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Molecular identification of *Giardia duodenalis* isolates from dogs in Moscow (Russia)

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Abstract

The purpose of the research is to genetically characterize *Giardia duodenalis* isolates from dogs in the city of Moscow (Russia).

Materials and methods. A total of 423 fecal samples from dogs were investigated for *G. duodenalis* cysts in 2025. Twenty-five isolates were detected, 19 of which were characterized. Genetic analysis was performed based on the *β-giardin* locus which is 569–581 base pairs long. The obtained sequences were compared with reference sequences in the GenBank database.

Results and discussion. In the present study, for the first time, genotyping of *G. duodenalis* isolates obtained from domestic dogs in Moscow (Russia) was carried out. Assemblage C was identified in 15.8% of dogs (n = 3/19), assemblage D in 68.4% of dogs (n = 13/19) and three isolates were found to be mixed C and D in 15.8% (n = 3/19). The study revealed the presence of dog-specific assemblages of *G. duodenalis* that are not zoonotic. However, it is possible that dogs may have other *Giardia* assemblages, so further studies with a larger number of isolates are needed.

Keywords: *Giardia duodenalis*, assemblages, genotyping, dogs, zoonotic infection

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Научная статья

Молекулярная идентификация изолятов *Giardia duodenalis*, выделенных от собак в г. Москве (Россия)

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Аннотация

Цель исследований – генетическая характеристика изолятов *Giardia duodenalis*, выделенных от собак в городе Москве (Россия).

Материалы и методы. Всего исследовано 423 пробы фекалий собак на наличие цист *G. duodenalis* в 2025 г. Было выделено 25 изолятов, 19 из которых были охарактеризованы. Генетический анализ проводили на основе локуса *β-гиардин* длиной 569–581 пар оснований. Полученные последовательности сравнивали с референсными последовательностями в базе данных GenBank.

Результаты и обсуждение. В настоящем исследовании впервые проведено генотипирование изолятов *G. duodenalis*, полученных от домашних собак в Москве. Группа C была выявлена у 15,8% собак (n = 3/19), группа D — у 68,4% собак (n = 13/19), а три изолята оказались смешанными группами C и D у 15,8% (n = 3/19). Исследование выявило наличие специфичных для собак групп *G. duodenalis*, не являющихся зоонозными. Однако возможно, что у собак могут быть и другие группы *Giardia*, поэтому необходимы дальнейшие исследования с большим числом изолятов.

Ключевые слова: *Giardia duodenalis*, группы, генотипирование, собаки, зоонозная инвазия

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Introduction

G. duodenalis is one of the main causes of intestinal infections worldwide [7, 15]. It is widely spread due to easy transmission and a high resistance of cysts to environmental conditions, especially in water [7, 12, 15, 45]. The infection tends to be the most common in regions with poor level of life where the population lives in unsanitary environments. Children are particularly susceptible to *Giardia* infection, especially when it combines with malnutrition or other pathogens [6, 13]. *G. duodenalis* is also a frequent cause of intestinal infections in various animal species [4, 15]. The increasing role of companion pets in

human life combined with sociocultural factors and sanitary conditions in some regions, may increase the risk of zoonotic infection [5, 12]. In this regard, special attention should be paid to dogs, especially young animals are often infected with the protozoon *G. duodenalis* [4, 5, 9, 11, 12, 19, 27, 32]. Periodically, dogs and cats can harbor giardiasis asymptomatically, without any clinical manifestations, such as various gastrointestinal disorders, but remain a zoonotic threat to its owners [3, 20, 31, 32].

