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Original article

DISTRIBUTION OF *ECHINOCOCCUS GRANULOSUS* G1 AND G3 GENOTYPE AMONG THE CATTLE IN WEST-KAZAKHSTAN REGION

Nurasyl Manapov[1*], Rabiga Uakhit[2,3], Sanzhar Zholaman[4], Ainura Smagulova[5], Lyudmila Lider[2,3], Vladimir Kiyan[2]

[1] National School on Agronomy and Food Industry (ENSAIA), University of Lorraine, 2 Avenue de la Forêt de Haye 54505 Vandoeuvre-lès-Nancy, France
[2] Laboratory of Biodiversity and Genetic Resources, National Center for Biotechnology, 13/5 Kurgalzhynskoye road, Astana, 010000, Kazakhstan.
[3] Laboratory of Parasitology, Department of Veterinary Medicine, S. Seifullin Kazakh Agrotechnical Research University, 62 Zhenis avenue, Astana, Kazakhstan
[4] Nazarbayev Intellectual School of Physics and Mathematics of Astana, 21 Hussein bin Talal, Astana, Kazakhstan
[5] Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences Krasnoobsk, Novosibirsk region, Russia

*Corresponding Author: Manapov N., Manapovbio@gmail.com.

ABSTRACT

This research focused on investigating the prevalence of echinococcosis in the West Kazakhstan region, particularly in livestock. A total of 55 samples were analyzed, leading to the identification of two genotypes, G1 and G3, reaffirming previous findings regarding the presence of the G3 genotype exclusively in the Aktobe region. The study revealed a high occurrence of *Echinococcus granulosus* sensu stricto (G1) in livestock animals. Phylogenetic analysis showed a strong similarity to reference samples of *E. granulosus* from the GenBank database, validating the species identification. The study also emphasized the urgent need for attention and action due to the alarming impact of echinococcosis on livestock, as well as its potential health risks to humans. Furthermore, the research shed light on the social and economic implications of echinococcosis, highlighting the importance of public awareness and education, effective control measures, and multi-faceted approaches involving community engagement, public health initiatives, and agricultural policies to address the issue.

Key words: *Echinococcus granulosus,* genotypes, phylogenetic analysis, livestock, health risks

1. INTRODUCTION

Cystic echinococcosis (CE) is a persistent parasitic disease found globally. CE impacts a wide range of animal species that serve as intermediate hosts, while humans are considered abnormal intermediate hosts [1].

Echinococcus parasites need carnivores as definitive hosts and herbivores as intermediate hosts to finish their life cycle. The adult stage lives in the small intestine of the carnivore, producing hundreds of worms that release thousands of infectious eggs in the host's feces. Once consumed by the intermediate host, the larvae travel through the bloodstream to internal organs, forming fluid-filled cysts. These cysts can produce thousands of protoscolices, which can develop into adult worms if consumed by a definitive host. Humans are accidental hosts and do not perpetuate the life cycle.

As of now, the *E. granulosus* complex comprises five species: *E. granulosus* sensu stricto (s.s.) (including genotypes G1, G2, and G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (comprising genotypes G6-G8 and G10), and *E. felidis* (G9) [2-5]. *E. granulosus* s.l. displays a high genetic diversity with 10 genotypes (G1-G10) that have been molecularly differentiated so far, mainly based on genetic variations in mitochondrial (mt) genes [6, 7]. *E. granulosus* s.s. (genotypes G1, G3) is the most widespread species of the E. granulosus s.l. complex [8, 9].

Limited information exists on the molecular identification of genotypes of the *E. granulosus* complex circulating in Kazakhstan. Overall, our aim in this research was to molecularly identify the genotypes of the *E. granulosus* complex infecting cattle in the West-Kazakhstan region.

