two lines of *E. canadensis* G7 were detected, differing in nucleotide sites 72 and 96 (Table 2). The major G7 line was composed of 19 isolates from Slovakia, one from north-western Ukraine (UKR3) and one from Poland, and exhibited one different nucleotide site (position 273) compared to the G7 reference sequence (GenBank Accession No. AB235847). The additional G7B line manifested two transition mutations and was detected in three samples, two (UKR-1 and UKR-2) of which originated from the same location in north-eastern Ukraine (Sumy region). The third isolate (SK-5) bearing these two nucleotide exchanges originated from eastern Slovakia (Veľký Horeš location in Trebišov district). This Slovak

Table 2. Nucleotide substitutions in the partial *cox1* gene (789 bp)

		Position							
Isolate	**Genotype**	**72**	**96**	**186**	**273**	**460**	**476**	**486**	**677**
G1ref-AB033407	G1	A	T	T	G	A	C	C	T
G7ref-AB235847	G7	A	G	C	A	A	T	T	C
ROM1	G1	.	.	.	.	A	C	C	T
HUN1	G1A	.	.	.	.	A	**T**	C	T
ROM2	G1B	.	.	.	.	**G**	C	**T**	**C**
G7-major haplotype (19 isolates)	G7	A	G	C	**G**	.	.	.	.
UKR1	G7A	**G**	**A**	C	G	.	.	.	.
UKR2	G7A	**G**	**A**	C	G	.	.	.	.
SK5	G7A	**G**	**A**	**T**	G	.	.	.	.

Point mutations are indicated by bold letters, dots indicate sites where sequences are not depicted with respect to reference bases due to different resulting genotypes (G1 or G7).

Table 3. Nucleotide substitutions in the partial *atp6* gene (513 bp)

		Position		
Isolate	**Genotype**	**65**	**238**	**261**
G1ref-AB031283	G1	T	G	T
G7ref-AB235847	G7	C	G	A
ROM1	G1	T	G	T
ROM2	G1	T	G	T
HUN1	G1	T	G	T
G7-major haplotype (17 isolates)	G7	C	G	A
SK5	G7A	C	**T**	A
SK13	G7A	C	**T**	A
SK15	G7A	C	**T**	A
SK6	G7B	**T**	G	A
SK11	G7C	C	G	**G**

Point mutations are indicated by bold letters.

sample also possessed a third, unique C/T mutation, at position 186.

All three isolates classified in this study as *E. granulosus* s.s. G1 were derived from humans and mutually displayed three slightly different *cox1* haplotypes (Table 2). Of these, one isolate from north-eastern Romania (ROM1) displayed sequences fully corresponding to the ref. G1 structure (Accession No. AB033407). A second isolate from Romania (ROM2) exhibited three transition mutations at positions 460, 486, and 677. This sequence type (denoted as G1B variant) was not previously recorded in Genbank. The HUN-1 human isolate from south-eastern Hungary (Békés county) displayed 476C/T substitution and was herein denoted as G1A following designations in the *Echinococcus* study by Šnábel *et al.* [20], in which the same pattern was recorded in four human and sheep isolates from Turkey.

In partial sequences of *atp6* gene (513 bp), the most frequently detected haplotype for samples determined as *E. canadensis* were matched with G7 ref. sequences (17 isolates). In the remaining G7 isolates, three different haplotypes were detected in five Slovak samples (Table 3). Specifically, polymorphic site 238 associated with G/T substitution was recorded in SK5 isolate obtained from eastern Slovakia (Trebišov district), and in two isolates from western Slovakia. These two isolates, SK13 (origin of Okánikovo, Komárno district) and SK15 (Okoč, Dunajská Streda district) were obtained from pig farms located 17 km apart. In addition, the SK6 isolate from eastern Slovakia (Košice district) and the SK11 isolate from central Slovakia (Lučenec district) had single nucleotide substitutions at 65T/C and 261A/G. These isolates had been provisionally designated as bearing G7B and G7C variants for the *atp6* gene. Sequences of human isolates from Romania and Hungary were homologous to G1 reference structure for *atp6* (GenBank Accession No. AB031283).

In the *nad1* gene all samples had a 100% identity with the globally distributed haplotype with reference sequence defined by Nakao *et al.* [18].