Currently, the genus *Giardia* comprises nine valid species, including *G. duodenalis* (syn. *G. intestinalis, G. lamblia*), *G. psittaci, G. agilis, G. ardeae, G. muris, G. cricetidarum, G. microti, G. peramelis,* and *G. varani*, distinguished by the morphology of their cysts and trophozoites, host specificity, and genetic uniqueness [8, 34, 35]. *G. duodenalis* among other species is uniquely genetically diverse, but all isolates of this species are morphologically similar to one another [10, 31, 42]. Molecular characterization of *G. duodenalis* is a useful and important tool in epidemiological studies. PCR analysis of *G. duodenalis* isolates targets polymorphic genes: the small subunit ribosomal RNA (ssu-rRNA), *β-giardin* (*bg*), *glutamate dehydrogenase* (*gdh*) and *triosephosphate isomerase* (*tpi*) genes [3, 25, 31, 33, 36]. Based on these loci, the *G. duodenalis* species was found to include eight major assemblages named from A to H. Assemblages C and D are specific for dogs, F is specific for cats, assemblage E is detected in livestock, G – in rodents, and assemblage H – in marine mammals [3]. Assemblages A and B are of the greatest interest. They are subdivided into subassemblages AI, AII, AIII, BIII and BIV with a high level of heterogeneity [32]. Isolates of assemblages A and B can be found in humans and many animal species (livestock, dogs, cats, guinea pigs, beavers) and can take parts in zoonotic transmission [4, 10, 11, 29].

Infected dogs may become a source of infection if they are in close proximity to humans. However, it depends on human living conditions and activities, the degree of contacts with animals, social factors and hygienic habits [1, 4, 5, 7]. Therefore, evaluating the prevalence of *G. duodenalis* assemblages among domestic dogs in a given region may provide useful information for the epidemiology of this infection [5, 11, 40].

Our previous studies have shown that *Giardia* is the most common protozoan in young dogs and cats in Moscow region. Thus, the prevalence of *G. duodenalis* reached 18.2% in dogs aged 1-12 months. Cases of asymptomatic cyst excretion were also noted [20]. Studies on the prevalence of *G. duodenalis* assemblages among domestic dogs in Russia have not been conducted yet, so the question of the presence of zoonotic assemblages in animals remained open until recently.

The aim of this study was to genetically characterize *G. duodenalis* isolates from dogs in the city of Moscow (Russia) for evaluation the current epidemiological situation and the potential for domestic animals to be a source of infection for humans.

Materials and methods

A total of 423 fecal samples from dogs were examined from February 2025 to August 2025. The fecal samples were provided by animal owners. All samples were collected and delivered in plastic containers labeled only with the species of the animal. Samples were stored at 4 °C and examined within 24 hours.

Direct smear was used to isolate *Giardia* cysts from the samples. A small amount of feces was mixed with a drop of normal saline on a slide, covered with a coverslip, and microscopy was performed using a Lomo microscope (Joint-stock company LOMO, Russia) at magnifications of 10× and 40× [47]. Results are shown in Fig.1. All *Giardia*-positive samples were stored at -20 °C until DNA extraction.

The *bg* genes were amplified using a single PCR. Primers developed by the authors were used in this study. The primer sequences are given below: GBGD-F: 5'-AGCTCAGCAACATGAACCAGC-3' and GBGD-R: 5'-ATCTCCGAGGCGACGTTCTC-3'. Amplification was performed on DT-96 thermal cycler (DNA-Technology LLC, Russia). The thermal profile consisted of an initial denaturation step of 95 °C for 3 min followed by 40 cycles of denaturation at 95 °C for 10 s, annealing and extension at 60 °C for 30 s, fluorescence signal collection. The length of the amplified PCR products was 569–581 bp. The intercalating dye SYBR Green was used to detect specific amplification; fluorescence signal detection was carried out through the corresponding channel.

PCR products were subjected to direct bidirectional Sanger sequencing with primers used for the initial detection. Sanger sequencing

was performed in Syntol laboratory (Moscow). The obtained sequences were initially processed using "Chromas 2.6.6." (Technelysium Pty Ltd) software. The obtained nucleotide sequences were analyzed using NCBI databases.

The GenBank accession Nos of the reference sequence for assemblages C were: PX454781, PX454782 and PX454786, for assemblages D were: PX454779, PX454780, PX454780-85, PX454787 and PX454788.