2. MATERIALS AND METHODS

Echinococcus cyst sample collection

The study defined an isolate as one hydatid cyst from each individual carcass. During the period, 55 isolates were collected from cattle in the West-Kazakhstan region (51°14′N 51°22′E) during one year (June 2022 – August 2024) during collection and visits to slaughterhouses in the West-Kazakhstan region. Isolates were preserved in ethanol (70% v:v) for further DNA extraction. Work with samples of echinococcus cyst of sheep was carried out in the parasitological laboratory of the Faculty of Veterinary Medicine and was approved by the Animal Ethics Committee of the National Center for Biotechnology (extract from protocol No. 1 dated April 01, 2022). All procedures were in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki) for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legal_en.htm).

2.1 Molecular analysis

The cysts exhibited a range of conditions, with some showing calcification and others containing pus. Consequently, we selected the most appropriate samples for ad-

ditional research and analysis. DNA was extracted using a GeneJet genomic DNA purification kit (Thermo Fisher, Cat.: K0701) with modifications. Briefly, extracted from organs, echinococcus cysts were homogenized in the Eppendorf tube using pestle, lyses buffer and Proteinase K.

Polymerase chain reaction (PCR) was applied to identify a fragment of the cox1 gene (446 bp) was amplified using the primer pairs previously used by Bowles et al. [10]. Reaction were performed in 15 µL 2^X GoTaq Hot Start Master-Mix, 9 µL nuclease-free water, 1 µL total primers, and 2.5 µL extracted DNA.

Following 1.0% agarose gel electrophoresis, PCR products were observed as single bands. The PCR-amplified target gene fragment was purified using a QIAquick PCR Purification Kit, (QIAGEN, Germany, Cat.: 28106), following the manufacturer's protocols. Sequencing was performed according to the manual for Seq Studio Genetic Analyzer (Thermo Fisher Scientific Applied Biosystems). The resulting nucleotide sequences were visually checked by the BioEdit program version 7.0. The nucleotide sequences of the studied species were compared with other sequences in the NCBI gene bank database by using the BLAST options. The nucleotide sequences of the studied species were deposited in NCBI GenBank database.

2.2 Phylogenetic analysis

The nucleotide sequences obtained in the current study were submitted to GenBank and published under accession numbers for cox1 gen presented in Table 1. 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 MUSCLE multiple sequence alignment program for cox1 partial genes. We used MEGA v11 software [11] to construct a maximum likelihood phylogenetic tree with the Tamura-Nei model, *Dipylidium caninum* (OQ281679) was used as an outgroup.

3. RESULTS

Genomic DNA was extracted from 55 cattle samples. Successful DNA extraction was achieved from 32 cattle samples, all of which showed a positive reaction with the cox1 primer. A total of 15 nucleotide sequences were obtained and analyzed. The amplified fragment length for the cox1 gene ranged from 310 to 390 base pairs for each sample (Fig. 1). Genotyping revealed the presence of G1 and G3 *E. granulosus* s.s. in cattle samples.

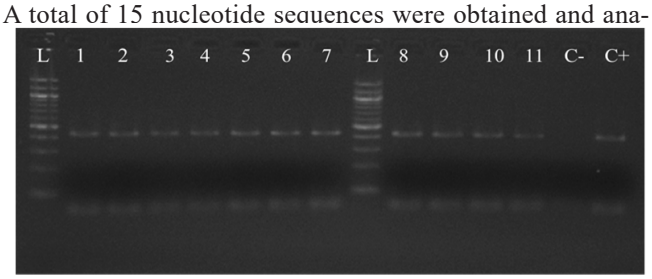

Figure 1 – Electrophoresis of partial COX gene
L – ladder (100-1000 bp), lines 1-11 studied isolates, C- - negative control, C+ - positive control

In table 1 presented data of nucleotide sequences of cattle *Echinococcus* isolates, with Genbank accession numbers. Out of the positive samples analyzed, 6 isolates were classified as G3 genotypes, and the remaining 9 isolates were classified as G1 genotypes based on the mitochondrial region of the COX1 gene.

