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Molecular identification of cystic echinococcosis in humans and pigs reveals the presence of both *Echinococcus granulosus* sensu stricto and *Echinococcus canadensis* G6/G7 in the hyperendemic focus of the Republic of Moldova

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Abstract
Cystic echinococcosis is caused by the parasitic species of the complex *Echinococcus granulosus* sensu *lato*. This disease is hyperendemic in the Republic of Moldova. Recent molecular analyses have revealed the exclusive presence of *E. granulosus* sensu stricto in sheep and cattle. Previous reports of prevalence in pigs suggest the potential presence of *Echinococcus canadensis* G6/G7, as this species is also reported in neighboring countries. The presence of cystic echinococcosis in pigs was specifically monitored at the slaughterhouse. In the meantime, human cases were genotyped for the first time. *E. canadensis* G6/G7 was identified in all ten pigs infected by *E. granulosus s.l.* One human case of infection by *E. canadensis* G6/G7 was also identified, while *E. granulosus* sensu stricto was found to be the cause for the 13 others. The description of one human case of *E. canadensis* G6/G7 has confirmed its zoonotic impact in the country. Future studies will be needed to estimate the relative proportion and distribution of both parasitic species in Moldova.

Keywords *Echinococcus granulosus* sensu stricto · *Echinococcus canadensis* G6/G7 · Cystic echinococcosis · Human · Pig

Introduction

Echinococcus granulosus sensu *lato* (*s.l.*) is a complex of parasitic species causing cystic echinococcosis (CE) in humans. This disease is present worldwide and was ranked second among foodborne parasites with the greatest global impact by the UN's Food and Agriculture Organization and the World Health Organization (2014). Humans are infected after orally ingesting eggs of the parasite released into the environment via the feces of infected carnivores following consumption of fertile metacestodes in the viscera of livestock or wildlife species. In humans, CE has a long asymptomatic period during which cysts develop mainly in the liver and lungs. The clinical spectrum ranges from asymptomatic infection to severe and, rarely, even fatal disease (Brunetti et al. 2010).

Of the five species of *E. granulosus s.l.*, *E. granulosus* sensu stricto (*s.s.*) and *E. canadensis* (G6/G7) are of key importance due to their zoonotic potential, representing respectively 88.44% and 11.07% of human CE cases worldwide (Alvarez Rojas et al. 2014). The lifecycles of both species are essentially domestic, with the dog as the main definitive

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host. In Europe, sheep are the main intermediate host for *E. granulosus s.s.*, while pigs are the main one for *E. canadensis* G6/G7. Human CE remains highly endemic in pastoral communities in Eastern European countries and Russia (Brunetti et al. 2010; Dezsenyi et al. 2018; Konyaev et al. 2013). From 1992 to 2001, 323 human cases of CE were recorded in Russia, but the actual figure could be as much as three times higher. Recently, the prevalence of abdominal CE was estimated by a cross-sectional ultrasound-based population study under the HERACLES project (Tamarozzi et al. 2018). The age- and sex-adjusted prevalence in this rural population was estimated as 0.41% in Bulgaria, 0.41% in Romania, and 0.59% in Turkey. The approach used in the HERACLES project revealed that a classical estimate based on hospital data underestimates the actual number of cases by 700 fold. Based on hospital data, a human incidence of 4.3 cases per 100,000 inhabitants per year was estimated in the Republic of Moldova, which considers the disease a high public health priority (Prisacari and Lungu 2014). In the late 1980s, a very high prevalence was reported in slaughtered livestock, 72.6% of sheep, 49.1% of cattle, and 18.2% of pigs being infected (Bondari 1992). A recent slaughterhouse survey concerning 5580 sheep, 1525 cattle, and 12,700 pigs has revealed a similar prevalence estimate, with CE observed in 61.9% of sheep and 59.3% of cattle. No infection, however, was reported in pigs (Chihai et al. 2016). The first molecular characterization of CE in sheep and cattle in the country has revealed the exclusive presence of *E. granulosus s.s.* (Umhang et al. 2014). As infection was previously reported in pigs (Bondari 1992), the presence of *E. canadensis* G6/G7 may be suspected, as this species has already been reported in Eastern European countries such as Romania, Hungary, Poland, and Slovakia (Bart et al. 2006; Bruzinskaite et al. 2009; Casulli et al. 2012; Dybicz et al. 2013; Šnábel et al. 2017; Šnábel et al. 2000; Turčeková et al., 2003).