Chromatograms were viewed in Chromas software to assess sequence quality. Only high-quality sequences were used for further analysis. These sequences were aligned with other sequences of the *bg* gene of *G. duodenalis* deposited in GenBank NCBI (https://www.ncbi.nlm.nih.gov/genbank/) by Clustal W in MEGA 7 using the default parameters. Phylogenetic tree was inferred using the Maximum Likelihood (ML) method under the GTR + I + G model of evolution using MEGA 7 [18]. To obtain an estimate of the support for each node, a bootstrap analysis using 1000 replicates was performed. Bootstrap support is given on appropriate clades for the ML tree.

Confidence intervals (CI) of the frequency of assemblage occurrence were calculated using IBM SPSS Statistics version 26.0 (IBM SPSS, NY, USA).

Results

G. duodenalis cysts were found in 25 (5,9%) of 423 samples from dogs.

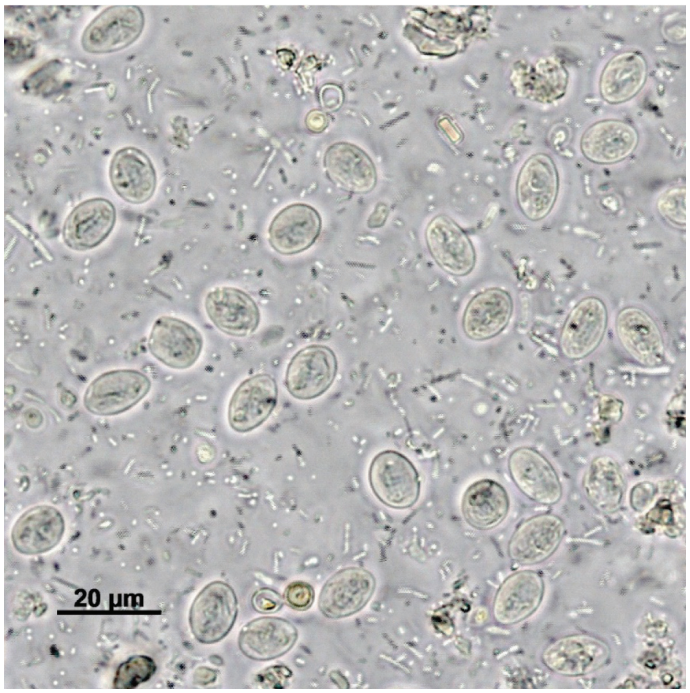

Fig. 1. Cysts of *Giardia duodenalis*. Scale bar: 20 µm
Рис. 1. *Giardia duodenalis* (масштабная линейка = 20 мкм)

Nineteen out of 25 *G. duodenalis* isolates obtained from dogs' feces were successfully sequenced and reliably compared. The study showed that assemblage C was identified in 15.8% of dogs (4.7–36.4%, CI), assemblage D in 68.4% (46.1–85.6%, CI) and three isolates were found to be mixed C and D in 15.8% (4.7–36.4%, CI). Assemblage D was the most prevalent. Statistical analysis showed that the distribution was significantly different from uniform (with equal shares) – $\chi2(1) = 10.526$, $p = 0.005$. The data are presented in Table No. 1.

A total of 19 sequences were obtained, constructed by superimposing the sequence from the forward primer onto the reverse complementary sequence of the reverse primer. Some sequences were identical to each other. Double peaks were observed in the chromatograms in three cases showing discrepancies in assemblages C and D, indicating that a mixture of assemblages C and D was present in these original samples.

