

GASTROINTESTINAL HELMINTHIASES OF DOGS WITH MOLECULAR GENETIC IDENTIFICATION OF ECHINOCOCCOSIS

Abdybekova A. M¹, Yalysheva S.V², Zhaksylykova A.A¹, Uakhit R.S.³, Kiyan V.S.^{4,*}

¹ Kazakh Scientific Research Veterinary Institute, 223, Raiymbek avenue, Almaty, 050016, Kazakhstan

² Al-Farabi Kazakh National University, 71, Al-Farabi avenue, Almaty, 050040, Kazakhstan

³ Kazakh Agrotechnical University named after S. Seifullin, 62, Zhenis avenue, Nur-Sultan, 010011, Kazakhstan.

⁴ National Center for Biotechnology, 13/5, Korgalzhin Highway, Nur-Sultan, 010011, Kazakhstan

*vskiyan@gmail.com

ABSTRACT

Echinococcosis is a serious zoonotic disease caused by a larval stage of the tapeworm from genus *Echinococcus*. Dogs act as definitive hosts and infect humans and farm animals. The article presents the results of a study of feces of 1020 dogs from Zhambyl, Kyzylorda, Turkestan, Almaty, Zhetysu, Karaganda, Aktobe, Pavlodar and Akmola regions for *Echinococcus granulosus*, collected in 2021-2022. Eggs of *Taenia spp.*, *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, *Ancylostoma caninum* were found using copro-ovoscopy methods. Helminth samples were differentiated by amplification and sequencing using the *Efla* translation elongation factor marker gene. Based on the analysis of the nucleotide sequences of 22 egg samples, two samples were identified as *E. granulosus*. Based on the G protein primer, the G1 genotype was established. These samples were deposited in the NCBI GenBank database (accession numbers ON630760 and ON630761). For the first time, molecular genetic characterization of the echinococcosis causative agent in the final hosts was carried out in Kazakhstan.

Key words: echinococcosis; dogs; coproovoscopy; helminths; PCR; sequencing; infection.

INTRODUCTION

Echinococcosis is a zoonosis caused by cestodes of the genus *Echinococcus* (family *Taeniidae*). This serious and near-cosmopolitan disease continues to be a significant public health issue [1]. The World Health Organization (WHO) has listed echinococcosis as one of the 17 neglected diseases targeted for control or elimination by 2050 [2]. In Kazakhstan the incidence rate continues to be high, especially in the southern part of the country [3].

Since dogs are widely used as service animals, they play an important role as disseminators of a number of dangerous helminthiases of humans and animals due to their predatory origin and peculiarities of use, maintenance, feeding, activity. In distant-pasture livestock rearing husbandries herd dogs with loose keeping form the packs of dogs, what contributes to the formation of particular parasitic communities on these farms depending on many natural and anthropogenic factors [4].

According to Abdybekova A.M. in the study of over a thousand dogs from the Almaty, Zhambyl and South Kazakhstan regions of Kazakhstan, including 418 herd dogs from 15 farms and 694 village dogs from 10 settlements, 8 species of helminths were registered, five of which are *Echinococcus granulosus*, *Taenia hydatigena*, *Multiceps multiceps*, *Dipylidium caninum*, *Toxocara canis* - met in mass quantities, the rest (*Taenia pisiformis*, *Taenia ovis*, *Toxocara leonina*) - sporadically, in the form of isolated cases. In the study of shepherd dogs in Almaty, Zhambyl and South Kazakhstan regions, the infection rate was *Taenia hydatigena* - 38.99%, *Echinococcus granulosus* - 25.36%, *Toxocara canis* - 14.83%, *Multiceps multiceps* - 13.40%. Low infection was noted by *Taenia ovis* - 0.24%, *Dipylidium caninum* - 5.50%. A high infestation of village dogs with taeniids was also noted: *Taenia hydatigena* - 21.61%, *Taenia pisiformis* - 1.44%, *Echinococcus granulosus* - 2.16%, *Multiceps multiceps* - 2.59%. In stray dogs living in anthropogenic zones, the most widespread helminths with a

high intensity of invasion were *D.caninum* (55.5-85.7%) and *T.canis* (18.1-33.3%). The study was carried out in 2002-2005 and the infection rate in dogs was determined using diagnostic deworming of dogs with a 1% aqueous solution of hydrobromic arecoline [5].

