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Genetic characterisation of *Echinococcus granulosus* sensu lato infecting ruminants in the Republic of North Macedonia

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ABSTRACT: This study aimed to characterize *Echinococcus* genotypes infecting ruminants in the Republic of North Macedonia and estimate their epidemiological significance and possible public health implications. Samples from 69 ruminants were collected between 2021 and 2022 from various locations across the Republic of North Macedonia, including Vinica, Sv. Nikole, Kichevo, Krushevo, Prilep, Skopje, Bogdanci, Mavrovo, Debar, Shtip, and Kumanovo. One cyst per animal was analyzed for genetic diversity by amplifying and sequencing the cytochrome c oxidase 1 (*cox1*) mitochondrial gene. Using the neighbor-joining method, phylogenetic analysis of the sequenced data was performed using MAFFT for sequence alignment, trimming, and tree construction with MEGAX. Phylogenetic analysis identified two major genotypes: G1 and G3. Among the 61 sheep samples, 38 (62.3%) were identified as G1, and 23 (37.7%) as G3. In cattle, 4 out of 8 isolates (50%) were G1, and the remaining 4 (50%) were G3. This study provides the first molecular characterization of *Echinococcus granulosus* sensu stricto (s.s.) genotypes (G1 and G3) in ruminants from the Republic of North Macedonia. The predominance of these highly zoonotic genotypes highlights significant public health risks and underscores the urgent need for integrated surveillance programs targeting both animal and human populations. These findings provide essential baseline data for implementing targeted control strategies in this endemic region.

Keyword: genotyping; echinococcosis; cox 1; cattle; sheep

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INTRODUCTION

Echinococcosis is a significant zoonotic parasitic disease hyperendemic in Asia, Africa, South America, and Australia. It is also present in the Mediterranean, Eastern European, and Balkan regions (Deplazes et al., 2017), posing a considerable threat to human and animal health. The etiologic agent of the disease is the larval stage of cestode parasites from the genus *Echinococcus*, which can cause either cystic or alveolar echinococcosis (Thompson, 2017; Haleem et al., 2018). Intermediate hosts, including wild and domestic ungulates, acquire the parasite by ingesting eggs shed by definitive hosts, primarily canids, which harbor adult tapeworms. As accidental dead-end hosts, humans become infected through environmental exposure to eggs, often via contaminated food, water, or direct contact with infected animals (Casulli et al., 2019).

The *Echinococcus* genus is divided into several species: *Echinococcus granulosus* sensu lato (s.l.), *Echinococcus multilocularis*, *Echinococcus shiquicus*, *Echinococcus vogeli*, and *Echinococcus oligarthra*. The *Echinococcus granulosus* (s.l.) group is genetically diverse and contains several strains based on mitochondrial DNA nucleotide sequence alterations. The strains include the common *Echinococcus granulosus* sensu stricto (s.s.) (G1-G3, with G2 as a microvariant of G3), *Echinococcus equinus* (G4, previously the horse strain), *Echinococcus ortleppi* (G5, previously the cattle strain), *Echinococcus felidis* (previously the lion strain), and *Echinococcus canadensis* (G6, previously the camel strain; G7, previously the pig strain; G8 and G10, cervid strains). G9 has recently been classified as a microvariant of G7 (Kinkar et al., 2017; Kinkar et al., 2018; Thompson, 2020; Khan et al., 2025). G1 (the sheep type) is the prevailing genotype in domestic animals globally, reportedly linked to the transmission cycle between domestic herbivores and dogs in endemic regions (Cucher et al., 2016; Kinkar et al., 2018; Bonelli et al., 2020).

