



## Nematode fauna in *Canis lupus familiaris* and *Felis catus* in Karakalpakstan and molecular genetic analysis of *Toxascaris leonina*

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### Article info

Received 01.10.2024

Received in revised form 14.11.2024

Accepted 05.12.2024

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### Introduction

There are numerous parasitological studies related to ecto- and endoparasites of agricultural, game and commercial animals in terrestrial biocoenoses. However, parasites of dogs and cats have received less attention. Domestic carnivores, such as the dog and cat, often become infected with parasites that are dangerous for both themselves and a number of agricultural animals, as well as humans. An accurate taxonomy of a parasite causing this or that disease in an animal is a prerequisite for successful implementation of anthelmintic measures in a particular region (Safarov et al., 2023).

The nematode *Toxascaris leonina* is a monoxenous parasite, whose hosts are domestic and wild canids and felids (Okulewicz et al., 2012; Fogt-Wyrwas et al., 2019; Jin et al., 2019). The hosts become infected with this helminth through eating its eggs (Sprenst, 1959). The larvae that hatch from these eggs penetrate into the mucous membrane of the small intestine, where they continue to develop. After several moults, these larvae return to the intestinal lumen, where they reach maturity, and the females lay eggs and exit into the external environment with faeces. Clinical symptoms include diarrhea, vomiting, abdominal discomfort, and even life-threatening intestinal obstruction (Lee et al., 2010). In addition, this nematode also infests humans, causing a zoonotic disease (Robertson &

*Akramova, F., Shakarbaev, U., Saidova, S., Mirzayeva, A., Saparov, K., Hamrokulova, Z., Toremuratov, M., Kaniyazov, A., & Azimov, D. (2024). Nematode fauna in *Canis lupus familiaris* and *Felis catus* in Karakalpakstan and molecular genetic analysis of *Toxascaris leonina*. Biosystems Diversity, 32(4), 518–521. doi:10.15421/012454*

The article analyses the current species diversity of nematodes of dogs and cats in Karakalpakstan. Our research team identified 11 species of nematodes in dogs: *Trichocephalus vulpis* (Froelich, 1789), *Diocotophyme renale* (Goeze, 1782), *Ancylostoma caninum* (Ercolani, 1859), *Uncinaria stenocephala* (Railliet, 1854), *Toxascaris leonina* (Linstow, 1902), *Toxocara canis* (Werner, 1782), *Spirocercera lupi* (Rudolphi, 1809), *Physaloptera praeputialis* (Linstow, 1888), *Rictularia affinis* (Jagerskiold, 1904), *Dirofilaria immitis* (Leidy, 1856), and *Dirofilaria repens* (Railliet et Henry, 1911). The prevalence of infection in the dog population ranged from 8.3% to 45.8%, with the intensity of infection ranging between 1 and 28 individuals. The nematode fauna of cats consisted of 9 species: *D. renale*, *A. caninum*, *U. stenocephala*, *T. leonina*, *T. canis*, *Ph. praeputialis*, *R. affinis*, *D. immitis*, and *D. repens*. In this group of animals, the prevalence ranged from 8.3% to 37.6% and the intensity of infection from 1 to 23 individuals. *Toxascaris leonina* was the predominant helminth species in both cats and dogs. *Toxocara canis* showed a prevalence of 37.6% and an infection intensity of 4 to 9 individuals in domestic Canidae, and 33.7% and 3–8 individuals in *Felis catus*. The research team conducted a molecular genetic analysis of *T. leonina*. The study showed that *Toxascaris leonina* from dogs differed from the same species of nematodes from cats and was characterised by two subclades. One clade identified in dogs (PP865013) is close to an isolate from China (MN329693) and Australia (NC023504), and the other one in cats (PP864705) to an isolate from the tiger (China, MW560284). In addition, the isolates are grouped together irrespective of hosts and location and are related to other species from the family Ascariidae.

**Keywords:** nematodes; *Canis lupus familiaris*; *Felis catus*; intensity of infection; prevalence; *Toxascaris leonina*.

