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The Cycle of Nematode *Dirofilaria Immitis* (Leidy, 1856) in the Ecological and Epizootological Chains of Canines in the Biocoenoses of Uzbekistan

Norkobilov Bakhromjon To'rayevich[1], Alisher Abdukahor Ugli Safarov[1, *], Firuza Djaloliddinovna Akramova[2], Djaloliddin Azimovich Azimov[2], Ulugbek Abulakimovich Shakarbayev[2], Abad Sultamuratovich Berdibayev[3]

[1]State Committee of Veterinary and Livestock Development of the Republic of Uzbekistan, Tashkent, Uzbekistan

[2]Institute of Zoology, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan

[3]Nukus State Pedagogical Institute, Nukus, Uzbekistan

Email address:
safarov-alisher@mail.ru (A. A. U. Safarov)

[*]Corresponding author

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Abstract: The importance of service dog breeding for humanity is difficult to overestimate. Infectious and invasive diseases are a limiting factor for breeding and improving dog breeds. They not only bring significant economic damage, do not allow the use of dogs in various sectors of the economy, sports, the army, law enforcement agencies, exchange of animals, and can also be dangerous to people. Such diseases include dirofilariasis-the only helminthiasis, which in the temperate continental climate of our country is transmitted by transmission through mosquitoes. The work-studies some issues related to the epizootology of dirofilariasis in domestic and wild canines and the distribution of *D. immitis* in domestic dog and common jackal. It also specifies the range of the intermediate hosts of this nematode. In the natural and urbanized territories of Uzbekistan four species of mosquitoes from the genera *Anopheles*, *Aedes* and *Culex* were identified as the nematode's intermediate hosts; the rate of their infection with *D. immitis* larvae was 2.4–5.6%.

Keywords: *Dirofilaria*, Distribution, Epizootology, Dog, Jackal, Mosquitoes, Uzbekistan

1. Introduction

Mammal predators are an essential biotic component of terrestrial ecosystems. In the biocoenoses of Uzbekistan, 33 species and subspecies from the families Canidae, Ursidae, Mustelidae, Hyaenidae and Felidae which form multispecies communities and act as hosts for various groups of ecto- and endoparasites represent them. In this context, special attention should be paid to Canidae consisting of diverse wild and synanthropic species. Various populations of these animals (dog, wolf, and fox) proved infected with various parasitic worms, including Dirofilaria [2-5]. The authors detected *Dirofilaria immitis* (Leidy, 1856) and *D. repens* Railliet and Henry, 1911 in domestic dogs, wolves and foxes. The information provided in the abovementioned works is of a purely faunistic character. It is fragmentary and quite outdated. According to recent research [12], representatives of the genus *Dirofilaria* Railliet et Henry, 1911 are increasingly more often recorded in domestic dog and wild canines in Uzbekistan, in both natural and urbanised environment.

The increasingly wide use of domestic dogs in security and investigation services has led to the growth of dog breeding activity in Uzbekistan. One of the most important issues in this area is to keep the animals in good health through development of diagnostic methods and treatment of sick dogs. This also refers to dirofilariasis of dogs.

Since Dirofilaria contribute to the development of

pathologies in dogs and humans [3, 14], we thought it necessary to study the biology and life cycle of *D. immitis* and the distribution of this nematode in the natural and urbanised ecosystems of Uzbekistan.

2. Material and Methods

2.1. Are of Study

The material used in this work consisted of *Dirofilaria* collected by the authors from domestic dogs from urban and rural populations and common jackals from the terrestrial biocoenoses of the Republic of Karakalpakistan. The research was carried out in 2016-2019 at the State Centre for the Diagnosis of Animal Diseases and Food Safety.

Dirofilaria were collected and studied following common methods [6].

2.2. Data Collection Methods

To establish the fact of being infected with helminthes, including *Dirofilaria*, the researchers carried out the complete helminthological dissection of 160 domestic dogs of different age and 186 individual organs from animals from the urban canine population of Tashkent, as well as 68 individuals of domestic dog from rural populations from various biocoenoses in Karakalpakistan. In addition, we examined 35 individuals of common jackal from natural populations.

To identify the intermediate hosts of Dirofilaria (*D. immitis*) the researchers studied mosquitoes (Culicidae) caught on dogs and near them. In total, 4,686 individuals of mosquito were studied in spring, summer and autumn following a common method [1].

To estimate the rate of infection with helminths in carnivores standard parasitological indicators were used-prevalence (%) and intensity (inds).

3. Results

3.1. Spontaneous Invasion of Predatory Carnivorous Dirofilariasis in Uzbekistan

Only *Dirofilaria immitis* (Leidy, 1856) was detected and identified in domestic dogs, jackals and foxes when 288 animals were examined. The invasiveness of the studied carnivores ranged from 7.7 to 18.6% (table 1, figure 1).