Cystic echinococcosis is mainly caused by Echinococcus granulosus (s.s.), especially in areas where sheep farming is important for the economy. Echinococcus granulosus (s.s.) has long been documented to affect humans and animals, causing cystic echinococcosis in Mediterranean and Balkan countries, where the parasite has circulated actively for over 20 years (Casulli et al., 2022). Such geographic regions are also reported to have high prevalence rates of echinococcosis in the domestic intermediate host, with studies reporting infection rates of 0.1%–10.5% in France (Umhang et al., 2020), 75% in Italy (Bosco et al., 2021), 17% in Turkey (Küçükyağlıoğlu and Uslu, 2022), and 30.4–53.8% in Greece (Chaligiannis et al., 2015). As for molecular epidemiology studies from surrounding countries, genotypes G1, G3, and G7 were identified in Bosnia and Herzegovina, followed by findings from Serbia and Greece (Debeljak et al., 2016; Roinioti et al., 2016; Hodžić et al., 2022). Genotypes G1 and G3 were detected in Romania and Italy, whereas genotype G1 was detected only in Bulgaria (Breyer et al., 2004; Dărăbuș et al., 2022; Bonelli et al., 2024).

Our earlier study in the Republic of North Macedonia documented a 60% prevalence of echinococcosis in slaughtered cattle and sheep and confirmed the endemicity of the disease in the country (Rashikj et al., 2022). However, molecular and phylogenetic analyses that are important for understanding the complex genetic diversity of the ruminant-infecting parasite were not conducted. Understanding local ruminant population diversity and transmission patterns requires identifying circulating genotypes, which can facilitate the implementation of alternative control strategies (Budke et al., 2017; Jesudoss Chelladurai et al., 2024). In line with this, the present study aimed to generate the first set of genotype-specific data on *Echinococcus granulosus* (s.l.) in the country, emphasizing the intraspecific variability and zoonotic potential. Identifying circulating genotypes is essential for understanding local transmission dynamics and assessing human infection risks. The data generated will improve understanding of the epidemiology of *Echinococcus granulosus* (s.l.) in the region and directly inform evidence-based public health interventions to reduce transmission between livestock, dogs, and humans.

MATERIALS AND METHODS

Sample Collection

Ethical approval was not required as offal samples were obtained with slaughterhouse consent from animals processed for consumption during routine procedures without experimental intervention. Post-mortem examinations of cystic lesions in organs were conducted on 849 domestic sheep and cattle at various slaughterhouses across the Republic of North Macedonia between April 2021 and July 2022. A subset of 69 animals was selected for genetic analysis (Figure 1). Infected organs were transferred to the Laboratory of Parasitology and Parasitic Dis-

Figure 1. Geographical distribution of the tested animals in the Republic of North Macedonia.

eases in the Faculty of Veterinary Medicine in Skopje, where they were dissected and examined as previously described (Rashikj et al., 2022). Sixty-nine cysts were randomly selected from 61 sheep and 8 cattle, excluding damaged or unsuitable cysts based on size or condition. One cyst per animal was examined. Selecting only one cyst per infected animal reduced the possibility of detecting multiple genotypes within the same host. Yet, this approach was chosen to maintain sample consistency and facilitate more precise genetic characterization. Cyst contents were examined by a light microscope for protoscoleces after puncturing the cyst and aspirating fluid. In cysts free of protoscoleces, the germinal layer was harvested. The protoscoleces and/or germinal layers were extracted from each cyst and preserved in 70% ethanol for subsequent molecular analysis. The collected materials were stored at -20°C until DNA extraction.

DNA Extraction and PCR

Nucleic acid extraction from protoscoleces and/or the germinal layer was performed on a KingFisher™ Flex Purification System (Thermo Fisher Scientific, Waltham, MA, USA) using the MagMax Core Nucleic Acid Extraction Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Nucleic acid quality and concentration were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). A specific set of primers (forward primer: 5'-TTT.TTT.GGC.CAT.CCT.GAG.GTT.TAT-3' and reverse primer: 5'-TAA.CGA.CAT.AAC.ATA.ATG.AAA.ATG-3') were used to amplify a 460-bp fragment of the mitochondrial cytochrome c oxidase 1 (*cox1*) gene (Bart et al., 2006). The total volume of the PCR mixture contained 25 µl of AmpliTaq Gold 360 Mastermix 2x (Applied Biosystems, Foster City, CA, USA), 0.6 µM of each primer, 3 µl of nucleic acid extract, and DNase/RNase-free water to obtain a final volume of 50 µl. The amplification was performed using SimpliAmp thermal cycler instrument (Applied Biosystems, Foster City, CA, USA) with the following conditions: initial denaturation at 95°C for 5 min, 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, followed by a final extension at 72°C for 7 min. Electrophoresis of PCR products on 1.5% agarose gels was followed by purification using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA).