The fragmentation of the Soviet Union allowed the Republic of Moldova to gain its independence in 1991. As a consequence, the proportion of animals from the public sector, slaughtered with an accompanying veterinary inspection, dropped drastically over a 10-year period from 1990 to 2000 as private backyard home slaughtering—which favors the infection of dogs (Erhan et al. 2001)—rose. Thus, about 84% of cattle and pigs and 40% of sheep and goats in 1990 were slaughtered with a veterinary inspection, compared with 10% and 4% respectively in 2000. From 2001 to 2012, this trend in favor of the private sector continued to increase. Farm type therefore appears to be an important factor in CE infection in Moldova. The private sector's extensive farming mainly combined with frequent home slaughtering resulted in a significantly higher prevalence of CE infection in cattle and sheep compared with that of the public sector, where intensive farming and meat inspection at the slaughterhouse limited the risk of exposure to and transmission of the parasite (Chihai et al. 2016). The intensive indoor farming system of the 12,700 pigs surveyed during the last slaughterhouse survey most probably explains the absence of parasitic infection (Chihai et al. 2016). As the presence of *E. canadensis* G6-G7 may be expected in Moldova, CE cases in pigs potentially infected by this CE species may also be expected in the private sector. Molecular investigations appear to be necessary to obtain a correct overview of CE epidemiology in order to identify not only the main intermediate host species involved but also which *E. granulosus s.l.* species infect humans.

In this study, both human and porcine cases of CE were genotyped for the first time in the country to evaluate the potential presence of *E. canadensis* G6/G7 and its relative zoonotic importance compared with *E. granulosus s.s.*

Material and methods

The presence of CE in pigs was specifically examined on two different days 1 month apart (in May and June 2014), at the abattoir in the Soroca District located at the northern border with Ukraine (Fig. 1). When CE cysts in the liver and/or lungs were suspected during meat inspection, all the cysts were collected and frozen. Concerning CE in humans, one cyst from each of 14 patients (5 males and 9 females) was collected during surgery in 2013 and frozen. All the patients had liver cysts except one, who had peritoneal cysts. The local review board approved the study protocol by following the rules of the National Ethics Commission. Each patient gave informed consent to be included in this study, according to Moldavian rules including anonymization. All the patients, between 29 and 72 years old, were from rural areas in the southern, central, or northern parts of Moldova (Fig. 1).

The fertility of the cysts was assessed by observing the presence of protoscoleces in the hydatid fluid. For pig samples, DNA was extracted from the cyst membrane using the iPrep purification instrument (Invitrogen, iPrep ChargeSwitch gDNATissue Kit). Human CE samples were subjected to disruption using ceramic beads followed by DNA extraction using NucleoSpin® Tissue (Macherey-Nagel, Düren, Germany) as previously described (Zait et al. 2016). A PCR targeting the partial sequence (444 bp) of the mitochondrial *cytochrome c oxidase subunit one* (*cox1*) gene was carried out as previously described (Bowles et al. 1992; Umhang et al. 2013). The PCR products from the pig samples were sequenced by a private company (GENEWIZ), while an ABI Prism 3130 DNA analyzer (Applied Biosystems) was used for human CE samples. The nucleotide sequences were aligned using Vector NTI (Life Technologies), and the sequence similarity to known sequences in the GenBank database was determined using the Basic Local Alignment Search Tool (BLAST) for nucleotide analysis. Previous nucleotide sequences described from sheep and cattle in Moldova (Umhang et al. 2014) were used for comparison with the sequences obtained in this study.