Thompson, 2002; Okulewicz et al., 2012). This necessitates diagnosing and identifying *T. leonina*, not only at the morphological level, but also at the molecular and genetic levels.

### Materials and methods

The research material was collected during 2023–2024 on farms and in residential areas in the city of Nukus and in Kegeyli, Khojeyli, Chimbai, and Karauzek Districts of Karakalpakstan. A total of 24 dogs and 24 cats from rural and urban areas were examined in all seasons using well-known parasitological methods. The helminths were also collected through deworming of the above-mentioned animals.

The helminths were studied according to a generally accepted method – nematodes were treated with a mixture of glycerine and lactic acid. The nematode species were identified according to well-known monographs (Kozlov, 1977; Anderson, 2000), using modern microscopic equipment (Olympus CK2-TR inverted microscope, Lomo research microscope, ML 2200 binocular microscope, and N 300 m Ningo Yongkin Optics trinocular microscope).

Common DNA was isolated from adult individuals of the nematode *Toxascaris leonina* from cats and dogs using a Blood & Tissue Kit (Qiagen, Germany) in accordance with the manufacturer's instructions. The

spectrophotometry of genomic DNA template was studied using an Implan NanoPhotometer N device (ThermoFisher Scientific, Dreieich, Germany), which confirmed the constancy of DNA concentration in the extracts. PCR settings were for amplification of 710 bp mitochondrial cytochrome oxidase subunit I (COI), where 1490 and 2198 are the mitochondrial cytochrome oxidase gene (Table 1).

**Table 1**  
Gene primers structure

Primer name	Sequence (5'-3')	Source
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al.
HCO2198	TAAACTTCAGGGTGACCAAAAATCA	(1994)

The reaction mixture for the PCR, 25 µL in volume, consisted of the following components: 12.5 µL of Platinum™ Hot Start PCR Master Mix (2x), 7.5 µL of water, 0.5 µL of primer F, 0.5 µL of primer R, and 4 µL of genomic DNA. Applied Biosystems ProFlex™ Thermo Fisher Scientific was used for PCR. PCR amplification was carried out using a proposed PCR programme consisting of three steps: step 1 – 20 s at 95 °C; step 2 (35 cycles) – 30 s at 95 °C, 30 s at 55 °C, 1 min at 72 °C; step 3 – 5 min at 72 °C.

The PCR products were analysed with the help of 1.5% agarose gel electrophoresis. The gel was prepared in a 1x tris-borate-EDTA buffer (TBE, Thermo Scientific) stained with ethidium bromide. 2 µL of marker were added to the first well for loading DNA gel (6X) and 1.3 µL of 50 bp DNA Ladder, then 5 µL of PCR products and 3 µL of marker were added to the other wells for loading DNA gel (6X). The electrophoresis was conducted on the SE-1 horizontal electrophoresis system (Helicon, Germany) at a voltage of 100 V for 100 minutes. The PCR products were visualised in UV light and photographed using the BK-AG100 gel document visualisation system (Biobase, China).

PCR products sequencing: one strip of PCR was cut with a razor blade, transferred to a 1.5 mL centrifuge tube and purified using a Pure-Link™ Quick Gel Extraction Kit (Invitrogen, USA), following the manufacturer's instructions. The cyclic sequencing reaction was conducted using the BigDye® Terminator v 3.1 kit (Applied Biosystems, USA). The sequencing reaction consists of BigDye – 1 µL, 5x Seg buffer – 2 µL, sequencing primer – 1.5 µL, and purified PCR product – 5.5 µL. LCO and HCO primers were used for sequencing.

To conduct the sequencing reaction, the following thermal cycling program was used: stage 1 (initial) – denaturation at 95 °C for 20 minutes; stage 2 – denaturation at 95 °C for 30 s, stage 3 – annealing at 53 °C for 30 s, stage 4 – elongation at 72 °C for 1 minute (35 consecutive cycles). The sequencing reaction product was stored at 4 °C.

The sequencing reaction products were purified from fluorescently labelled terminator nucleotides using the Dynabeads Sequencing Clean-Up Kit (Applied Biosystems, USA).