Sequencing and Phylogenetic Analysis

Amplicons were obtained for sequencing at LGC Genomics (Germany), and both forward and reverse strand sequences were determined. The nucleotide sequences from this work were submitted to GenBank. Consensus sequences were obtained using the Staden 2.0 software package. The resulting sequences were compared against reference and closely related sequences retrieved from the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). MAFFT v.7 was used to perform multiple sequence alignment. MEGAX was used for trimming and phylogenetic analysis (Kumar et al., 2018). The phylogenetic tree was inferred using the neighbor-joining method (Saitou and Nei, 1987), with 1000 bootstrap replicates taken (Felsenstein, 1985). Evolutionary distances were computed using the Jukes-Cantor model (Jukes and Cantor, 1969).

Table 1. Distribution of *Echinococcus granulosus* (s.s.) genotypes in sheep and cattle in the Republic of North Macedonia

Host Species	Number of Isolates	G1 Genotype	G3 Genotype
Sheep	61	38 (62.3%)	23 (37.7%)
Cattle	8	4 (50%)	4 (50%)

Statistical Analysis

Descriptive statistics were performed to estimate the prevalence of the disease. Prevalence was calculated as the proportion of positive cases out of the total number of tested animals. The 95% confidence intervals (CIs) for genotype proportions were calculated using the standard normal approximation method for binomial proportions in Microsoft Excel.

RESULTS

Among the 61 sheep isolates, 38 (62.3%, 95% CI: 49.7% - 73.5%) were G1, and 23 (37.7%, 95% CI: 26.5% - 50.3%) were G3. Both genotypes were evenly represented in cattle, with 4 of 8 isolates (50%, 95% CI: 21.8% - 78.2%) identified as G1 and the other 4 (50%, 95% CI: 21.8% - 78.2%) as G3 (Table 1).

The geographical distribution of the identified G1 and G3 genotypes across the sampled regions of the Republic of North Macedonia is presented in Figure 2.