The PX454788 sequence obtained and the most common in this study was identical to sequences

Table 1

Prevalence of *Giardia duodenalis* assemblages in dogs

Таблица 1

Распространение *Giardia duodenalis* у собак

G. duodenalis assemblages	Prevalence		95.0 % confidential intervals
	n	%	
C	3	15.8	4.7–36.4
D	13	68.4	46.1–85.6
C+D	3	15.8	4.7–36.4

deposited in GenBank from Spain (PV153057), Egypt (OQ787091), China (KY979501) and Brazil (KT728467). The PX454786 sequence obtained in this study had 99.83% similarity to *G. duodenalis* assemblage C sequence from Brazil (JF422718) deposited in GenBank. Another sequence obtained in this study also had a high degree of similarity to G. duodenalis assemblage C sequence from Brazil (JF422718) deposited in GenBank. The PX454782 sequence had 99.82% similarity, while the PX454781 sequence had 99.65% similarity.

The phylogenetic relationships between *G. duodenalis* assemblages isolated from dogs are presented in Fig. 2.

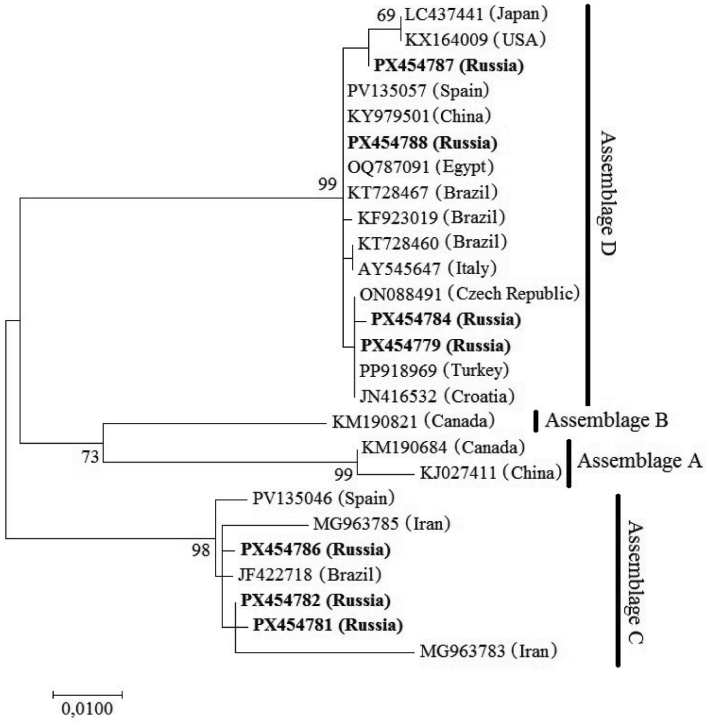

Fig. 2. Phylogenetic relationships of *Giardia duodenalis* from Moscow, Russia with other populations of this species as inferred from the ML method using the *beta-giardin* (*bg*) gene sequences under the GTR + I + + G model. Bootstrap support is given on appropriate clades. The new sequences are indicated in bold

Рис. 2. Филогенетическая взаимосвязь *Giardia duodenalis* из Москвы с другими изолятами этого вида, полученными методом ML с использованием последовательностей генов бета-гиардина (bg) в рамках модели GTR + I+ G. Новые последовательности выделены жирным шрифтом

Discussion

Population, habitat, and region have an influence on the assemblages circulating in dogs. Thus, the distribution of assemblages varies across regions and the zoonotic risk varies greatly [1, 4, 5, 11, 32].