Table 1 – *Echinococcus granulosus* isolates Genbank accession numbers and samples ID from the West-Kazakhstan region

Geographical origin	Host	Sample ID	GB number	Genotype
West-Kazakhstan	cattle	7-22-1	OR136387	G1
		7-22-2	OR136397	G1
		7-22-3	OR136457	G1
		7-22-4	OR139966	G1
		7-22-5	OR139967	G1
		7-22-6	PQ034666	G3
		7-22-7	PQ034667	G3
		7-22-9	PQ034668	G3
		7-22-10	PQ034669	G3
		7-22-14	PQ045256	G1
		7-22-15	PQ045257	G3
		7-22-16	PQ045258	G1
		7-22-17	PQ045259	G1
		7-22-19	PQ045260	G1
		7-22-25	PQ045261	G3

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 MUSCLE multiple sequence alignment algorithms for cox1 partial gene. The evolutionary history of 15 nucleotide sequences was examined using the Maximum Likelihood method and the Tamura-Nei model. Notably, the isolates with the G3 genotype formed distinct clades within the group, while isolates with the G1 genotype were closely grouped with the reference samples of *E. granulosus* as shown on Fig. 2. The phylogenetic tree was rooted using *Dipylidium caninum* as an outgroup.

4. Discussion

Echinococcus granulosus sensu stricto G1 (s.s.) is widely known to be one of the most common genotypes responsible for cystic *Echinococcus* (CE) on a global scale [12]. The mitochondrial cytochrome c oxidase subunit 1 (cox1) gene is an essential marker for distinguishing between inter- and intra-specific variants. The larval stage of *Echinococcus* spp. involves asexual reproduction, leading to numerous genetic mutations and variations at both the genus and species levels [13].

The research aimed to investigate the prevalence of echinococcosis in the West Kazakhstan region. A total of 55 samples were analyzed, leading to the identification of two genotypes, G1 and G3. This study reaffirms previous findings, highlighting the presence of the G3 genotype exclusively in the Aktobe region [14]. To date, few studies have explored deeply the genetic diversity of *E. granulosus* s.l. in Kazakh-