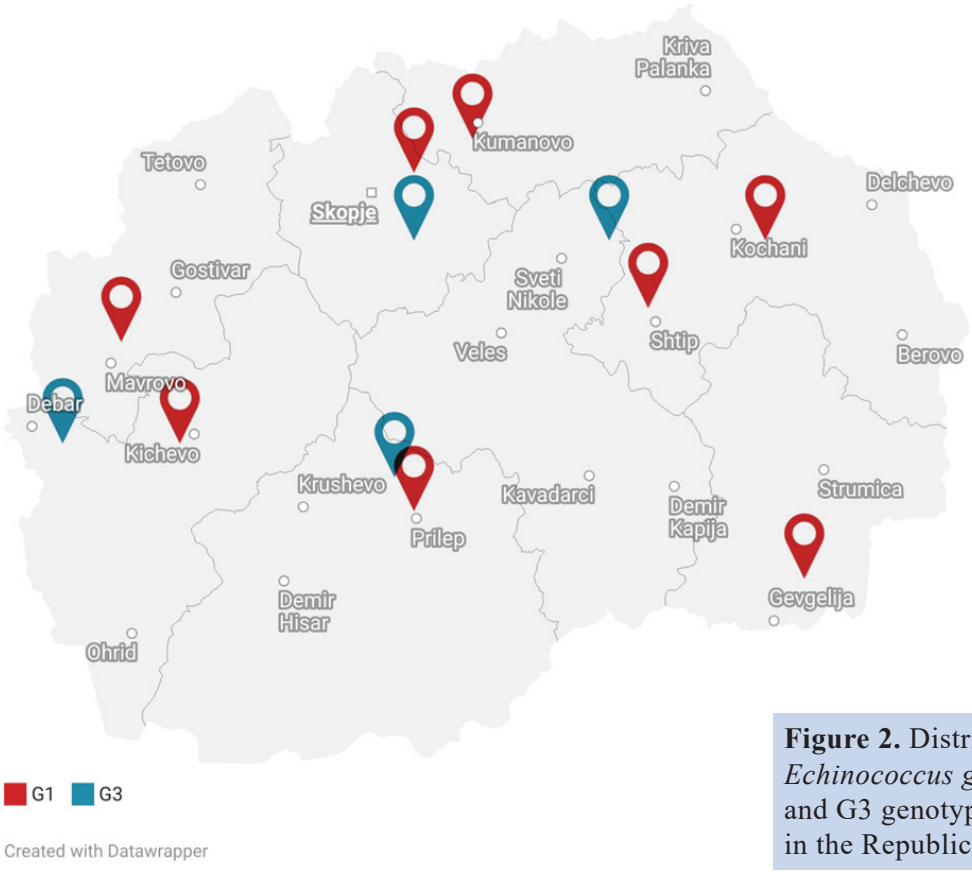

Figure 2. Distribution of *Echinococcus granulosus* (s.s.) G1 and G3 genotypes in cattle and sheep in the Republic of North Macedonia.

Nucleotide sequences for the *cox1* gene have been deposited at GenBank with the accession numbers PV094083–PV094140. Phylogenetic analyses showed that the clade samples were closely related to the *Echinococcus granulosus* s.s. (G1–G3) complex (Figure 3). Sequence PV094083 (MKD1 22 - internal laboratory identification number assigned to each sample for tracking and reference purposes) was grouped with sequences from Greece, Italy, Turkey, and Spain into the G1 clade, which had a low bootstrap support of 38. Sequence cluster analysis: The sequences PV094131 (MKD126 21), PV094133 (MKD132 21), and PV094128 (MKD85 21) were grouped with sequences from Albania and Romania in a subgroup of a larger clade, but with a moderate bootstrap value of 46. In addition, sequences PV094106 (MKD18 21) and PV094104 (MKD13 22) clustered with the same larger clade (moderate support, bootstrap 51). The PV094136 (MKD149 21), PV094139 (MKD159 21), and PV094092 (MKD6 21) sequences clustered with members of the G3 genotype clade with a bootstrap value of 99 (very high support). BLAST analysis confirmed that the sequences above belong to *Echinococcus granulosus* (s.s.), the only species of the genus infecting ruminants in the Republic of North Macedonia.

DISCUSSION

This study provides the first molecular evidence of *Echinococcus granulosus* (s.s.) infection in ruminants in the Republic of North Macedonia, using the *cox1* gene sequencing, a well-established marker for differentiating genotypes (Raissi et al., 2021). The findings confirm that *E. granulosus* (s.s.) is the only species circulating in the country. G1 emerged as the dominant genotype, found in 38 of 61 sheep and 4 of 8 cattle isolates, while G3 was identified in 23 sheep and 4 cattle. This distribution reflects a