In 2017, to determine the epizootological and epidemiological role of carnivores in the dissemination of helminthiases that are dangerous for both animals and humans, scientists from the Kazakh Scientific-Research Veterinary Institute studied the feces of dogs from various rural districts of 12 regions of the republic (Almaty, Kyzylorda, Zhambyl, South Kazakhstan, Aktobe, West Kazakhstan, Kostanay, North Kazakhstan, Pavlodar, Akmola, Karaganda, East Kazakhstan). According to the results of studies the infected with *Taenia sp.* village dogs were found in 4 regions of the republic: Almaty region - (EI-35%), Karaganda (EI 16.66 - 41.66%), North Kazakhstan (EI 5 - 16.66%), Kostanay (EI 3.03 - 40%) [5]. In 2019, out of 271 investigated samples, 15 (15.46%) samples contained eggs of *Taenia sp.* with AI 1 - 188 eggs [6].

The aim of this study is to investigate the infection of dogs with taeniids by coproovoscopic methods and to identify *Echinococcus granulosus* using PCR and DNA sequencing.

MATERIALS AND METHODS

Parasitological methods

In order to evaluate the incidence of dog intestinal helminths 820 samples of dog feces were taken from Zhambyl, Kyzylorda, Turkestan, Almaty, Karaganda, Aktobe, Pavlodar and Akmola regions (8 regions of the Republic of Kazakhstan) in 2021.

In the spring of 2022 feces from 200 dogs of various service purposes were taken in 13 rural districts (Ulkenshygan, Pinzhim, Ulkenagash, Baskunchi, Birlik, Koktal, Usharal, Chulakai, Taldy, Sarybel, Zhaskent, Aydarly, Konyrolen), in the city of Zharkent, Panfilov district, Zhetysu region and in the city of Taldykorgan, Zhetysu region.

A total of 1020 samples of dog feces were studied using the Darling method. Species identification of eggs was carried out according to V.F. Kapustin [7], S.N. Boev et al. [8], N.V. Demidov [9].

Extensiveness of invasion counted using the formula:

$$p = \frac{m}{n} \cdot 100\% \quad (3),$$

where, p is the proportion of infected animals;

m is the number of infected animals;

n - sampling size.

The invasion intensity was determined by counting the average number of eggs in each test sample of dog feces.

Molecular methods

DNA extraction and PCR analysis

To isolate DNA, taeniid eggs were collected from contaminated faecal samples by serial dilution in water and centrifugation. The obtained fraction with eggs was incubated in artificial gastric juice for 30 min at 50°C. Next, an alkaline treatment was carried out to destroy the protective wall of the eggs [10]. After that, further DNA isolation was carried out



Figure 1 - Eggs of *Taenia* spp.

using a commercial kit Qiamp, Qiagen.

The amount and purity of the isolated DNA could be determined by measuring the absorbance at 260 and 280 nm using a NanoDrop 2000 instrument (Thermo Scientific, USA). DNA was dissolved in ddH₂O and stored at -70°C.

PCR was performed in 25 µl reaction mixture containing 10× Taq buffer with (NH₄)₂SO₄, 2.5 mM MgCl₂, 1 U Taq DNA polymerase and 200 µM dNTP (Thermo Scientific, Carlsbad, CA, USA), 10 pmol of each primer and 20 ng of the extracted taeniid sample DNA as a template. Thermal reactions were carried out for 25 cycles of denaturation (94°C, 30 s), annealing (56°C, 30 s), and elongation (72°C, 30 s). The resulting restriction fragments were separated by electrophoresis on ethidium bromide containing 1.5% agarose gel using 1x TAE buffer.

DNA obtained from parasite eggs was used as a template for amplification of the mitochondrial gene *Efla* extension factor 1 alpha. PCR was set using primers *E. granulosus sensu stricto EflaF* (F: TCCTAACATGCCTTGGTAT) and *EflaR* (R: GTTACAGCCTTGATCACG) (Boubaker, 2013) to determine the G1-G3 genotype [11].