Table 1. *Spontaneous invasion of predatory carnivorous dirofilariasis in Uzbekistan.*

Speces	Investigated, instance	Infected		
		Ex. i, %	In. i, instance.	Localization
*Canisfamiliaris*dom.	228	9.6	1-17	Heart, pulmonary artery
Canisaureus	35	18.6	11-19	Heart
Canislupus	13	-	-	-
Vulpesvulpes	12	7.7	1-2	peritoneal cavity

The greatest infection with dirofilariae was observed in the Jackal (EI-18.6%) and the lowest in the Fox (EI-7.7%). Therefore, the indicator, dogs occupy an intermediate position, where the invasiveness was 9.6%.

The submissions show that dirofilaria fairly common in domestic dogs in both the urban and rural population. It turned out that the nematodes of *D. immitis* in Uzbekistan are found in dogs regardless of the methods of their maintenance. They are most often registered in stray and domestic dogs of urban populations (EI-5.0%) and in service shepherds, farms of rural population (EI-13.2%). The intensity of infestation in both populations ranged from 1-17 copies. the greatest infection was observed in dogs at the age of 3-5 years. The extent of infestations of dogs of this age group reached up to 16.5%, with the intensity of infestations of 4-17 specimens. the least infestation was observed in dogs under the age of 1 year (EI-1.2%, AI-1-2 specimens).

We examined 263 individuals and, additionally, 186 organs and separated mature males and females of *Dirofilaria immitis* (Leidy, 1856) detected in the right ventricle and pulmonary artery of dogs from rural and urban populations and common jackals (figure 1). We collected 196 individuals of Dirofilaria, 83♂ and 113♀. The males-to-females ratio was 42.3%♂ to 57.7%♀.

In Uzbekistan, nematodes *D. immitis* are detected in any dogs, regardless of the conditions they live in. Most often, they occur in stray and domestic dogs from urban populations (prevalence-5.0%) and herding dogs from rural populations at farms (prevalence-13.2%). The intensity of infection in both populations ranged between 1 and 17 inds. The highest infection rate was recorded in dogs aged 3-5 years. The prevalence recorded in this age group reached 16.5%, while the intensity of the infection was 4-17 inds. The lowest rate of infection was recorded in dogs aged below 1 year (prevalence-1.2%, intensity-1-2 inds).

3.2. Morphological Signs of Dirofilaria Immitis and Conducted Laboratory Analyzes

Figure 1. *D. immitis (Leidy, 1856): mature Dirofilaria detected in a dog's heart.*

The researchers also estimated the seasonal dynamics of infection with nematode *D. immitis* in dogs: the recorded prevalence was 13.1% in spring, 14.2% in summer, 16.9% in autumn and 15.4% in winter, which indicates low variation of prevalence depending on the season.

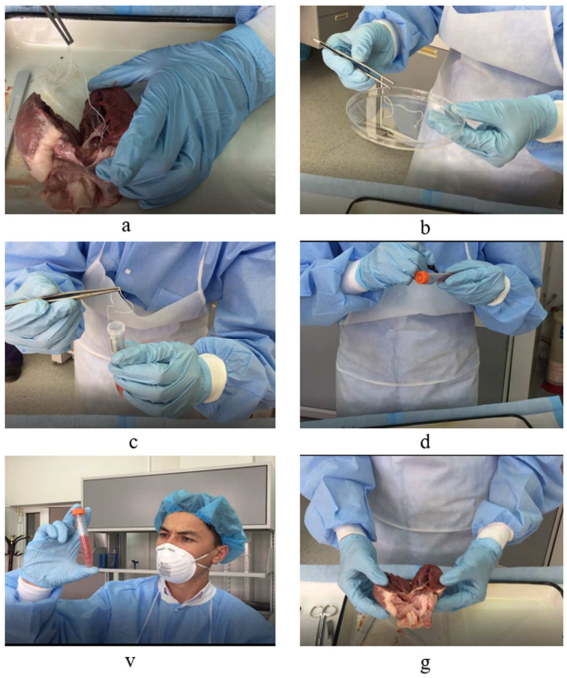

Figure 2. *Laboratory analyses of Dirofilaria immitis (Leidy, 1856).*

The age structure of *D. immitis* populations varies greatly depending on the season. In summer and spring, only mature males and females were recorded in dogs. In autumn and spring, both mature and immature individuals of this nematode were recorded in the organisms of infected dogs.

We also recorded *D. immitis* in common jackal-*Canis aureus* L., 1758-from populations in Shumanay and Kanlykul Districts of the Republic of Karakalpakistan. They concentrated in the right ventricle and major pulmonary arteries. The prevalence was 18.6%. The intensity was 11-19 inds. The highest prevalence was recorded in late autumn (20.5%).