The DNA sequencing reaction products were separated and analysed using capillary gel electrophoresis with laser-induced fluorescence on an Applied Biosystems 3500 (Thermo Fisher Scientific) genetic analyser.

Sequence alignment and phylogenetic analysis: Geneious 4.8.5 and MEGA 11 programmes were used to edit the source data obtained using the DNA sequence analysis system.

The goal of the research was to study the species diversity of nematodes in domestic cats and dogs in Karakalpakstan and the association of *Toxascaris leonina* with its hosts using a molecular genetic analysis.

## Results

Our research team identified 11 species of nematodes from 8 families in dogs in Karakalpakstan: *Trichocephalus vulpis* (Froelich, 1789), *Diocetophyme renale* (Goeze, 1782), *Ancylostoma caninum* (Ercolani, 1859), *Uncinaria stenocephala* (Railliet, 1854), *Toxascaris leonina* (Linstow, 1902), *Toxocara canis* (Werner, 1782), *Spirocerca lupi* (Rudolphi, 1809), *Physaloptera praeputialis* (Linstow, 1888), *Rictularia affinis* (Jagerskiold, 1904), *Diofiliaria immitis* (Leidy, 1856), and *D. repens* (Railliet et Henry, 1911). 9 species of nematodes from 6 families were registered in cats: *D. renale*, *A. caninum*, *U. stenocephala*, *T. leonina*, *T. canis*, *Ph. praeputiale*, *R. affinis*, *D. immitis* and *D. repens*.

Given below is the taxonomy of nematodes of dogs and cats.

Class Nematoda Rudolphi, 1808

Subclass Adenophorea (Linstow, 1905)

Order Trichocephalida (Skrjabin et Schulz, 1928)

Suborder Trichocephalata Skrjabin et Schulz, 1928

Family Trichocephalidae Baird, 1853

Genus *Trichocephalus* Schrank, 1788

*Trichocephalus vulpis* Froelich, 1789

Definitive hosts: domestic dog.

Localisation: intestine.

Discovery areas: Karakalpakstan – the city of Nukus, Cimbai and Kegeyli Districts.

Biology: develops without the participation of an intermediate host.

Prevalence: 16.7%, Infection intensity: 1–4 individuals.

Order Diocetophymida (Skrjabin, 1927)

Family Diocetophymidae Railliet, 1915

Genus *Diocetophyme* Collet-Meygret, 1802

*Diocetophyme renale* (Goeze, 1782)

Definitive hosts: domestic dog, domestic cat.

Localisation: kidneys.

Discovery areas: Karakalpakstan – Khojeyli, Cimbai, and Karauzek Districts.

Biology: intermediate hosts – oligochaetes (earthworms), reservoir hosts – fish (Kozlov, 1977). Prevalence: 12.5% in dogs, 8.3% in cats. Infection intensity: 1–3 individuals in dogs, 1–2 individuals in cats.

Subclass Secernentea (Linstow, 1905)

Order Strongylida Railliet et Henry, 1913

Family Ancylostomatidae Looss, 1905

Genus *Ancylostoma* (Dubinin, 1843)

*Ancylostoma caninum* (Ercolani, 1859)

Definitive hosts: domestic dog, domestic cat.

Localisation: intestine.

Discovery area: Karakalpakstan – city of Nukus.

Biology: develops in a direct way. Animals become infected either orally or percutaneously (Kozlov, 1977). Prevalence: 12.5% in dogs, 16.7% in cats. Infection intensity: 1–3 individuals in dogs, 1–4 individuals in cats.

Genus *Uncinaria* Froelich, 1789

*Uncinaria stenocephala* (Railliet, 1884)

Definitive hosts: domestic dog, domestic cat.

Localisation: intestine.

Discovery areas: Karakalpakstan – Kegeyli and Karauzek Districts.

Biology: life cycle is same as in *Ancylostoma* (Kozlov, 1977). Prevalence: 20.8% in dogs, 29.2% in cats. Infection intensity: 1–5 individuals in dogs, 1–7 individuals in cats.