Bioinformatics analysis

Multiple alignment of the obtained sequences was performed using the ClustalW algorithm in the MEGA (v.11) program [12]. The phylogenetic analysis was constructed by the maximum likelihood (ML) method using the MEGA soft-

ware (v.11).

RESULTS

In Zhambyl region from 100 collected dog faecal samples none of helminth eggs were found. In Kyzylorda region from 100 collected dog faecal samples in 13 (13%) samples *Toxocara canis* eggs were detected with the invasion intensity of 3-41 eggs in one field of view of the microscope. In Turkistan region from 100 collected dog faecal samples none of helminth eggs were found.

In Almaty region from 200 collected dog faecal samples 18 were infected with various types of helminths. The extensiveness of invasion was 9%, the invasion intensity was 1-17 eggs. Of the 18 infected dogs, 10 (55.55%) had eggs of *Taenia* spp. with the invasion intensity 1-17 eggs (Figure 1), 7 (38.88%) dogs had *Toxocara canis* eggs with the invasion intensity 3-41 eggs, 2 (11.11%) dogs had *Toxascaris leonina* eggs with the invasion intensity 1-14.

In Karaganda region from 50 collected samples in 15 (30%) samples *Toxocara canis* eggs with the invasion intensity 1-51 were found, eggs of *Taenia* spp. were found in 1 sample with the invasion intensity 25 eggs.

In Aktobe region from 70 collected dog faecal samples

none of helminth eggs were found.

In Pavlodar region from 100 collected dog faecal samples *Toxocara canis* eggs with the invasion intensity 1 egg were found in 1 (1%) sample, larvae of nematodes with the invasion intensity 2-3 larvae were detected in 2 samples (2%).

In the Akmola region from 100 the invasion intensity *Toxocara canis* eggs with the invasion intensity 1-25 eggs were found in 4 (4%) samples, 1 egg of *Taenia* spp. in 1 sample.

As a result of a scatological study, out of 200 dogs (from the Zhetysu region), 20 were infected with various species of helminths. The extensiveness of invasion was 10%, the the invasion intensity was 1-35 eggs. Of 20 infected dogs, 14 (70%) had eggs from . with the invasion intensity 1-35 eggs, in 3 dogs (15%) *Toxascaris leonina* with the invasion intensity 4-14 eggs (Figure 2A), in 2 (10%) *Toxocara canis* eggs with the invasion intensity 1-4 (Figure 2B), in 2 (10%) *Trichuris vulpis* eggs with the invasion intensity 1-13 (Figure 2C), in 2 (10%) *Ancylostoma caninum* with the invasion intensity 1-3 eggs (Figure 2D). Thus, according to the results of a scatological study of 200 dogs, 14 (EI 7%) were found to have eggs of helminths of the family *Taeniidae*.

To identify *Echinococcus granulosus* by PCR, 22 samples of dog feces from Zhetysu, Almaty, Karaganda and Akmola regions were studied, in which eggs of helminths of the *Taeniidae* family were found (table 1).