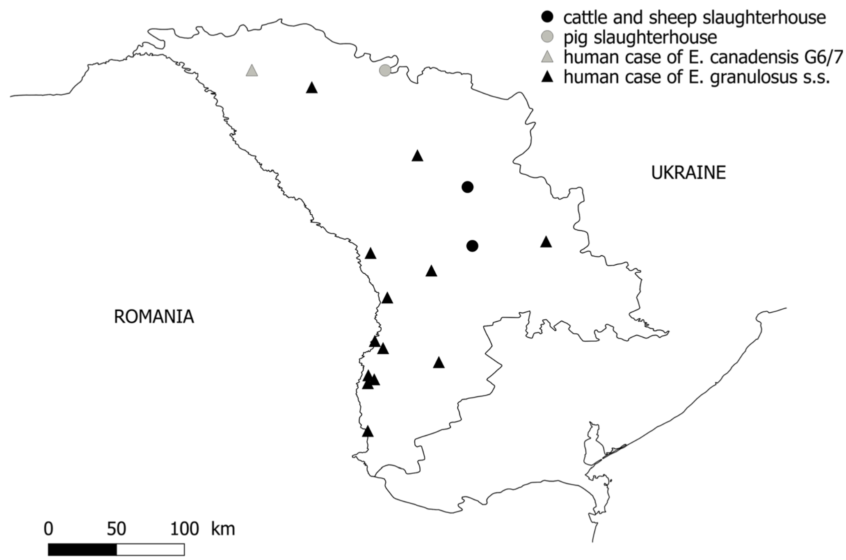

Fig. 1 Geographic origin of *E. granulosus s.l.* samples from humans and livestock at the slaughterhouse included in this study. The sheep and cattle samples were from Umhang et al. (2014)

Results and discussion

The survey of CE in pigs at the slaughterhouse identified 12 pigs with suspicious CE cysts aged from 21 to 46 months. All the infected pigs were from private households except one that came from a public farm. One to 11 cysts per pig were sampled, all from the liver. Two samples from the same pig from a private farm were identified as *Taenia hydatigena*. The partial nucleotide sequences of the *cox1* gene obtained from the other 11 pigs were the same for all the cysts (GenBank MG548754) and corresponded to *E. canadensis* G6/G7. No protoscoleces were observed in any of the 36 cysts examined.

A fertile *E. canadensis* G6/G7 cyst (GenBank MG548752) also infected one female CE patient originating from the North. Its nucleotide sequence was the same as those obtained from the pigs. *E. granulosus s.s.* (GenBank MG548740-MG548751 and MG548753) was systematically identified in the other 13 patients. Protoscoleces were observed in all but one cyst.

The comparison of haplotypes for *E. granulosus* between livestock (sheep, $n = 51$; cattle, $n = 15$) and human cases revealed the presence of 18 different haplotypes due to the presence of 15 points of mutation corresponding only to substitutions. Seven haplotypes (Mol03, Mol04, Mol06, and Mol11 to Mol14) were not previously referenced in GenBank before their description in Moldova. The three main haplotypes (Mol01, Mol10, and Mol16) were grouped in ten to 33 samples from the three intermediate host species. The main haplotype, Mol01 ($n = 33$), corresponds to the founder haplotype of *E. granulosus s.s.* identified in Italy and Eastern European countries (Bulgaria, Hungary, and Romania) (Romig et al. 2015). Eight different haplotypes were identified from the 13 human samples, three of which were not identified in livestock.