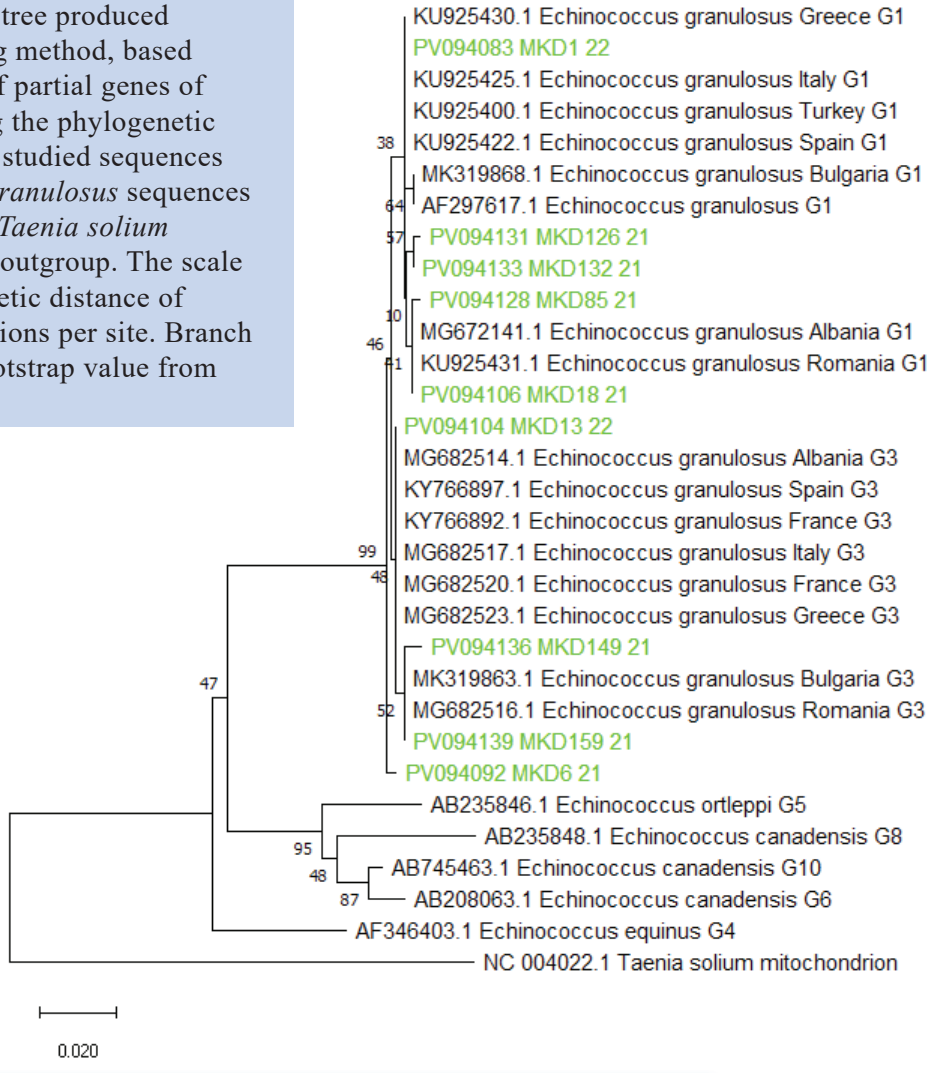

Figure 3. A phylogenetic tree produced using the neighbor-joining method, based on combined sequences of partial genes of cox 1 (460bp), illustrating the phylogenetic relationships between the studied sequences and other *Echinococcus granulosus* sequences retrieved from GenBank. *Taenia solium* sequence was used as the outgroup. The scale bar represents a phylogenetic distance of 0.020 nucleotide substitutions per site. Branch numbers represent the bootstrap value from 1000 replicated datasets.

broader regional pattern also reported in neighboring Balkan countries, including Serbia, Bosnia and Herzegovina, Greece, and Bulgaria, where shared livestock management practices, cross-border animal movement, and suitable environmental conditions support parasite persistence (Breyer et al., 2004; Debeljak et al., 2016; Roinioti et al., 2016; Hodžić et al., 2022). These results fill a key epidemiological gap and highlight the importance of coordinated control strategies across the region.

Although G3 has shown higher prevalence in some areas (Espinoza et al., 2014), our study found both G1 and G3 equally represented in cattle. However, the limited cattle sample size (n = 8) restricts broader conclusions about genotype distribution in this host. The absence of genotype G7 may also be attributed to this limited sampling, as G7 has been reported in cattle and other intermediate hosts in nearby regions (Hodžić et al., 2022). G1 remains the globally dominant zoonotic genotype (Romig et al., 2015), while G3 is less frequently detected (Kinkar et al., 2018). Both genotypes carry significant public health implications (Casulli et al., 2022), and their presence in livestock suggests ongoing transmission risk, especially via the sheep–dog cycle. Genotypic data can thus guide targeted interventions, such as dog deworming and livestock vaccination, to disrupt these cycles.

Phylogenetic analysis showed that G1 sequences from the Republic of North Macedonia clustered with those from Greece, Italy, and Turkey, though with low bootstrap support, suggesting regional transmission links. In contrast, G3 sequences formed a well-supported cluster with samples from Albania, France, and Spain (bootstrap value 99), indicating limited genetic diversity and possible dissemination through livestock trade (Nakao et al., 2010; Hodžić et al., 2022). These molecular findings reinforce the need for regional cooperation in surveillance and control.