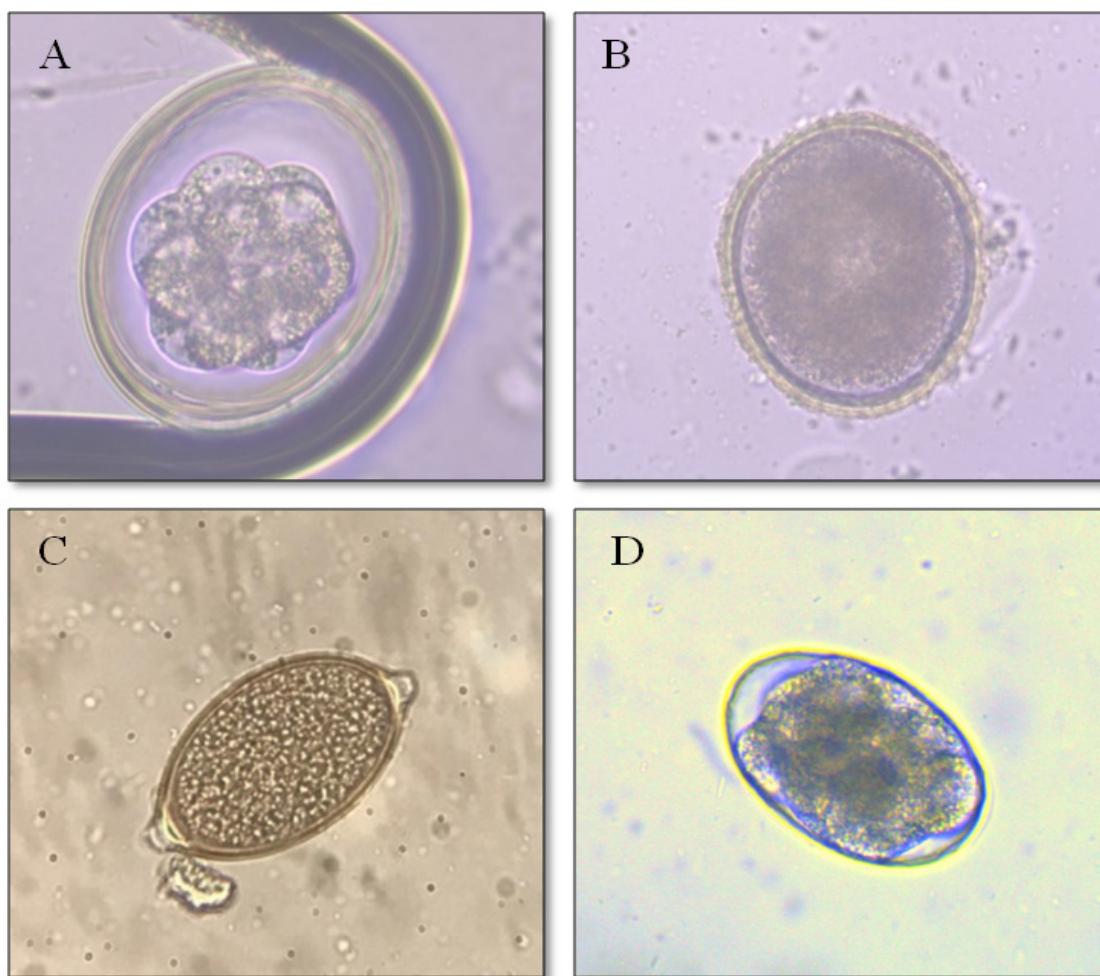


Figure 2 - Helminth eggs in the feces of dogs in the Zhetysu region

Table 1 – Dog faecal samples with eggs of helminths of *Taeniidae* family

| № of sample | Locality | Region | The number of taeniid eggs |
|-------------|-----------------------------|-------------------------------------|----------------------------|
| 1 | Taldykorgan | Zhetysu region | 2 |
| 2 | Taldykorgan | Zhetysu region | 1 |
| 3 | Taldykorgan | Zhetysu region | 15 |
| 4 | Taldykorgan | Zhetysu region | 1 |
| 5 | Birlik rural district | Zhetysu region Panfilovsky district | 3 |
| 6 | Sarybel rural district | Zhetysu region Panfilovsky district | 2 |
| 7 | Koktal rural district | Zhetysu region Panfilovsky district | 1 |
| 8 | Ulken Agash rural district | Zhetysu region Panfilovsky district | 9 |
| 9 | Taldykorgan | Zhetysu region | 1 |
| 10 | Taldykorgan | Zhetysu region | 13 |
| 11 | Ulken Shagan rural district | Zhetysu region Panfilovsky district | 35 |
| 12 | Taldykorgan | Zhetysu region | 15 |
| 13 | Taldykorgan | Zhetysu region | 4 |

| | | | |
|----|--------------------------|-------------------------------------|----|
| 14 | Zharkent | Zhetysu region Panfilovsky district | 2 |
| 15 | Boleksaz rural district | Almaty region Raimbek district | 2 |
| 16 | Boleksaz rural district | Almaty region Raimbek district | 1 |
| 17 | Karatogan rural district | Almaty region Raimbek district | 11 |
| 18 | Boleksaz rural district | Almaty region Raimbek district | 1 |
| 19 | Zharkent | Zhetysu region Panfilovsky district | 2 |
| 20 | Kegen rural district | Almaty region Raimbek district | 8 |
| 21 | Sadovoye village | Karagandy region | 25 |
| 22 | Nur-Sultan | Akmola region | 1 |

According to the results of PCR analysis using specific primers, DNA of *Echinococcus granulosus sensu stricto* was isolated in 2 samples - 8 and 11 (Figure 3). The molecular weight of the PCR products was 706 bp.

