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EPIDEMIOLOGICAL INVESTIGATION OF DIROFILARIA IMMITIS IN MOSQUITOES FROM SAMARKAND REGION, UZBEKISTAN: PRELIMINARY RESULTS

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Abstract

Dirofilaria immitis, commonly known as heartworm, poses a substantial threat to canines in the Samarkand region, Uzbekistan. This study aims to comprehensively investigate the prevalence and transmission dynamics of Dirofilaria immitis, considering the distinctive geographical and climatic factors influencing disease spread. A cross-sectional survey conducted from January 2022 to December 2023, involved the Knott's test, the presence of D. immitis microfilariae was assessed. Simultaneously, 4828 female mosquitoes were sampled in June, July and September 2023 to investigate their potential role as vectors. A total of 41 (25.7%) dogs tested positive for D. immitis microfilariae. District-wise analysis highlighted significantly higher infection rates in Urgut 10 (55.5%), Jomboy 6 (42.8%), and Payariq 11 (42.3%) districts. Concurrently, an infection rate of 4.9% was observed in 237 mosquitoes, with the Jomboy mosquitoes having the highest infection rate of 130 (54.8%). The prevalence of D. immitis larvae in mosquitoes is notably elevated, ranging from 3.8% to 10.2% in the studied areas of the Samarkand region. The intensity of infestation varied from 16-26 larvae in Culex pipiens and 10-16 larvae in Anopheles maculipennis.

Keywords: Dirofilaria immitis, Mosquitos, Epidemiology, Samarkand, Uzbekistan

1. Introduction

Uzbekistan boasts a rich and diverse fauna, a result of its strategic geographical location and the varied topography and natural environments within the republic.

The expansive territory of Uzbekistan encompasses three distinct landscape zones, plains, foothills, and mountains, each hosting a unique array of habitats with distinct ecological characteristics. The diverse fauna is attributed to the abundance of these habitats, providing a home for a wide range of vertebrates, including predatory mammals (Safarov et al., 2021a, Deak et al., 2022).

Understanding the parasitic helminth fauna in companion animals is not only of profound theoretical interest, but also holds significant practical implications, as these parasites can pose a threat to human health. Dogs, being the most prevalent companion animals globally, and maintaining intimate proximity to humans, play a crucial role in this context (Safarov et al., 2022a). Consequently, vigilant monitoring of parasitic diseases in dogs should be a top priority for parasitologists. The parasitic nematode Dirofilaria immitis, commonly referred to as heartworm, predominantly infects dogs but can also be found in felids, wild canids, and, albeit extremely rarely, humans. In a survey Dogs, both domestic and wild canids, serve as the primary reservoirs for this parasite (Ciucă et al., 2016), while mosquitoes act as the exclusive known intermediate host and vector for D. immitis (Slocombe et al., 1989; McGill et al., 2019).

Recent research has significantly updated and supplemented information on heartworms (D. immitis and D. repens) in dogs and some wild predator species (Saparov, 2016; Azimov et al., 2019; Norkobilov et al., 2020; Safarov et al., 2021a, 2022a,b; Berdibaev, 2021). However, there has been insufficient research on dirofilariasis in domestic and stray dogs, which is prevalent in urban and rural areas of the Samarkand region, one of the central regions of Uzbekistan. Additionally, existing literature is outdated. According to literary sources, human and animal dirofilariasis is endemic in the Samarkand region (Avdyuhina et al., 1997; Safarov et al., 2023a).

The Samarkand region holds significant tourist importance in Uzbekistan, attracting more than 5 million tourists in 2023, with an expected increase to 10 million in 2024. Moreover, the region shares a border with the Republic of Tajikistan and is traversed by the Zarafshan River. These indicators underscore the need to study the epizootic situation of D. immitis, the causative agent of dirofilariasis, which is zoonotic in all regions of the area, and to enhance countermeasures. In this context, the aim of this study is to investigate D. immitis in mosquitoes in the Samarkand region, Uzbekistan.

2. Materials and methods

2.1. Mosquito sampling and processing

Mosquito sampling was conducted in the Jomboy, Bulungur, and Okdaryo districts of Samarkand, Uzbekistan during June, July, and September 2023. A total of 4,828 female mosquitoes were captured over this period.