The previous description of CE in pigs in Moldova (Bondari 1992), associated with reports of *E. canadensis* G6/G7 in pigs from neighboring countries, gave rise to the suspicion that this *Echinococcus* species was present in Moldova. Nevertheless, only molecular analyses could definitively prove its presence, especially as *E. granulosus s.s.* was also known to frequently infect pigs. In this study, all the CE infections in sampled pigs were due to *E. canadensis* G6/G7. Unfortunately, as the slaughterhouse survey only focused on detecting CE cases in pigs, no information about non-infected pigs was recorded, making it impossible to estimate CE prevalence in this animal species. The detection of CE in pigs, previously free from this parasite when from the public sector, suggests a higher prevalence of *E. canadensis* G6/G7 on farms from the private sector, as already observed for *E. granulosus s.s.* in Moldova (Chihai et al. 2016) and in Hungary (Dán et al. 2018). As for many countries of the former Soviet Union (e.g., Kazakhstan, Kyrgyzstan, and Tajikistan), the abandonment of large collectivized farming systems and the neglect or collapse of public veterinary health services have resulted in an increase in CE prevalence not only among livestock but also among humans (Torgerson 2013). The current geographic distribution of *E. canadensis* G6/G7 in Moldova is unknown, but it may be correlated with pig-breeding on private farms.

No data are currently available concerning the definitive hosts of *E. granulosus s.l.* in the country. The absence of fertile cysts among the 36 cysts from the 11 infected pigs was surprising, but the fertility of *E. canadensis* G6/G7 cysts in pigs is recognized (Cardona and Carmena 2013), so even if it is highly variable, the status of pigs as a main intermediate host is not questioned. The potential infection of wild boar, present throughout the country, also needs to be considered in the future as it may help maintain the parasite's lifecycle with a prevalence similar to that of pigs due to hazardous hunting practices with equally hazardous elimination of viscera (Onac et al. 2013; Umhang et al. 2014).

Despite the small number of CE patients included in this study, the presence of *E. canadensis* G6/G7 was also identified among human cases. This very low proportion of *E. canadensis* G6/G7 among human CE cases was also reported around the south-eastern border with Romania, where one case of *E. canadensis* G6/G7 was identified out of 59 other CE cases caused by *E. granulosus s.s.* (Piccoli et al. 2013). The predominance of *E. granulosus s.s.* complies with the genotyped CE cases published worldwide, where *E. canadensis* appears to be the second most involved species, the others representing only a minor proportion (< 0.5%) (Alvarez Rojas et al. 2014). Today, some differences are observed at clinical level between *E. granulosus s.s.* and *E. canadensis* G6/G7 concerning smaller cysts for *E. canadensis* G6/G7 (Schneider et al. 2010) and an affinity for the brain (Sadjjadi et al. 2013), but these do not have any impact on the management of patients. The most frequent haplotypes of *E. granulosus s.s.* in humans mainly correspond to those identified in livestock. A more global sampling of CE cases in intermediate hosts and humans both in terms of numbers and geographic distribution would probably show that all the haplotypes observed in humans are present in livestock. Considering only the partial *cox1* gene locus (396 bp), many different haplotypes were identified in comparison with the number of samples concerned, arguing for high genetic diversity fostered by the very high prevalence observed for *E. granulosus s.s.* while the same haplotype was reported for all the *E. canadensis* G6/G7 cysts examined. Longer nucleotide sequences targeting different genes are required before any relevant conclusions may be drawn on the genetic diversity of CE in Moldova. All the pig samples and even the one human sample infected by *E. canadensis* G6/G7 came from the North of Moldova. Knowing the infections reported in neighboring countries (Casulli et al. 2012; Onac et al. 2013; Piccoli et al. 2013), it may be useful to investigate the genetic diversity of *E. canadensis* G6/G7 in the Republic of Moldova by analyzing samples from other areas and using longer nucleotide sequences.

The presence of both *E. granulosus s.s.* and *E. canadensis* G6/G7 in Moldova, like in other Eastern European countries, highlights the importance of molecular diagnosis for a correct and precise identification of CE species, which could affect their control, although no concrete evidence of this effect has yet been demonstrated (Craig et al. 2007). Furthermore, countries impacted by huge changes in livestock production, such as those from the former Soviet Union, are of great epidemiological interest for evaluating the evolution of endemic *E. granulosus s.l.* situations. Future studies will be needed to estimate the relative proportion and distribution of both these parasitic species in Moldova.