From the data obtained, it can be concluded that *Echinococcus granulosus* was detected only in dogs in the rural dis-

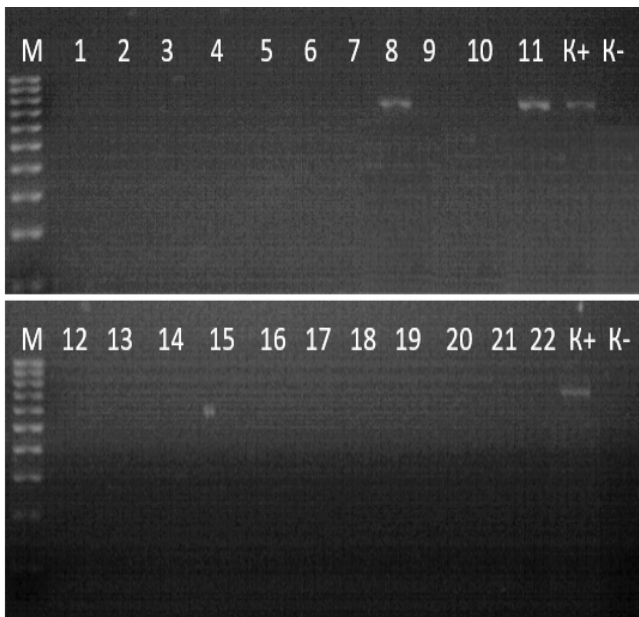


Figure 3 – M – marker (Gene Ruler 100 bp Plus DNA Ladder), 1-22 - studied samples from dogs, K- - negative control, K+ - positive control.

tricts of Ulken-Agash and Ulken Shagan, Panfilov district, Zhetysu region.

Subsequently, the obtained positive amplicons of PCR analysis with a molecular weight of 706 bp. were used for Sanger sequencing (Applied Biosystems SeqStudio) followed by bioinformatic analysis of the resulting nucleotide sequences. Data entered into the GenBank database (access numbers ON630760, ON630761)

The evolutionary history was inferred by using the Max-

imum Likelihood method and Tamura-Nei model [13]. The tree with the highest log likelihood is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with a superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. There were a total of 651 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [12].

Thus, according to the results of scatological studies, taeniid eggs were found in dogs in Almaty, Karaganda, Ak-mola and Zhetysu regions. In total, taeniid eggs were found in 22 faecal samples out of 1020 examined in 9 regions of the Republic of Kazakhstan. *Echinococcus granulosus* was confirmed by PCR in 2 dog feces samples from Zhetysu region.

Discussion

According to the results of the study, it was found that the infection rate of dogs from Zhambyl, Kyzylorda, Turkestan, Almaty, Karaganda, Aktobe, Pavlodar and Akmola regions (8 regions of the Republic of Kazakhstan) with gastrointestinal parasites is at a low level. One of the probable reasons for the decrease in infection of domestic dogs with echinococcosis is the regular deworming of dogs. This is supported by the fact that infection with other species of helminths is also low. According to the latest statistical studies, there is a downward trend in the human incidence of echinococcosis in the country. However, there is an increase in certain regions of the country [3].

In this study, the molecular genetic characterization of the causative agent of echinococcosis in domestic dogs was carried out for the first time. The genotypes found in two samples belong to the species *Echinococcus granulosus sensu stricto* (genotypes G1 and G3), which is the most common in the