According to our research, in Uzbekistan rural and urban populations of dog and populations of common jackal show high rate of infection with *D. immitis* causing dirofilariasis in carnivores. This infection contributes to the development of pathologies in humans, which is supported by numerous publications [2, 3, 13].

Since this infection causes serious problems in working dogs and humans, on the one hand, while the biology of its development in the organism of the definitive and intermediate hosts is not yet studied properly, on the other hand, we thought it important to specify the range of intermediate hosts and their rate of infection with nematode larvae in Tashkent.

The results of parasitological research into blood sucking Diptera show that *D. immitis*'s intermediate hosts are four species of mosquitoes-*Anopheles maculipennis, Aedes caspius, Culex modestus* and *C. pipiens* (table 2).

Table 2. *Rate of infection with D. immitis larvae in Tashkent.*

No.	Species	Examined, inds	Infected	
			No. of inds	Prevalence, %
1	*Anopheles maculipennis* Mg.	1010	37	3.6
2	*A. superpictus* Grassi	920	-	-
3	*Aedes caspius* Edw.	1018	49	4.8
4	*Culex modestus* Fic.	1002	25	2.4
5	*C. pipiens* L.	1034	58	5.6
6	*Culex pusillus* Macq.	980	-	-

In Tashkent, mosquitoes attack dogs in warm seasons, usually, between April and October. The largest populations are recorded in July, with slightly smaller numbers in August and September. In some years, this period continues until the second half of October.

3.3. Stages of Development of Microfilaria and Distributors of Dirofilaria Immitis (Survey of Districts of Tashkent Megapolis Area)

The table shows that the rate of infection with Dirofilaria larvae varies depending on the mosquito species. The highest rate was recorded in *Culex pipiens* and *Aedes caspius*. The infection intensity was 22-25 and 13-17 inds, respectively. The lowest rate was in *Culex modestus* (2.4%).

Overall, the rate of infection with *D. immitis* larvae in mosquitoes in the studied districts (Bektemir, Sergeli, Yunusabad and Almazar districts) of Tashkent was quite high (2.4-5.6%), which indicates that the conditions in urban biotopes are also favourable for the development of larval stages.

D. immitis microfilariae were recorded in the intestines of some individuals of mosquitoes at different time after they had been caught on or near dogs that were known to be infected. Most of the blood-sucking individuals had developing larvae in their Malpighian tubes, where 3rd-stage infectious larvae were also recorded migrating towards the mosquitoes' heads. According to our observations, in a laboratory (at a temperature of 28-30°C and relative humidity of 70-80%) *D. immitis* develop into the infectious stage in mosquitoes *Culex pipiens* within 10-13 days. Given below are the comparative morphometric parameters of microfilariae from the intestines of mosquitoes *C. pipiens* (45 minutes after their extraction), a fixed smear stained with the Romanovsky-Giemsa method and microfilariae from the blood of a Central Asian shepherd dog named Alabay (Almazar District, Tashkent).

The microfilariae are unsheathed. The head end is rounded, the tail is pointed. Dimensions: between 0.168 mm

and 0.224 mm (0.196 mm on average) in length and between 0.004 mm and 0.006 mm (0.005 mm on average) in width (figure 3). Similar parameters were recorded in microfilariae from the peripheral blood of a dog, with only one exception. The body length ranged between 0.166 mm and 0.225 mm, with an average of 0.195 mm, which had no differential meaning. We identified the recorded microfilariae as *D. immitis*.

As was already noted, infectious *D. immitis* larvae develop in mosquitoes, their intermediate hosts, and concentrate in the insects' heads, from where, when the latter bite dogs, the larvae are inoculated into the definitive host's blood vessels. On the 13th day of staying in mosquitoes they reach a length of 1.32 mm to 1.84 mm (1.58 mm on average) and a width of 0.028 mm to 0.048 mm (0.038 mm on average) (figure 3). The infectious larvae are highly mobile. The front end of their body is cylindrical, and the tail is short and conic. The esophagus is 1/3 of the length of the larva's body. The intestine is well developed. A nerve ring and rudimentary genitals can be discerned. Further, the larvae develop in the organism of a definitive host. The results of our observations almost entirely correspond with those of the research made earlier in other countries [2, 3, 13, 14].