Order Ascaridida Skrjabin, 1915

Family Ascarididae Baird, 1853

Genus *Toxascaris* Leiper, 1907

*Toxascaris leonina* (Linstow, 1902)

Definitive hosts: domestic dog, domestic cat.

Localisation: intestine.

Discovery areas: Karakalpakstan – all studied districts.

Biology: develops in a direct way. Animals become infected when they ingest infectious eggs with food. Prevalence: 45.8% in dogs, 37.6% in cats. Infection intensity: 3–28 individuals in dogs, 3–23 individuals in cats.

Genus *Toxocara* Stiles, 1905

*Toxocara canis* (Werner, 1782)

Definitive hosts: domestic dog, domestic cat.

Localisation: intestine.

Discovery areas: Karakalpakstan – all studied districts.

Biology: develops in a direct way. Prevalence: 37.6% in dogs, 33.3% in cats. Infection intensity: 4–9 individuals in dogs, 3–8 individuals in cats.

Order Spirurida (Railliet, 1914)

Family Spiruridae Oerleg, 1885

Genus *Spirocerca* Railliet et Henry, 1911

*Spirocerca lupi* (Rudolphi, 1809)

Definitive hosts: domestic dog.

Localisation: stomach.

Discovery areas: Karakalpakstan – city of Nukus, Karauzek District.  
 Biology: intermediate hosts are beetles from the families Scarabaeidae and Tenebrionidae, in which the larvae reach the invasive stage. We have established that all classes of vertebrates participate in the life cycle as reservoir hosts (Kozlov, 1977).

Family Physolopterae Leiper, 1908

Genus *Physaloptera* Rudolphi, 1819

*Physaloptera praeputialis* Linstow, 1888

Definitive hosts: domestic dog, domestic cat.

Localisation: stomach, intestines, abdominal cavity.

Discovery areas: Karakalpakstan – Khojeyli, Chimbai, and Karauzek Districts.

Biology: not studied. Prevalence: 25% in dogs, 20.8% in cats. Infection intensity: 3–5 individuals in dogs, 2–4 individuals in cats.

Family Rictulariidae Railliet, 1916

Genus *Rictularia* Froelich, 1802

*Rictularia affinis* Jägerskiöld, 1904

Definitive hosts: domestic dog, domestic cat.

Localisation: intestine.

Discovery areas: Karakalpakstan – city of Nukus, Khojeyli and Chimbai Districts.

Biology: not studied. However, data is available (Kabilov, 1983) on intermediate hosts for another species – *Rictularia amurensis* Schulz, 1927, which are darkling beetles in southern and north-eastern Uzbekistan.

Probably, these beetle species are also intermediate hosts to the *Rictularia* species in question. Prevalence: 29.2% in dogs, 25% in cats. Infection intensity: 1–7 individuals in dogs, 2–5 individuals in cats.

Family Filariidae Cobbold, 1819

Genus *Dirofilaria* Raill et Henry 1911

*Dirofilaria immitis* (Leidy, 1856)

Definitive hosts: domestic dog, jackal, wolf, fox, jungle cat, domestic cat.

Localisation: right ventricle of the heart.

Discovery area: Karakalpakstan – city of Nukus.

Biology: blood-sucking dipterans from the genera *Aedes* and *Culex* are intermediate hosts, in which invasive larvae develop. Predatory mammals become infected through mosquitoes, which, when biting, inject invasive larvae into the blood (Kozlov, 1977, Safarov et al., 2021). Prevalence: 12.5% in dogs, 8.3% in cats. Infection intensity: 3–6 individuals in dogs, 2–4 individuals in cats.

*Dirofilaria repens* Railliet et Henry, 1911

Definitive hosts: domestic dog, domestic cat.

Localisation: subcutaneous tissue.

Discovery areas: Karakalpakstan – city of Nukus, Chimbai and Karauzek Districts.

Biology: same as in *D. immitis* (Kozlov, 1977, Safarov et al., 2021). Prevalence: 16.7% in dogs, 12.5% in cats. Infection intensity: 2–7 individuals in dogs, 2–4 individuals in cats (Table 2).