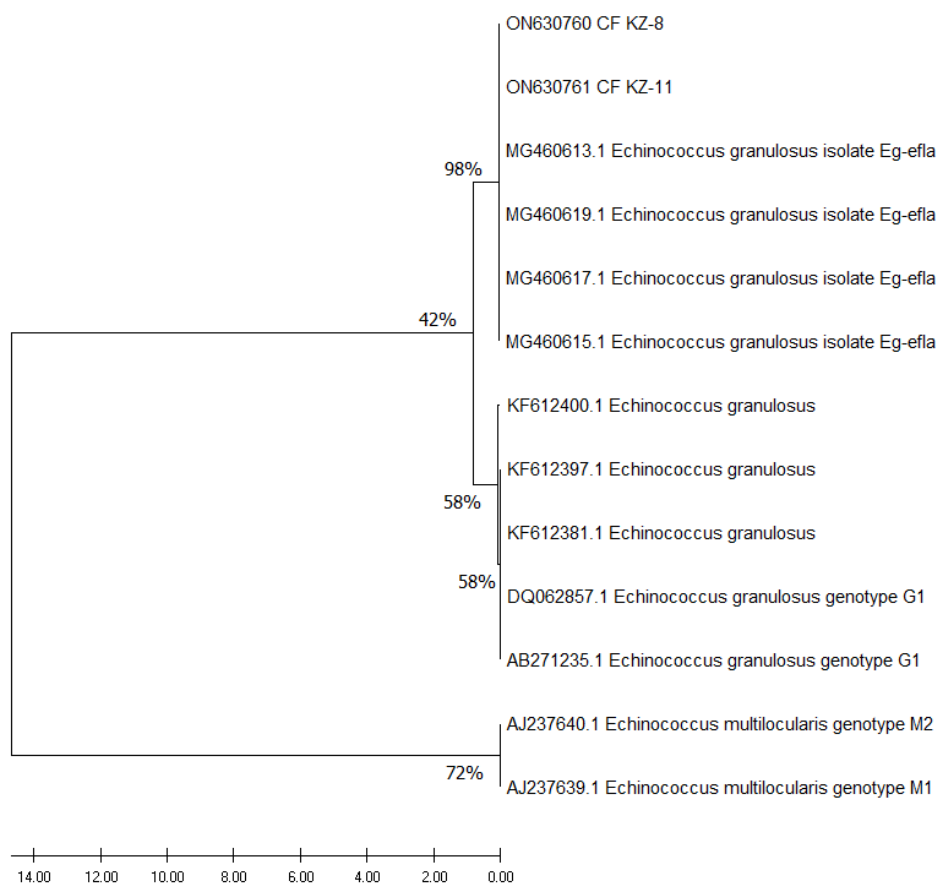


Figure 4 - Evolutionary analysis by the maximum likelihood method. Scale bar shown (14.00 discrepancy).

world and causes the vast majority of cases of echinococcosis in humans [14]. This species is widespread globally, particularly in rural livestock-raising areas. In Kazakhstan genotypes were previously studied but in intermediate hosts only – the presence of *Echinococcus granulosus sensu stricto* G1 and G3 in cattle, sheep and *Echinococcus canadensis* (G6/G7 strain) in sheep was described [15]. Further study of *Echinococcus granulosus* genotypes in definitive and intermediate hosts is needed.

Conclusion

In conclusion, dogs pose a potential public health hazard by infecting humans with parasitic diseases. Close human contact with dogs, lack of deworming practices, and favorable climatic conditions are factors promoting the survival of infective stages of canine helminth parasites. The general public and dog owners should be aware of the danger of infection and take the necessary precautions. In order to control echinococcosis, veterinary services should monitor and deworm dogs regularly.

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ГАСТРОИНТЕСТИНАЛЬНЫЕ ГЕЛЬМИНТОЗЫ СОБАК С МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКОЙ ИДЕНТИФИКАЦИЕЙ ЭХИНОКОККОЗА

Абдыбекова А. М.¹, Ялышева С.В.², Жаксылыкова А.А.¹, Уахит Р.С.³, Киян В.С.^{4,*}

¹Казахский научно-исследовательский ветеринарный институт, пр. Райымбека, 223, Алматы, 050016, Казахстан

²Казахский национальный университет имени аль-Фараби, проспект Аль-Фараби, 71, Алматы, 050040, Казахстан

³Казахский агротехнический университет им. С.Сейфуллина, проспект Женис, 62, Нур-Султан, 010011, Казахстан.