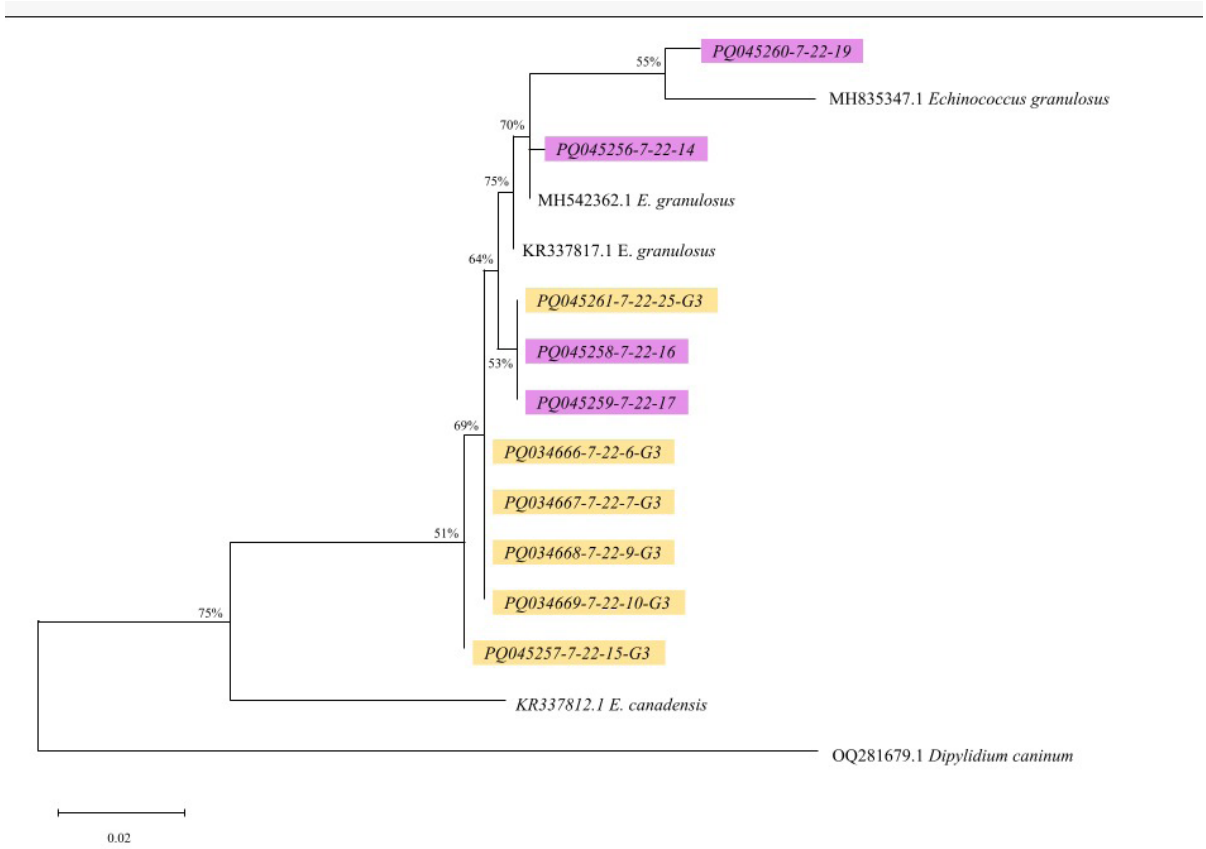

Figure 2 – Maximum Likelihood evolution tree of *E. granulosus* based on COX1 gene (*yellow highlighting e. granulosus isolates g3 genotype, purple highlighting g1 genotype)

stan, especially in livestock. This study revealed the main occurrence of *E. granulosus* s.s. (principally G1 90.0 %) livestock animals [15-17].

The research findings significantly contribute to our knowledge of genotype distribution and underline the necessity for continued monitoring throughout Kazakhstan. Out of the 15 successfully obtained nucleic acid sequences, six isolates were classified as G3, while nine were categorized as G1. Phylogenetic analysis (Fig.2) revealed a strong similarity to reference samples of *Echinococcus granulosus* from the GenBank database, validating the species identification.

The social aspect of the prevalence of echinococcosis in the West Kazakhstan region is significant. Echinococcosis not only affects livestock but also poses potential health risks to humans [18], especially those in close contact with infected animals [19]. This highlights the importance of public awareness and education regarding the transmission, prevention, and treatment of echinococcosis [20]. Additionally, the economic impact of echinococcosis on the livestock industry emphasizes the need for effective control measures and veterinary interventions to safeguard animal health and ensure food safety [21,22].

CONCLUSION

Overall, addressing the social implications of echinococcosis requires a multi-faceted approach involving community engagement, public health initiatives, and agricultural policies. Furthermore, this study sheds light on the prevalence of echinococcosis in cattle, highlighting the urgent need for attention and action due to its alarming impact on livestock.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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РАСПРОСТРАНЕНИЕ ECHINOCOCCUS GRANULOSUS ГЕНОТИПА G1 И G3 СРЕДИ КРУПНОГО РОГАТОГО СКОТА В ЗАПАДНО-КАЗАХСТАНСКОЙ ОБЛАСТИ

Нурасыл Манапов[1*], Рабига Уахит[2,3], Санжар Жоламан[4], Айнура Смагулова[2], Людмила Лидер[2,3], Владимир Киян[2]

[1]Национальная школа агрономии и пищевой промышленности (ENSAIA), Университет Лотарингии, 2 Avenue de la Forêt de Haye 54505 Вандёвр-ле-Нанси, Франция
[2]Лаборатория биоразнообразия и генетических ресурсов, Национальный центр биотехнологии, Кургальжинское шоссе, 13/5, Астана, 010000, Казахстан.
[3]Лаборатория паразитологии, Кафедра ветеринарной медицины, Казахский агротехнический исследовательский университет им. С. Сейфуллина, проспект Женис, 62, Астана, Казахстан
[4]Назарбаев Интеллектуальная школа физико математического направления города Астана, Хусейн бен Талал, 21, Астана, Казахстан

*Автор-корреспондент, Манапов Н., Manapovbio@gmail.com.