The resulting N-J dendrogram derived from concatenated sequences of *cox1*, *atp6* and *nad1* is presented in Fig. 2. For samples with G7 genotype three different

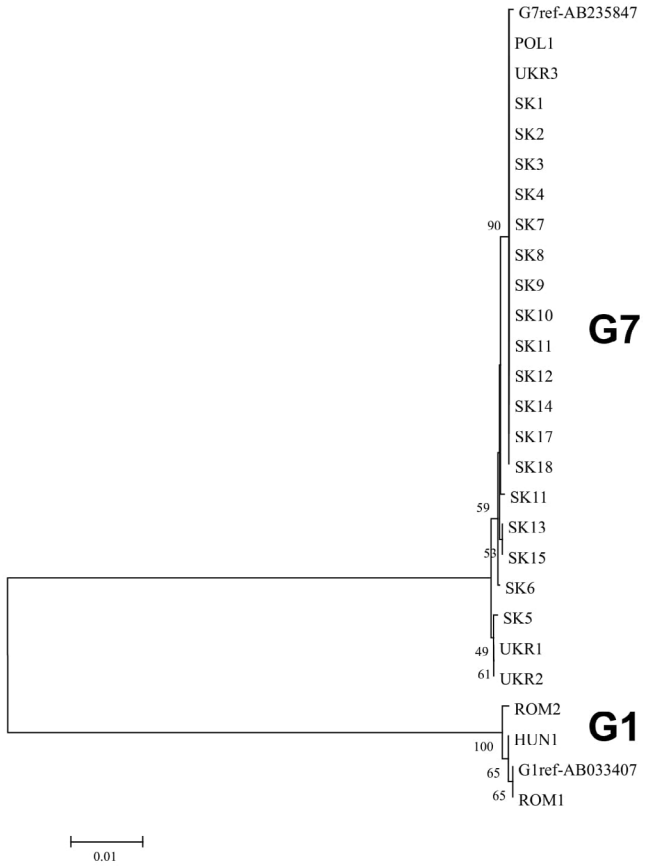

Figure 2. Dendrogram inferred for examined central-eastern European isolates from concatenated DNA sequences in mitochondrial genes *cox1*, *atp6, nad1* using the neighbor-joining (N-J) method. Numbers at nodes of branches refer to bootstrap support values in 1,000 replications. Only bootstrap values higher than 40% are shown.

haplotypes were identified in *cox1* and four haplotypes in *atp6*. For G1 samples, three haplotypes were detected in *cox1* and one shared haplotype in *atp6*. The average nucleotide diversity (π) at individual sites was 0.0327. Two clusters in G7 samples were obtained in the resulting tree, with minor group consisting of two isolates from eastern Ukraine and the eastern Slovak isolate SK5. The latter isolate was the most distinguished sample in a G7 sample set as it possessed nucleotide substitutions relative to the most common type in both polymorphic genes *cox1* and *atp6*. Both human samples from Slovakia had sequences corresponding to G7 sequence unlike human samples from Hungary and Romania that displayed G1 sequence pattern.

4 Discussion

The results obtained in this study support the circumstantial evidence that *E. canadensis* G7 is highly prevalent (or exclusive) in Poland, Slovakia and the forest-steppe zone of Ukraine, while *E. granulosus* s.s. is the primary causative agent for CE in Romania. In Hungary, the first case of human CE infection with the highly infectious *E. granulosus* s.s. was genetically confirmed, coupled to previous findings of the species encountered in domestic ungulates [21].

The highest number of isolates (18) was examined from various regions of Slovakia. In all 16 pig isolates and 2 human isolates, G7 genotype of *E. canadensis* was detected. Five of the 18 Slovak isolates displayed 1-2 polymorphic sites compared to most common variant circulating in the region, and have been provisionally designated as G7A-C microvariants. Consistently with this study, only *E. canadensis* G7 was documented in Slovak sheep, cattle and humans in previous reports [22-24].