Studies conducted in many countries have shown varying distributions of assemblages in dogs. Thus, Bahramdoosta et al. (2021) showed the presence of C (15 %), A (20 %) and B (30 %) assemblages in dogs, with zoonotic assemblages being reported more frequently than species-specific ones in Iran [4]. Esmailzadeh et al. (2023) detected assemblages C (1,21 %), D (0,83 %) and zoonotic assemblage A (0,83 %), but non-zoonotic assemblages were predominant in Iran [9]; similar results were obtained by Uiterwijk et al. (2020) in the Netherlands – assemblages C (9 %), D (25,9 %) and zoonotic assemblage A (1,1 %) [44], Mateo et al. (2023) in Spain – C (36,6 %), D (27,3 %) and A (18,2 %) [26], Murnik et al. (2023) in Germany – C (38,4 %), D (35,7 %) and zoonotic assemblage A (8 %) [27]. In our study, only assemblages C (17,6 %) and D (82,3 %) were detected in dogs. Studies conducted by Osmari et al. (2021) showed the presence of only species-specific assemblages in dogs in Brazil [29], while a year earlier Trevisan et al. (2020) showed the presence of assemblages C (30 %) and A (10 %) [42]. A similar combination of assemblages C and A in dogs was shown in Argentina [22] and in Germany [27]. Gultekin et al. (2017) showed only zoonotic assemblages A (42 %) and B (100 %) in dogs in Turkey [11]. In addition, only zoonotic assemblage A in dogs was detected by Sotiriadou et al. (2013) in Germany [36] and Krumrie et al. (2022) in the UK [17]. In Italy, Agresti et al. (2021) found that assemblages A (64 %) and B (24 %) were predominant in dogs and also identified assemblage C (8 %), but to a lesser extent [2]. Species-specific assemblages C and D have been exclusively identified in dogs in Taiwan [43], Poland [31, 32], China [23, 24, 38, 46], Portugal [30] and Thailand [39].

This wide distribution of species-specific assemblages in dogs is thought to be a consequence of their better adaptation to these animal species than those of A and B assemblages isolated from humans [23, 44].

Transmission of assemblages common to animals and humans is possible from animal to animal, animal to human, and human to animal; however, the frequency and ratio of their occurrence are unknown [13, 41]. Two routes of Giardia transmission may be present in urban environment: transmission of dog-specific assemblages circulating among these animal species and potential transmission of zoonotic assemblages between animals and humans. Transmission of dog-specific assemblages is thought to be facilitated by intense contact between dogs in large communities competing with transmission of other assemblages. In our study, information on the origin of animals was not available, but we suspect, as it has been reported in several studies, that animals' environment is significantly contaminated with dog-specific assemblages in kennels and shelters [44]. In addition, the friendly nature of domestic dogs facilitates their close communication leading to the spread of dog-specific C and D assemblages; young animals with coprophagia are especially at risk [12]. Uiterwijk M. et al. (2020) report a similar assumption about Giardia circulation, in particular the high prevalence of assemblages D and C/D in hunting dogs, associated with living conditions and behavior favorable for feco-oral transmission of species-specific assemblages [44].

Giardiasis is a widespread disease among people and household and shelter dogs in Russia. According to Stepanova et al. [37] a total of 604 673 cases were registered in all constituent entities of the Russian Federation in 2010-2021, of which 70.1 % were among children of 0-17 years old, 423 596. However, at the same time, the dynamics of morbidity rate were characterized by a pronounced downward trend from 58.5 cases per 100 thousand population in 2010 to 12.7 in 2023, respectively, in children aged 3-6 years from 121.2 to 56 cases per 100 thousand children in this age group. However, in 2023, an increase in the incidence rate was noted, amounting to 15.8 cases per 100 thousand population, and in children – 62 cases per 100 thousand children in this age group. The water route of transmission among humans is considered to be the main route [28, 37]. Giardia infection in dogs is registered in different regions of Russia, but the prevalence varies. In Moscow, for example, the infection rate was 17.1% in young dogs [21]. In Novosibirsk region, the prevalence was 14.4 % in dogs [16]. *G. duodenalis* isolates circulating among humans and animals in Russia have not been previously characterized. Our study is the first one and it characterizes *G. duodenalis* isolates of domestic dogs only in the territory of Moscow city. Thus similar studies are needed in

other regions to fully access the epizootic situation of Giardia in animals.

Conclusion

G. duodenalis isolates from domestic dogs in Moscow were found to belong to assemblages C, D, and mixed assemblages C+D, which are species-specific and pose no risk of transmission to humans. However, it is possible that other *G. duodenalis* assemblages may be identified in dogs by studying a larger number of isolates. Therefore, further monitoring of *G. duodenalis* prevalence among pets in Moscow is necessary.

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