Compliance with ethical standards The local review board approved the study protocol by following the rules of the National Ethics Commission.

Conflict of interest The authors declare that there is no conflict of interest.

References

Alvarez Rojas CA, Romig T, Lightowlers MW (2014) Echinococcus granulosus sensu lato genotypes infecting humans - review of current knowledge. Int J Parasitol 44:9–18. https://doi.org/10.1016/j.ijpara.2013.08.008

Bart JM, Morariu S, Knapp J, Ilie MS, Pitulescu M, Anghel A, Cosoroaba I, Piarroux R (2006) Genetic typing of Echinococcus granulosus in Romania. Parasitol Res 98(2):130–137. https://doi.org/10.1007/s00436-005-0015-9ISTEX

Bondari L (1992) Echinococcosis in animals in the Republic of Moldova (biology, epizootology, chemotherapy and prevention). PhD thesis, Academy of Sciences of Chisinau, Moldova

Bowles J, Van Knapen F, McManus D (1992) Cattle strain of Echinococcus granulosus and human infection. Lancet 339(8805):1358. https://doi.org/10.1016/0140-6736(92)92003-XISTEX

Brunetti E, Kern P, Vuitton DA (2010) Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop 114(1):1–16. https://doi.org/10.1016/j.actatropica.2009.11.001

Bruzinskaite R, Sarkunas M, Torgerson PR, Mathis A, Deplazes P (2009) Echinococcosis in pigs and intestinal infection with Echinococcus spp. in dogs in southwestern Lithuania. Vet Parasitol 160(3–4):237–241. https://doi.org/10.1016/j.vetpar.2008.11.011

Cardona GA, Carmena D (2013) A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. Vet Parasitol 192(1–3):10–32. https://doi.org/10.1016/j.vetpar.2012.09.027

Casulli A, Interisano M, Sreter T, Chitimia L, Kirkova Z, La Rosa G, Pozio E (2012) Genetic variability of Echinococcus granulosus sensu stricto in Europe inferred by mitochondrial DNA sequences. Infect Genet Evol 12(2):377–383. https://doi.org/10.1016/j.meegid.2011.12.014

Chihai O, Umhang G, Erhan D, Boué F, Talambuta N, Rusu S, Zamornea M (2016) Slaughterhouse survey of cystic echinococcosis in cattle and sheep from the Republic of Moldova. J Helminthol 90(3):279–283. https://doi.org/10.1017/S0022149X15000103

Craig PS, McManus DP, Lightowlers MW, Chabalgoity JA, Garcia HH, Gavidia CM, Gilman RH, Gonzalez AE, Lorca M, Naquira C, Nieto A, Schantz PM (2007) Prevention and control of cystic echinococcosis. Lancet Infect Dis 7(6):385–394. https://doi.org/10.1016/S1473-3099(07)70134-2>

Dán Á, Rónai Z, Széll Z, Sréter T (2018) Prevalence and genetic characterization of Echinococcus spp. in cattle, sheep, and swine in Hungary. Parasitol Res 117(9):3019–3022. https://doi.org/10.1007/s00436-018-5977-5

Dezsenyi B, Somoracz A, Danka J, Kucsera I, Barth TFE, Casulli A (2018) Human cystic echinococcosis in Hungary (2000-2014): a retrospective case series analysis from a single-center study. Infection 46(4):477–486. https://doi.org/10.1007/s15010-018-1146-0

Dybicz M, Gierczak A, Dabrowska J, Rdzanek L, Michalowicz B (2013) Molecular diagnosis of cystic echinococcosis in humans from central Poland. Parasitol Int 62(4):364–367. https://doi.org/10.1016/j.parint.2013.03.005