АБСТРАКТ

В данном исследовании основное внимание уделялось изучению распространенности эхинококкоза в Западно-Казахстанской области, в частности среди крупного рогатого скота. Всего было проанализировано 55 образцов, что привело к идентификации двух генотипов, G1 и G3, что подтвердило предыдущие выводы относительно присутствия генотипа G3 исключительно в Актюбинской области. Исследование выявило высокую встречаемость Echinococcus granulosus sensu stricto (G1) у сельскохозяйственных животных. Филогенетический анализ показал сильное сходство с контрольными образцами E. granulosus из базы данных GenBank, что подтверждает идентификацию вида. Исследование также подчеркнуло срочную необходимость внимания и действий в связи с тревожным воздействием эхинококкоза на домашний скот, а также его потенциальными рисками для здоровья человека. Кроме того, исследование пролило свет на социальные и экономические последствия эхинококкоза, подчеркнув важность осведомленности и просвещения общественности, эффективных мер контроля и многогранных подходов, включающих вовлечение сообщества, инициативы общественного здравоохранения и сельскохозяйственную политику для решения этой проблемы.

Ключевые слова: *Echinococcus granulosus*, генотипы, филогенетический анализ, домашний скот, риски для здоровья.

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БАТЫС ҚАЗАҚСТАН ОБЛЫСЫНДАҒЫ ІРІ ҚАРА МАЛ АРАСЫНДА *ECHINOCOCCUS GRANULOSUS* G1 ЖӘНЕ G3 ГЕНОТИПТІҢ ТАРАЛУЫ

Нұрасыл Манапов[1*], Рабиға Уахит[2,3], Санжар Жоламан[4], Айнура Смағұлова[2], Людмила Лидер[2.3], Владимир Киян[2]

[1]Ұлттық агрономия және тамақ өнеркәсібі мектебі (ENSAIA), Лотарингия университеті, 2 Avenue de la Forêt de Haye 54505 Vandoevre-le-Nancy, Франция
[2]Биоәртүрлілік және генетикалық ресурстар зертханасы, Ұлттық биотехнология орталығы, Кургалжинское тас жолы, 13/5, Астана, 010000, Қазақстан.
[3]Қазақ агротехникалық зерттеу университетінің ветеринария кафедрасы паразитология зертханасы. С.Сейфуллина, Жеңіс даңғылы, 62, Астана, Қазақстан
[4]Астана қаласындағы физика-математика бағытындағы Назарбаев Зияткерлік мектебі, Хусейн бин Талал, 21, Астана, Қазақстан

*Корреспондент автор, Манапов Н., Manapovbio@gmail.com.

АҢДАТПА

Бұл зерттеу Батыс Қазақстан облысында, атап айтқанда, ірі қара мал арасында эхинококкоздың таралуын зерттеуге бағытталған. Барлығы 55 сынама талданды, бұл G1 және G3 екі генотипті анықтауға әкелді, бұл G3 генотипінің тек Ақтөбе облысында болуына қатысты бұрынғы мәліметтерді растады. Зерттеу нәтижесінде ауыл шаруашылығы жануарларында Echinococcus granulosus sensu stricto (G1) көп кездесетіні анықталды. Филогенетикалық талдау GenBank дерекқорынан алынған E. granulosus үлгілеріне қатты ұқсастығын көрсетті, бұл түрлердің сәйкестендірілуіне қолдау көрсетеді. Сондай-ақ зерттеуде эхинококкоздың малға дабыл әсеріне, сондай-ақ оның адам денсаулығына ықтимал қауіп-қатерлеріне қатысты шұғыл назар аудару және әрекет ету қажеттілігі айтылды. Сонымен қатар, зерттеу эхинококкоздың әлеуметтік және экономикалық әсеріне жарық түсіріп, халықты хабардар ету мен білім берудің, тиімді бақылау шараларының және проблеманы шешу үшін қоғамдастықтың қатысуын, қоғамдық денсаулық сақтау бастамаларын және ауыл шаруашылығы саясатын қоса алғанда, көп қырлы тәсілдердің маңыздылығын көрсетеді.

Түйінді сөздер: *Echinococcus granulosus*, генотиптер, филогенетикалық талдау, мал шаруашылығы, денсаулыққа қауіп.