**Table 2**

Infection of cats and dogs in Karakalpakstan with nematodes

No.	Nematode species	Localisation		Prevalence		Infection intensity			
		<i>Canis familiaris</i>	<i>Felis catus</i>	<i>Canis familiaris</i>	<i>Felis catus</i>	min		max	
						<i>Canis familiaris</i>	<i>Felis catus</i>	<i>Canis familiaris</i>	<i>Felis catus</i>
1	<i>Trichocephalus vulpis</i>	large intestine	–	4/16.7%	–	1	–	4	–
2	<i>Diocotophyme renale</i>	kidneys, abdominal cavity	kidneys, abdominal cavity	3/12.5%	2/8.3%	1	1	3	2
3	<i>Ancylostoma caninum</i>	intestine	small intestine	3/12.5	4/16.7	1	1	3	4
4	<i>Uncinaria stenocephala</i>	intestine	small and large intestines	5/20.8	7/29.2	1	1	5	7
5	<i>Toxascaris leonina</i>	intestine	intestine	11/45.8	9/37.6	3	3	28	23
6	<i>Toxocara canis</i>	intestine	intestine	9/37.6	8/33.3	4	3	9	8
7	<i>Spirocerca lupi</i>	stomach	–	2/8.3%	–	1	–	2	–
8	<i>Physoloptera praeputeiale</i>	stomach, intestines, abdominal cavity	oesophagus, stomach	6/25%	5/20.8	3	2	5	4
9	<i>Rictularia affinis</i>	intestine	intestine	7/29.2	6/25%	1	2	7	5
10	<i>Dirofilaria immitis</i>	right ventricle of the heart	pulmonary artery, right ventricle of the heart	3/12.5	2/8.3%	3	2	6	4
11	<i>Dirofilaria repens</i>	subcutaneous tissue	subcutaneous tissue	4/16.7%	3/12.5	2	2	7	4

The identified nematodes are typical representatives of the helminthofauna of both dogs and cats. During the study, the nematodes *Trichocephalus vulpis* and *Spirocerca lupi* were not detected in cats. In terms of prevalence and infection intensity, the dominant species are *Toxascaris leonina* and *Toxocara canis*, which concentrate in the intestine. *Toxascaris leonina* showed a prevalence of 45.8% and infection intensity of 3–28 individuals in dogs, and 37.6% and 3–23 individuals in cats.

*Toxocara canis* showed a prevalence of 37.6% and an infection intensity of 4 to 9 individuals in domestic Canidae, and 33.7% and 3–8 individuals in *Felis catus*.

The lowest prevalence in dogs was shown by *Spirocerca lupi* (8.3%), and in cats by 2 species of nematodes – *Diocotophyme renale* and *Dirofilaria immitis* (both 12.5%), with the infection intensity of 1–2 and 2–4 individuals, respectively. To establish the relation of the dominant nematode *Toxascaris leonina* to its hosts and compare it with the results of NCB, we conducted a molecular genetic analysis of this worm, which we repeated five times (Fig 1.).

## Discussion

By their life cycle, the discussed nematodes can be divided into 3 groups.

1. The life cycle of the first group does not include the changing of hosts, but their definitive hosts become infected in two ways. The first is an oral one, when the host ingests eggs with infectious larvae. This sub-