Konyaev SV, Yanagida T, Nakao M, Ingovatova GM, Shoykhet YN, Bondarev AY, Odnokurtsev VA, Loskutova KS, Lukmanova GI, Dokuchaev NE, Spiridonov S, Alshinecky MV, Sivkova TN, Andreyanov ON, Abramov SA, Krivopalov AV, Karpenko SV, Lopatina NV, Dupal TA, Sako Y, Ito A (2013) Genetic diversity of Echinococcus spp. in Russia. Parasitology 140(13):1637–1647. https://doi.org/10.1017/S0031182013001340

Onac D, Gyorke A, Oltean M, Gavrea R, Cozma V (2013) 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 193(1–3):289–291. https://doi.org/10.1016/j.vetpar.2012.11.044

Piccoli L, Bazzocchi C, Brunetti E, Mihailescu P, Bandi C, Mastalier B, Cordos I, Beuran M, Popa LG, Meroni V, Genco F, Cretu C (2013) Molecular characterization of Echinococcus granulosus in south-eastern Romania: evidence of G1–G3 and G6–G10 complexes in humans. Clin Microbiol Infect 19(6):578–582. https://doi.org/10.1111/j.1469-0691.2012.03993.x

Prisacari V, Lungu V (2014) Human cystic echinococcosis in the republic of Moldova - an epidemiological study. Curierul Medical 57(1):59–65

Romig T, Ebi D, Wassermann M (2015) Taxonomy and molecular epidemiology of Echinococcus granulosus sensu lato. Vet Parasitol 213(3–4):76–84. https://doi.org/10.1016/j.vetpar.2015.07.035

Sadjjadi SM, Mikaeili F, Karamian M, Maraghi S, Sadjjadi FS, Shariat-Torbaghan S, Kia EB (2013) Evidence that the Echinococcus granulosus G6 genotype has an affinity for the brain in humans. Int J Parasitol 43:875–877. https://doi.org/10.1016/j.ijpara.2013.06.008

Schneider R, Gollackner B, Schindl M, Tucek G, Auer H (2010) Echinococcus canadensis G7 (pig strain): an underestimated cause of cystic echinococcosis in Austria. Am J Trop Med Hyg 82(5):871–874. https://doi.org/10.4269/ajtmh.2010.09-0639

Šnábel V, D'Amelio S, Mathiopoulos K, Turčeková L, Dubinský P (2000) Molecular evidence for the presence of a G7 genotype of Echinococcus granulosus in Slovakia. J Helminthol 74(2):177–181. https://doi.org/10.1017/S0022149X00000251ISTEX

Šnábel V, Calma C, Georgescu SO, Cavallero S, D'Amelio S, Vasilkova Z, Ito A (2017) Genetic survey of alveolar and cystic echinococcoses in Romania: first molecular evidence of Echinococcus multilocularis in humans in the country. Helminthologia 54(3):189–198. https://doi.org/10.1515/helm-2017-0025

Torgerson PR (2013) The emergence of echinococcosis in central Asia. Parasitol 140(13):1667–1673. https://doi.org/10.1017/S0031182013000516

Turčeková L, Šnábel V, D'Amelio S, Busi M, Dubinský P (2003) Morphological and genetic characterization of Echinococcus granulosus in the Slovak Republic. Acta Trop 85(2):223–229. https://doi.org/10.1016/S0001-706X(02)00229-2

Umhang G, Richomme C, Boucher JM, Hormaz V, Boué F (2013) Prevalence survey and first molecular characterization of Echinococcus granulosus in France. Parasitol Res 112(4):1809–1812. https://doi.org/10.1007/s00436-012-3245-7ISTEX

Umhang G, Chihai O, Boué F (2014) Molecular characterization of Echinococcus granulosus in a hyperendemic European focus, the Republic of Moldova. Parasitol Res 113(12):4371–4376. https://doi.org/10.1007/s00436-014-4112-5

Zait H, Kouidri M, Grenouillet FE, Umhang G, Millon L, Hamrioui B, Grenouillet F (2016) Molecular characterization of Echinococcus granulosus sensu stricto and Echinococcus canadensis in humans and livestock from Algeria. Parasitol Res 115(6):2423–2431. https://doi.org/10.1007/s00436-016-4994-5