The widespread detection of G1 and G3 genotypes is consistent with known high-risk factors in the region, including stray dog populations, poor slaughter practices, lack of regular deworming, and environmental contamination (Torgerson and Budke, 2003; Bosco et al., 2021). As definitive hosts, dogs shed eggs that remain viable in the environment for extended periods, posing risks to livestock and humans. Mitigation strategies such as proper offal disposal, public education on hygiene practices, and routine deworming are essential (Budke et al., 2017). Long-term pasture contamination and inadequate animal management also sustain transmission (Eckert et al., 2001; Khan et al., 2025).

Cystic echinococcosis is a serious public health concern, often requiring prolonged treatment and leading to significant economic losses. The World Health Organization classifies it as a major foodborne parasitic disease (Hodžić et al., 2022). Transmission to humans occurs mainly through contact with infected dogs or contaminated environments (Alvarez Rojas et al., 2018), and anthelmintic treatment of dogs has proven effective in reducing infection risk (Larrieu et al., 2019). In the Republic of North Macedonia, where close contact between humans and dogs is common, integrated veterinary and public health initiatives are needed to mitigate risk. Given the epidemiological complexity of the Balkans, regional coordination in surveillance and control, including monitoring of definitive hosts and potential wildlife reservoirs, is essential for long-term impact (Hodžić et al., 2022).

While this study analyzed only one cyst per animal to maintain sampling consistency, this approach may have missed mixed-genotype infections. Such cases have been documented in intermediate hosts, including humans (Oudni-M'rad et al., 2016; Nungari et al., 2019), and could provide insights into recombination and co-infection dynamics. Future studies should expand host diversity and sample size to better characterize the genetic structure of circulating parasite populations.

CONCLUSIONS

In this study, we identified the *Echinococcus granulosus* (s.s.) genotypes (G1 and G3) in cattle and sheep from the Republic of North Macedonia by combining PCR-based amplification of the *cox1* gene with phylogenetic analysis. The G1 genotype was the most prevalent among sheep, whereas cattle host both genotypes in equal proportions. However, the smaller sample size of cattle limits definitive conclusions about prevalence in this host species. Despite methodological limitations, including the analysis of single cysts per animal and regional sampling constraints, our findings provide the first molecular evidence of these highly zoonotic genotypes circulating in livestock in the Republic of North Macedonia. The genetic similarities with isolates from neighboring countries suggest regional transmission patterns that have implications for cross-border control efforts. These data highlight the significant zoonotic risks

posed by both genotypes and provide a scientific foundation for the development and implementation of integrated surveillance and control programs targeting the complete transmission cycle involving livestock, dogs, and humans in the Republic of North Macedonia.

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REFERENCES

Alvarez Rojas CA, Mathis A, Deplazes P (2018) Assessing the contamination of food and the environment with Taenia and *Echinococcus* eggs and their zoonotic transmission. Curr Clin Microbiol Rep 5:154–163.

Bart JM, Morariu S, Knapp J, Ilie MS, Pitulescu M, Anghel A, et al. (2006) Genetic typing of *Echinococcus granulosus* in Romania. Parasitol Res 98(2):130–137.

Bosco A, Alves LC, Cociancic P, Amadesi A, Pepe P, Morgoglione ME, et al (2021) Epidemiology and spatial distribution of *Echinococcus granulosus* in sheep and goats slaughtered in a hyperendemic European Mediterranean area. Parasit Vectors 14(1):421. Bonelli P, Loi F, Cancedda MG, Peruzzu A, Antuofermo E, Pintore E, et al (2020) Bayesian analysis of three methods for diagnosis of cystic echinococcosis in sheep. Pathogens 9(10):1–9.

Bonelli P, Serra E, Dei Giudici S, Peruzzu A, Crotti S, Danesi P, et al (2024) Molecular phylogenetic analysis of *Echinococcus granulosus* sensu lato infecting sheep in Italy. Acta Trop 252:105782.