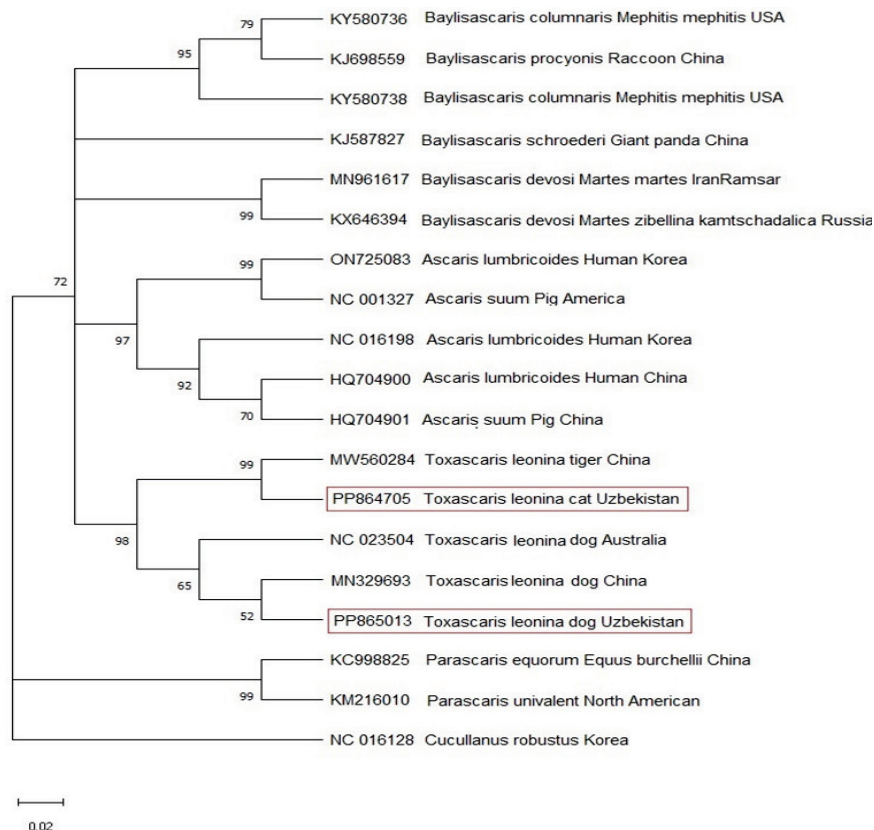
group includes representatives of the genera *Trichocephalus* and *Toxascaris*. The other one includes species of the genera *Ancylostoma* and *Uncinaria*, whose infectious larvae the hosts ingest with food or water. Predatory mammals can also become infected through the skin, or percutaneously, in the case of species from the genus *Ancylostoma*.

2. In group 2, parasites can either change hosts in their life cycles or develop completely in one host. This is typical of the genus *Toxocara*. Mouse-like rodents may also participate in the life cycle of the genus *Toxocara* as reservoir hosts. Carnivorous animals become infected, when they eat mice infested with eggs with stage 2 larvae.

3. In group 3, the life cycle necessarily includes intermediate hosts. This group includes most species of nematodes, representatives of the genera *Diocotophyme*, *Dirofilaria*, *Spirocerca*, *Physoloptera*, and *Rictularia*. The life cycles of a number of species from these families include reservoir hosts, which are food for predatory mammals.

The described types of nematodes' life cycles are extremely important from ecological and epizootological aspects.

The phylogenetic tree shows that *Toxascaris leonina* from dogs differs from the same species of nematodes from cats. The figure shows that isolates taken from different hosts are characterised by two subclades. One clade identified in dogs (PP865013) is close to an isolate from China (MN329693) and Australia (NC023504), and another one in cats (PP864705) to an isolate from the tiger (China, MW560284). In addition, the isolates are grouped together irrespective of hosts and location and are related to other species from the family Ascarididae.



**Fig.** Phylogenetic tree of the family Ascarididae (comparison of nucleotide sequences, analysis of a DNA section of *Toxascaris leonina* (from 5' to 3' – last three directions))

## Conclusion

Our research identified 11 species of nematodes from 8 families in dogs in Karakalpakstan. In cats, we identified 9 species of nematodes from 6 families. By their life cycles, the identified nematodes can be divided into three groups: 1 – those developing without changing hosts; 2 – those who in their life cycles may change hosts or can develop without changing hosts; 3 – those whose life cycles include the participation of intermediate hosts.

Our observations show that domestic dogs and cats in Karakalpakstan feature very similar nematode species diversities. Among the recorded nematode species, the most common are *Toxascaris leonina* and *Toxocara canis*, which cause serious diseases in the studied mammals. The nucleotide sequences of *T. leonina* from dogs differ from those of the nematodes recorded in cats and are characterised by two subclades.

The research work was carried out within the framework of the budget programme “Ways of the development of vertebrates’ helminthofauna, taxonomy and improvement of control measures” implemented in 2020–2024.

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