The two Romanian patients originated from Iasi and Vaslui counties in eastern Romania and were farmers, 39- and 62-year-old, respectively. The finding of *E. granulosus* s.s. in these patients is in agreement with previous studies which provided evidence of almost all human CE records in Romania infected with this species [25-27]. However, in a survey by Piccoli *et al.* [28] a single human case of *E. canadensis* G7 was reported, apart from 59 cases of *E. granulosus* s.s. in patients from south-eastern Romania. The authors stated that the main risk factor for acquiring CE infection seemed to be the close contact with stray dogs reported for 97% of patients. Over the last decades, the free movement of stray dogs has been an increasing public health problem in Romania that might contribute to maintaining the high prevalence of human CE infections both in humans and animals [11]. Amongst domestic animals, sheep and cattle were primarily infected with *E. granulosus* s.s. [21,25,27,29]. In addition, a single cattle isolate (2.3% of cattle isolates in the respective study) was typed as *E. canadensis* G7 [21]. Unlike this, in pigs solely G7 genotype was documented [25] and both *E. granulosus* s.s. and *E. canadensis* in similar proportions were identified in wild boars (15 specimens with *E. granulosus* s.s. and 15 specimens with *E. canadensis* G7) [21,30]. Iasi and Vaslui counties are located in the easternmost part of Romania, bordering the Republic of Moldova to the west, where hyperendemic focus of *Echinococcus granulosus* with high prevalences of sheep (82.5%) and cattle (78.9%) was recently described [31]. Consistently with the present diagnosis of *E. granulosus* s.s. in the two Romanian patients, solely this species was detected in 33 sheep and 15 cattle specimens from the Moldavian region in the above report.

In the current study, *E. granulosus* s.s. was detected in liver and lung cysts of a 13-year-old male patient from Hungary (Szeghalom town in Békés county). In Békés county, the natural vegetation has been originally derived from the forest-grove steppe, with significant amount of livestock farming being present (sheep, cattle, pigs, etc.) that facilitates *Echinococcus* transmission to humans [32]. Greater probablility of *E. granulosus* s.s. detection compared to G7 was enhanced by the proximity of Békés county (south-eastern Hungary) to Romania where there is a significant predominance of *E. granulosus* G1-G3 complex. Previously, *E. canadensis* was found in an Hungarian immigrant that was being treated in an Austrian hospital [26]. Among domestic ungulates, Casulli *et al.* [21] documented in Hungary 21 cattle specimens as *E. granulosus* s.s., whereas in pig *E. canadensis* G7 prevailed (ten of 12 specimes G7, two of 12 specimens *E. granulosus* s.s.). In sheep, one isolate of *E. granulosus* s.s. and one isolate of *E. canadensis* G7 were documented in the above study.

The G1A microvariant of *E. granulosus* s.s., identified in the Hungarian patient, has C/T non-synonymous substitution responsible for replacement of the alanine with valine at position 476 in the 789-bp *cox1* fragment. This sequence type is the most commonly recorded G1 variant in southern Palearctic and it was previously recorded in China (Qinghai province) in sheep [33], in Russia (Altai region) in human [34], in Mongolia in human (GenBank Accession No. AB787538, unpublished), in Turkey in sheep, cattle and human [20,35], in Iran in sheep, human, camel and goat [36] and GenBank Accession No. KP751431 (unpublished), in Jordan in sheep [36], and in Tunisia in cattle, sheep and donkey [37]. The

476C/T mutation compared to the reference G1 structure corresponds to the frequently found the 35C/T mutation in a shorter, 366-bp fragment of the *cox1*, most commonly analyzed for *Echinoccoccus* typing with primers defined by Bowles *et al.* [38]. On the other hand, in South America which has a common perpetuation of *E. granulosus* G1, this sequence pattern has been so far only found in sheep from Brazil [39], and in cattle from Argentina [40].

In Ukraine, the prevalence of *E. granulosus* s.l. varies substantially in different geographical zones. The highest occurrence in humans has been documented in the southern steppe zones associated with sheep-raising regions (89.4% of cases), whereas only 10.6% of cases were found in forest-steppe zones with 11% of pigs being infected and low infection rates in sheep [8]. Three isolates (two from pigs and one from human) examined in the current study originated from the latter forest-steppe zone. As presumed, *E. canadensis* G7 was genetically confirmed to circulate in this territory in examined samples. The human isolate was recovered from 21-year-old woman from Volyn oblast in north-western Ukraine and pig isolates were recovered from Sumy oblast in north-eastern Ukraine. Interestingly, both pig isolates differed in two nucleotides from the common G7 genotype, and the same base exchanges were identified in one of the Slovak isolates from the eastern part of the country. Thus, this G7 microvariant (G7A) was detected in 13.6% (3 of 22) of the G7 isolates from the two distant sites and therefore is likely more commonly distributed in central-eastern Europe. In previous studies with Ukrainian isolates, *E. canadensis* G7 was detected in two pigs and in one wild boar from the Lebedyn district of Sumy oblast, the same region where our isolates originated [42,43].