Breyer I, Georgieva D, Kurdova R, Gottstein B (2004) *Echinococcus granulosus* strain typing in Bulgaria: The G1 genotype is predominant in intermediate and definitive wild hosts. Parasitol Res 93(2):127–130.

Budke CM, Casulli A, Kern P, Vuitton DA (2017) Cystic and alveolar echinococcosis: Successes and continuing challenges. PLoS Negl Trop Dis 11(4):e0005363.

Casulli A, Siles-Lucas M, Tamarozzi F (2019) *Echinococcus granulosus* sensu lato. Trends Parasitol 35(8):663–664.

Casulli A, Massolo A, Saarma U, Umhang G, Santolamazza F, Santoro A (2022) Species and genotypes belonging to *Echinococcus granulosus* sensu lato complex causing human cystic echinococcosis in Europe (2000–2021): A systematic review. Parasit Vectors 15(1):109. Chaligiannis I, Maillard S, Boubaker G, Spiliotis M, Saratsis A, Gottstein B, et al (2015) *Echinococcus granulosus* infection dynamics in livestock of Greece. Acta Trop 150:64–70.

Cucher MA, Macchiaroli N, Baldi G, Camicia F, Prada L, Maldonado L, et al (2016) Cystic echinococcosis in South America: Systematic review of species and genotypes of *Echinococcus granulosus* sensu lato in humans and natural domestic hosts. Trop Med Int Health 21(2):166– 175.

Dărăbuș G, Buşe A, Oprescu I, Morariu S, Mederle N, Ilie M, et al (2022) Investigation on descriptive epidemiology, geographical distribution, and genotyping of *Echinococcus granulosus* s.l. in bovine from Romania. Vet Sci 9(12):678.

Debeljak Z, Boufana B, Interisano M, Vidanovic D, Kulisic Z, Casulli A (2016) First insights into the genetic diversity of *Echinococcus granulosus* sensu stricto (s.s.) in Serbia. Vet Parasitol 223:57–62.

Deplazes P, Rinaldi L, Alvarez Rojas CA, Torgerson PR, Harandi MF, Romig T, et al (2017) Global distribution of alveolar and cystic echinococcosis. Adv Parasitol 95:315–493.

Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, World Health Organization (2001) WHO/OIE manual on echinococcosis in humans and animals: A public health problem of global concern. World Organisation for Animal Health. Accessed March 2021.

Espinoza S, Salas AM, Vargas A, Freire V, Diaz E, Sánchez G, et al. (2014) Detection of the G3 genotype of *Echinococcus granulosus* from hydatid cysts of Chilean cattle using *COX1* and ND1 mitochondrial markers. Parasitol Res 113(1):139–147.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution (N Y) 39(4):783–791.

Haleem S, Niaz S, Qureshi NA, Ullah R, Alsaid MS, Alqahtani AS, et al (2018) Incidence, risk factors, and epidemiology of cystic echinococcosis: A complex socioecological emerging infectious disease in Khyber Pakhtunkhwa, province of Pakistan. Biomed Res Int. 5042430:15.

Hodžić A, Alić A, Spahić A, Harl J, Beck R (2022) Genetic diversity of *Echinococcus granulosus sensu* lato from animals and humans in Bosnia and Herzegovina. Parasit Vectors 15(1).

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Mammalian Protein Metabolism. Elsevier, pp 21–132.

Jesudoss Chelladurai JRJ, Quintana TA, Johnson WL, Schmidt C, Righter D, Howey E (2024) Cystic echinococcosis in cattle and sheep caused by *Echinococcus granulosus* sensu stricto genotypes G1 and G3 in the USA. Parasit Vectors 17(1):128.

Khan S, Cable J, Masud N, Hailer F, Younus M, Hussain N, et al (2025) Epidemiological and genotypic assessment of cystic echinococcosis in ruminant populations of Northern Punjab, Pakistan: A neglected zoonotic disease. Parasitol Res 124(1):7.