⁴Национальный центр биотехнологии, Коргалжинское шоссе, 13/5, Нур-Султан, 010011, Казахстан

*vskiy@gmail.com

АННОТАЦИЯ

Эхинококкоз — тяжелое зоонозное заболевание, вызываемое личиночной стадией ленточного червя из рода *Echinococcus*. Собаки выступают в роли окончательных хозяев и заражают людей и сельскохозяйственных животных. В статье представлены результаты исследования фекалий 1020 собак из Жамбылской, Кызылординской, Туркестанской, Алматинской, Жетысуской, Карагандинской, Актюбинской, Павлодарской и Акмолинской областей на *Echinococcus granulosus*, собранных в 2021-2022 гг. При помощи методов копроовоскопии были обнаружены яйца *Taeniia spp.*, *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, *Ancylostoma caninum*. Образцы гельминтов дифференцировали путем амплификации и секвенирования с использованием маркерного гена фактора элонгации трансляции *Efla*. По результатам анализа нуклеотидных последовательностей 22 проб яиц, два образца были идентифицированы как *E. granulosus*. На основе праймера, представляющего собой G-белок, был установлен генотип G1. Данные образцы были депонированы в базе данных NCBI GenBank (инвентарный номер ON630760 и ON630761). Впервые в Казахстане проведена молекулярно-генетическая характеристика возбудителя эхинококкоза у окончательных хозяев.

Ключевые слова: эхинококкоз; собаки; копроовоскопия; гельминты; ПЦР; секвенирование; зараженность.

ИТТЕРДІҢ АСҚАЗАН-ШЕК ГЕЛЬМИНТОЗДАРЫ ЭХИНОКОККОЗҒА МОЛЕКУЛЯРЛЫҚ-ГЕНЕТИКАЛЫҚ ИДЕНТИФИКАЦИЯСЫМЕН

Абдыбекова А. М.¹, Ялышева С.В.², Жаксылыкова А.А.¹, Уахит Р.С.³, Киян В.С.^{4,*}

¹Қазақ ветеринария ғылыми-зерттеу институты, Райымбек даңғылы, 223, Алматы, 050016, Қазақстан

²Әл-Фараби атындағы қазақ ұлттық университеті, Аль-Фараби даңғылы, 71, Алматы, 050040, Қазақстан

³С. Сейфуллин атындағы қазақ агротехникалық университеті, Жеңіс даңғылы, 62, Нұр-Сұлтан, 010011, Қазақстан.

⁴Ұлттық биотехнология орталығы, Коргалжын тас жолы, 13/5, Нұр-Сұлтан, 010011, Қазақстан

*vskiy@gmail.com

ТҮЙІН

Эхинококкоз - *Echinococcus* тұқымдасына жататын дернәсіл сатысынан туындаған ауыр зоонозды ауру. Собаки выступают в роли окончательных хозяев и заражают людей и сельскохозяйственных животных. Иттер ақтық ие болып табылады және адамдар мен ауылшаруашылық жануарларын жұқтырады. В статье представлены результаты исследования фекалий 1020 собак из Жамбылской, Кызылординской, Туркестанской, Алматинской, Жетысуской, Карагандинской, Актюбинской, Павлодарской и Акмолинской областей на *Echinococcus granulosus*, собранных в 2021-2022 гг. Мақалада 2021-2022 жылдары Жамбыл, Қызылорда, Түркістан, Алматы, Жетісу, Қарағанды, Ақтөбе, Павлодар және Ақмола облыстарынан жиналған 1020 иттің нәжісін *Echinococcus granulosus*-қа зерттеу нәтижелері келтірілген. Копроовоскопия әдісімен *Taeniia spp.*, *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, *Ancylostoma caninum* жұмыртқалары анықталды. Гельминттердің үлгілері *Efla* трансляциясының элонгация факторының маркерлік генин қолдана отырып, күшейту және жүйелеу арқылы сараланды. 22 жұмыртқа сынамасына нуклеотидтер тізбегін талдау нәтижелері бойынша екі үлгісі *E. granulosus* ретінде анықталды. G - ақуызы болып табылатын праймер негізінде G1 генотипі анықталды. Бұл үлгілер NCBI GenBank деректер базасында (инвентарлық нөмірі ON630760 және ON630761) сақталды. Қазақстанда алғаш рет эхинококкоздың ақтық иелерінде эхинококкоз қоздырғышының молекулярлық-генетикалық сипаттамасы жүргізілді.

Кілт сөздер: эхинококкоз; иттер; копроовоскопия; гельминттер; ПТР; секвенирование; инфекция.