Human *Echinococcus* protoscoleces from south-eastern part Poland were examined in our study and were found to have homologous sequences to *E. canadensis* G7. Previously, this genotype has been documented in Poland in 38 pigs and five humans [42]. G7 was also found in a European beaver from north-eastern Poland [43]. In surveys focused on humans infected with CE, Pawlowski and Stefaniak [44] reported 16 human cases with G7, as was reported also by Dybicz *et al.* [45] for 30 patients from central Poland. This was followed by another report of Dybicz *et al.* [46], documenting seven cases of G7-infected patients and two cases of G1-infected patients. Nevertheless, the authors stated that patients may have acquired infections with *E. granulosus* s.s. G1 outside Poland (Kazakhstan and Turkey, respectively), and thus they cannot be unambiguously regarded as indigenous. However, the first unequivocal presence of *E. granulosus* s.s. has been recently genetically corroborated in sheep from Podhale region in southern Poland [47].

Further studies are needed to more precisely elucidate the geographic distribution of *E. granulosus* s.s., especially in samples from the southern steppe zones of Ukraine from which genetic data are absent. In addition, evaluation of isolates from a wider spectrum of domestic and sylvatic intermediate hosts are required to obtain a more precise overview of the transmission cycles in *E. granulosus* s.l. in endemic foci of central-eastern Europe.

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Conflicts of interest: Authors declare nothing to disclose.

References

[1] Torgerson P.R., Macpherson C.N.L. The socioeconomic burden of parasitic zoonoses: global trends, Vet. Parasitol., 2011, 182, 1, 79-95

[2] Nakao M., McManus D.P., Schantz P.M., Craig P.S., Ito A., A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes, Parasitology, 2007, 134, 5, 1-10

[3] Hüttner M., Nakao M., Wassermann T., Siefert L., Boomker J.D., Dinkel A., et al., Genetic characterization and phylogenetic position of *Echinococcus felidis* (Cestoda: Taeniidae) from the African lion, Int. J. Parasitol. 2008, 38, 7, 861-868

[4] McManus D.P., Current status of the genetics and molecular taxonomy of *Echinococcus* species, Parasitology, 2013, 140, 13, 1617-1623

[5] Romig T., Dinkel A., Mackenstedt U., The present situation of echinococcosis in Europe, Parasitol. Int., 2006, 55, Suppl. S187-191

[6] Bruzinskaite R., Sarkūnas M., Torgerson P.R., Mathis A., Deplazes P., Echinococcosis in pigs and intestinal infection with *Echinococcus* spp. in dogs in southwestern Lithuania, Vet. Parasitol., 2009, 160, 3-4, 237-241

[7] Berger S., Echinococcosis: Global Status, 2016 Edition, GIDEON Informatics, Los Angeles, 2016

[8] Malczewski A., CE and AE in Eastern Europe, (In: Cestode Zoonoses: Echinococcosis and Cysticercosis: An Emergent and Global Problem), IOS, Amsterdam, 2002, 81- 89

[9] Romig T., Ebi D., Wassermann M., Taxonomy and molecular epidemiology of *Echinococcus granulosus* sensu lato, Vet. Parasitol., 2015, 213, 3-4, 76-84

[10] Breyer I., Georgieva D., Kurdova R., Gottstein B., *Echinococcus granulosus* strain typing in Bulgaria: the G1 genotype is