Kinkar L, Laurimäe T, Sharbatkhori M, Mirhendi H, Kia EB, Ponce-Gordo F, et al. (2017) New mitogenome and nuclear evidence on the phylogeny and taxonomy of the highly zoonotic tapeworm *Echinococcus granulosus* sensu stricto. Infect Genet Evol 52:52–58.

Kinkar L, Laurimäe T, Acosta-Jamett G, Andresiuk V, Balkaya I, Casulli A, et al. (2018a) Distinguishing *Echinococcus granulosus* sensu stricto genotypes G1 and G3 with confidence: A practical guide. Infect Genet Evol 64:178–184.

Kinkar L, Laurimäe T, Acosta-Jamett G, Andresiuk V, Balkaya I, Casulli A, et al. (2018b) Global phylogeography and genetic diversity of the zoonotic tapeworm *Echinococcus granulosus* sensu stricto genotype G1. Int J Parasitol 48(9):729–742.

Kinkar L, Laurimäe T, Balkaya I, Casulli A, Zait H, Irshadullah M, et al. (2018c) Genetic diversity and phylogeography of the elusive, but epidemiologically important *Echinococcus granulosus* sensu stricto genotype G3. Parasitol 145(12):1613–1622.

Küçükyağlıoğlu A, Uslu U (2022) Prevalence and economic significance of hidatidosis in cattle slaughtered at an abattoir in Konya, Turkey. Turkiye Parazitoloji Dergisi 46(3):207–212.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549.

Larrieu E, Gavidia CM, Lightowlers MW (2019) Control of cystic echinococcosis: Background and prospects. Zoonoses Public Health 66:889–899.

Nakao M, Yanagida T, Okamoto M, Knapp J, Nkouawa A, Sako Y, et al. (2010) State-of-theart *Echinococcus* and *Taenia*: Phylogenetic taxonomy of human-pathogenic tapeworms and its application to molecular diagnosis. Infect Genet Evol 10(4):444–452.

Nungari L, Mbae C, Gikunju J, Mulinge E, Kaburu T, Zeyhle E, Magambo J. (2019) Prevalence and genotyping of *Echinococcus* species from livestock in Kajiado County, Kenya. Biomed Res Int 2019:4798906.

Oudni-M'rad M, M'rad S, Ksia A, Lamiri R, Mekki M, Nouri A, et al. (2016) First molecular evidence of the simultaneous human infection with two species of *Echinococcus granulosus* sensu lato: *Echinococcus granulosus* sensu stricto and *Echinococcus canadensis*. Parasitol Res 115:1065–1069.

Raissi V, Etemadi S, Sohrabi N, Raiesi O, Shahraki M, Salimi-Khorashad A, et al. (2021) Molecular characterization and phylogeny of

Taenia hydatigena and *Echinococcus granulosus* from Iranian sheep and cattle based on *COX1* gene. Curr Microbiol 78(4):1202–1207.
Roinioti E, Papathanassopoulou A, Theodoropoulou I, Simsek S, Theodoropoulos G (2016) Molecular identification of *Echinococcus granulosus* isolates from ruminants in Greece. Vet Parasitol 226:138–144.
Romig T, Ebi D, Wassermann M (2015) Taxonomy and molecular epidemiology of *Echinococcus granulosus* sensu lato. Vet Parasitol 213(3–4):76–84.
Rashikj L, Cvetkovikj A, Nikolovski M, Cvetkovikj I, Stefanovska J (2022) Cystic echinococcosis in slaughtered cattle and sheep from Republic of North Macedonia. Mac Vet Rev 45(1):35–41.
Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–425.
Thompson RC (2017) Biology and systematics of *Echinococcus*. Adv Parasitol 95:65–109. Thompson RC (2020) The molecular epidemiology of *Echinococcus* infections. Pathogens 9:1–9.
Torgerson PR, Budke CM (2003) Echinococcosis – an international public health challenge. Res Vet Sci 74(3):191–202.
Umhang G, Richomme C, Bastid V, Boucher JM, Peytavin De Garam C, Itié-Hafez S, et al (2020) National survey and molecular diagnosis of *Echinococcus granulosus* sensu lato in livestock in France, 2012. Parasitology 147(6):667–672.