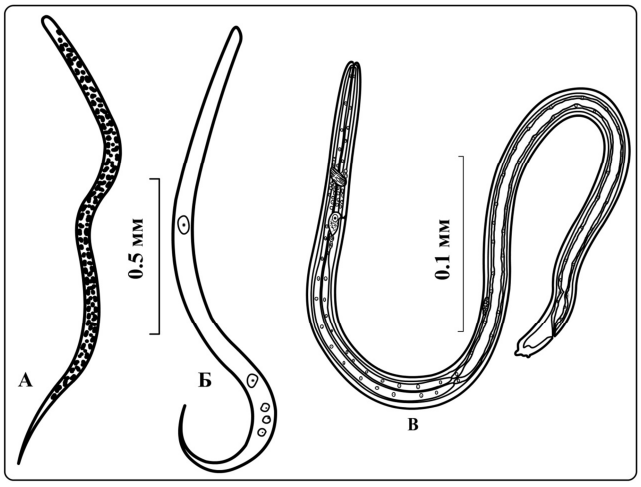

Figure 3. *D. immitis (Leidy, 1856): A-microfilariae, B-1st-stage larva, C-infectious larva.*

Thus, in the city of Tashkent and in the natural biocoenoses of Uzbekistan nematode *D. immitis* has the following cycle pattern: definitive hosts (dogs, foxes, wolves, jackals) → intermediate hosts (mosquitoes *Culex pipiens*, *C. modestus, Aedes caspius, Anopheles maculipennis*) → definitive hosts (pic. 4).

4. Discussion

D.immitis was recorded in rural and urban populations of dog and populations of common jackal from the study areas-the city of Tashkent and Republic of Karakalpakstan.

The highest rate of infection was recorded in dogs from rural populations and working dogs from some districts of Karakalpakstan.

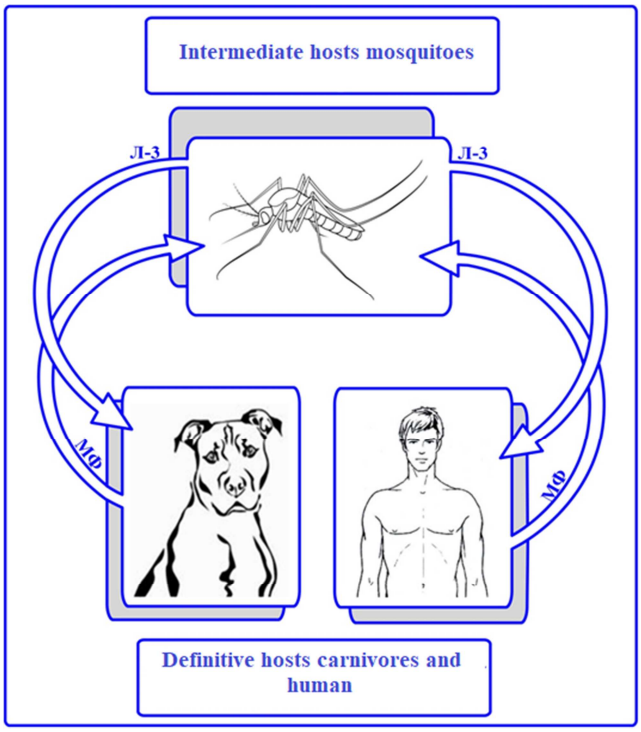

Figure 4. *D. immitis (Leidy, 1856) life cycle pattern: MF-microfilariae, L-3-infectious larvae.*

Intermediate hosts mosquitoes Definitive hosts carnivores and human. МФ-MF, Л-3-L-3.

Wild and domestic predators, such as dog, jackal, wolf and fox, are the most important supporters of Dirofilaria's cycle (figure 4).

A large portion of mosquitoes (genera *Culex, Aedes, Anopheles*) in the study area was infected with *D. immitis* larvae.

Thus, in the biocoenoses of Uzbekistan nematode *D. immitis* has the following cycle pattern: definitive hosts (dogs, foxes, wolves, jackals) → intermediate hosts (mosquitoes *Culex pipiens*, *C. modestus, Aedes caspius, Anopheles maculipennis*) → definitive hosts.

5. Conclusion

D. immitis was detected in dogs of rural and urban populations and common Jackal, Fox of the surveyed territories of Uzbekistan-the megalopolis of Tashkent and the Republic of Karakalpakstan.

Jackals from the regions of Karakalpakstan characterize the highest infection.

Domestic and wild predators such as the dog, Jackal, and Fox are of paramount importance in maintaining the circulation of dirofilaria.

Significant infection of mosquitoes (Culex, Aedes, Anopheles) with *D. immitis* larvae was observed in the studied territory.

Thus, the circulation of the nematode *D. immitis* in the biocenoses of Uzbekistan occurs according to the scheme: definitive hosts (dogs, jackals, wolves, foxes) → intermediate

hosts (mosquitoes *Culex modestus, C. pipiens, Aedescaspius, Anopheles maculipennis*) → definitive hosts.

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