predominant in intermediate and definitive wild hosts, Parasitol. Res., 2004, 93, 2, 127-130
[11] Neghina R., Neghina A.M., Marincu I., Iacobiciu I., Epidemiology and Epizootology of Cystic Echinococcosis in Romania 1862-2007, Foodborne Pathog. Dis., 2010, 7, 6, 613-618.
[12] Xiao N., Qiu J., Nakao M., Nakaya K., Yamasaki H., Sako Y., et al., Short report: Identification of *Echinococcus* species from a yak in the Qinghai-Tibet plateau region of China, Am. J. Trop. Med. Hyg., 2003, 69, 4, 445-446
[13] Xiao N., Qiu J., Nakao M., Li T., Yang W., Chen X., et al., *Echinococcus shiquicus*, a new species from the Qinghai-Tibet plateau region of China: discovery and epidemiological implications, Int. J. Parasitol., 2005, 35, 693-701
[14] Bowles J., McManus D.P., NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*, Int. J. Parasitol., 1993, 23, 7, 969-997
[15] Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., et al., Clustal W and Clustal X version 2.0, Bioinformatics, 2007, 23, 2947-2948
[16] Tamura K., Stecher G., Peterson D., Filipski A., Kumar S., MEGA6: molecular evolutionary genetics analysis version 6.0, Mol. Biol. Evol., 2013, 30, 12, 2725-2729
[17] Tamura K., Nei M., Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, Mol. Biol. Evol., 1993, 10, 3, 512-526
[18] Nakao M., Sako Y., Yokoyama N., Fukunaga M., Ito A., Mitochondrial genetic code in cestodes, Mol. Biochem. Parasitol., 2000, 111, 2, 415-424
[19] Rozas J., Sanchez-Delbarrio J.C., Messeguer X., Rozas R., DnaSP, DNA polymorphism analyses by the coalescent and other methods, Bioinformatics, 2003, 19, 18, 2496-2497
[20] Šnábel V., Altintas N., D'Amelio S., Nakao M., Romig T., Yolasigmaz A., et al., Cystic echinococcosis in Turkey: genetic variability and first record of the pig strain (G7) in the country, Parasitol. Res., 2009, 105, 1, 145-154
[21] Casulli A., Interisano M., Sréter T., Chitimia L., Kirkova Z., La Rosa G., et al., Genetic variability of *Echinococcus granulosus* sensu stricto in Europe inferred by mitochondrial DNA sequences, Infect. Genet. Evol., 2012, 12, 2, 377-383
[22] Šnábel V., D'Amelio S., Mathiopoulos K., Turčeková Ľ., Dubinský P., Molecular evidence of the presence of a G7 genotype in Slovakia, J. Helminthol., 2000, 74, 2, 177-181
[23] Turčeková Ľ., Šnábel V., D´Amelio S., Busi M., Dubinský P., Morphological and genetic characterization of *Echinococcus granulosus* in the Slovak Republic, Acta Trop., 2003, 85, 2, 223-229
[24] Turčeková Ľ., Šnábel V., Dudiňák V., Gašpar V., Dubinský P., Prevalence of cystic echinococcosis in pigs from Slovakia, with evaluation of size, fertility and number of hydatid cysts, Helminthologia, 2009, 46, 3, 151-158
[25] Bart J.M., Morariu S., Knapp J., Ilie M.S., Pitulescu M., Anghel A., et al., Genetic typing of *Echinococcus granulosus* in Romania, Parasitol. Res., 2006, 98, 2, 130-137
[26] Schneider R., Gollackner B., Schindl M., Tucek G., Auer H. *Echinococcus canadensis* G7 (Pig Strain): An Underestimated Cause of Cystic Echinococcosis in Austria, Am. J. Trop. Med. Hyg., 2010, 82, 5, 871-874
[27] Mitrea I.L., Ionita M., Costin I.I., Predoi G., Avram E., Rinaldi L., et al., Occurrence and genetic characterization of *Echinococcus granulosus* in naturally infected adult sheep and cattle in Romania, Vet. Parasitol., 2014, 206, 3-4, 159-166
[28] Piccoli L., Bazzocchi C., Brunetti E., Mihailescu P., Bandi C., Mastalier B., et al., Molecular characterization of *Echinococcus granulosus* in south-eastern Romania: evidence of G1-G3 and G6-G10 complexes in humans, Clin. Microbiol. Infect., 2013, 19, 6, 578-582
[29] Mitrea I.L., Ionita M., Wassermann M., Solcan G., Romig T., Cystic echinococcosis in Romania: an epidemiological survey of livestock demonstrates the persistence of hyperendemicity, Foodborne Pathog. Dis., 2012, 9, 11, 980-985
[30] Onac D., Győrke A., Oltean M., Gavrea R., Cozma V., First detection of *Echinococcus granulosus* G1 and G7 in wild boars (*Sus scrofa*) and red deer (*Cervus elaphus*) in Romania using PCR and PCR-RFLP techniques, Vet. Parasitol., 2013, 193, 1-3, 289-291
[31] Umhang G., Chihai O., Boué F., Molecular characterization of *Echinococcus granulosus* in a hyperendemic European focus, the Republic of Moldova, Parasitol. Res., 2014, 113, 12, 4371-4376
[32] Molnár Z., Classification of pasture habitats by Hungarian herders in a steppe landscape (Hungary), J. Ethnobiol. Ethnomed., 2012, 8, 28
[33] Nakao M., Li T., Han X., Ma X., Xiao N., Qiu J., et al., Genetic polymorphisms of *Echinococcus* tapeworms in China as determined by mitochondrial and nuclear DNA sequences, Int. J. Parasitol., 2010, 40, 3, 379-385
[34] Konyaev S.V., Yanagida T., Ingovatova G.M., Shoikhet Y.N., Nakao M., Sako Y., et al., Molecular identification of human echinococcosis in the Altai region of Russia, Parasitol. Int., 2012, 61, 4, 711-714
[35] Kinkar L., Laurimäe T., Simsek S., Balkaya I., Casulli A., Manfredi M.T., et al., High-resolution phylogeography of zoonotic tapeworm *Echinococcus granulosus* sensu stricto genotype G1 with an emphasis on its distribution in Turkey, Italy and Spain, Parasitology, 2016, 143, 13, 1790-1801
[36] Yanagida T., Mohammadzadeh T., Kamhawi S., Nakao M., Sadjjadi S.M., Hijjawi N., et al., Genetic polymorphisms of *Echinococcus granulosus* sensu stricto in the Middle East, Parasitol. Int., 2012, 61, 4, 599-603
[37] Boufana B., Lahmar S., Rebaï W., Ben Safta Z., Jebabli L., Ammar A., et al., Genetic variability and haplotypes of *Echinococcus* isolates from Tunisia, Trans. R. Soc. Trop. Med. Hyg., 2014, 108, 11, 706-714
[38] Bowles J., Blair D., McManus D.P., Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing, Mol. Biochem. Parasitol., 1992, 54, 2, 165-173
[39] Beato S., Parreira R., Roque C., Gonçalves M., Silva L., Maurelli M.P., et al., *Echinococcus granulosus* in Portugal: the first report of the G7 genotype in cattle, Vet. Parasitol., 2013, 198, 1-2, 235-239
[40] Laurimae T., Kinkar L., Andresiuk V., Haag K.L., Ponce-Gordo F., Acosta-Jamett G., et al., Genetic diversity and phylogeography of highly zoonotic *Echinococcus granulosus* genotype G1 in the Americas (Argentina, Brazil, Chile and Mexico) based on 8279bp of mtDNA, Infect. Genet. Evol., 2016, 45, 290-296
[41] Kędra A.H., Swiderski Z., Tkach V.V., Dubinský P., Pawlowski Z., Stefaniak J., et al., Genetic analysis of *Echinococcus granulosus* from humans and pigs in Poland, Slovakia and

Ukraine. A multicenter study, Acta Parasitol., 1999, 44, 4, 248-254

[42] Kędra A.H., Tkach V.V., Swiderski Z.P., Pawlowski Z., Emets A., Pawlowski J., Molecular characterisation of *Echinococcus granulosus* from a wild boar, Acta Parasitol., 2000, 45, 2, 121-122

[43] Tkach V.V., Swiderski Z., Drozdz J., Demiaszkiewicz A.W., Molecular identification of *Echinococcus granulosus* from wild European beaver, *Castor fiber* (L.) from North-Eastern Poland, Acta Parasitol., 2002, 47, 2, 173-176

[44] Pawłowski Z.S., Stefaniak J., Bąblowica wywołana przez *Echinococcus granulosus* w Wielkopolsce w latach 1990-2000. [Cystic echinococcosis in Poznan Region, Poland in the years 1990-2000], Przegl. Epidemiol., 2003, 57, 4, 579-586 (In Polish, English abstract)

[45] Dybicz M., Gierczak A., Dąbrowska J., Rdzanek Ł., Michałowicz B., Molecular diagnosis of cystic echinococcosis in humans from central Poland, Parasitol. Int., 2013, 62, 4, 364-367

[46] Dybicz M., Borkowski P.K., Dąbrowska H., Chomicz L., Cases of *Echinococcus granulosus* Sensu Stricto Isolated from Polish Patients: Imported or Indigenous?, BioMed Res. Int., 2015, Article ID 728321, 5 pp

[47] Sałamatin R., Kaczmarek A., Kowal J., Nosal P., Kornaś S., Cielecka D., et al., Bąblowica jednojamowa w Polsce: *Echinococcus granulosus* czy *Echinococcus canadenensis*? [Cystic echinococcosis in Poland: *Echinococcus granulosus* or *Echinococcus canadensis?*]. In: Proceedings of International Scientifical Conference „Trichinellosis and other parasitic zoonozes associated with the sylvatic environment (October 5-7, 2015, Puławy-Zaborek, Poland), 2015, 24-25 (In Polish)