These districts were chosen for the study because they are located on the banks of the Zarafshan River. Mosquito capture employed Biogents® Sentinel 2 traps (Biogents AG, Regensburg, Germany), utilizing carbon dioxide and BG-Lure™ (Biogents AG, Regensburg, Germany) or octanol (Biogents AG, Regensburg, Germany) as attractants. Each trap, positioned approximately 1.5 m above the ground, was deployed at 17:00 and retrieved four days later around 10:00. Subsequent to capture, individual mosquitoes were morphologically identified to the species level following established keys (Severini et al., 2009). The specimens were then grouped based on species, sampling dates, and province.

For each mosquito pool, a 10-min bead-based mechanical lysis was performed in the TissueLyser apparatus. The lysis was performed in the presence of 800 µl of MEM medium (Sigma Aldrich). Mosquito lysates were then centrifuged at 13,000g for 3 min, and genomic DNA was extracted from 200 µl of the supernatant in the presence of 100 µl of lysis buffer. The extraction was performed using the QIAcube kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. DNA was eluted in a final volume of 100 µl and stored at -20 °C until analysis. Finally, the extracted genomic DNA was analysed for the presence of Dirofilaria spp. using a multiplex real-time qPCR assay as elsewhere by Laidoudi et al. (2020).

2.2. Statistical analysis

The statistical analysis was conducted utilizing EpiInfo 7 software (CDC, USA). Prevalence and 95% confidence intervals (CI) were calculated for each parasite species globally, as well as stratified by environment and region. Group differences were evaluated using the chi-square test, with significance set at a p-value <0.05.

3. Results

Morphological identification revealed the presence of at least six mosquito species, dominated by Aedes caspius (21.9%), followed by Anopheles maculipennis (20.9%), Culex modestus (18.8%), Anopheles superpictus (15.4%), Culex pipiens (14.0%), and C. pusillus (8.6%). Culex pipiens (10.3%; 95%±CI 10.3±0.023) and Anopheles maculipennis (6.6%; 95%±CI 6.6±0.015) exhibited the highest

infestation rates, with invasion intensity ranging between 10-26 and 10-16 larvae respectively. Aedes caspius displayed minimal infestation (3.9%; 95%±CI 3.9±0.011). Overall, mosquito infestation with D. immitis larvae in the studied areas (Jomboy, Bulungur, and Okdarya) in the Samarkand region ranged from 3.8% to 10.2% (Table 1).

Table 1: Infection rates of mosquitoes with *Dirofilaria immitis* larvae in overall and according to each district of Samarkand region, Uzbekistan.

Mosquito species	Studied districts of Samarkand region, Uzbekistan							
	Jomboy		Bulungur		Okdaryo		Total	
	Positive/total (Rate, %±C.I.[1])	P value	Positive/total (Rate, %±C.I.[1])	P value	Positive/total (Rate, %±C.I.[1])	P value	Positive/total (Rate, %±C.I.[1])	P value
A. maculipennis	39/580 (6.7±0.019)	0.000*	11/318 (3.5±0.019)	0.000*	17/112 (15.2±0.066)	0.000*	67/1010 (6.6±0.015)	0.000*
A. superpictus	0/220 (0)		0/188 (0)		0/340 (0)		0/748 (0)	
A. caspius	27/610 (4.4±0.015)		5/233 (2.1±0.017)		9/217 (4.1±0.027)		41/1060 (3.9±0.011)	
C. modestus	23/175 (13.1±0.050)		18/286 (6.3±0.027)		18/449 (4.0±0.017)		59/910 (6.5±0.015)	
C. pipiens	41/202 (20.3±0.054)		2/238 (0.8±0.011)		27/240 (11.3±0.039)		70/680 (10.3±0.023)	
C. pusillus	0/190 (0)		0/112 (0)		0/118 (0)		0/420 (0)	
Total	130/1977 (6.6±0.011)		36/1375 (2.6±0.007)		71/1476 (4.8±0.011)		237/4828 (4.9±0.005)	

Abbreviations: [1]: C.I.: 95% confidence interval, *: Statistically significant, $p < 0.05$.

Parasitological studies of blood-sucking dipterans identified four mosquito species as intermediate hosts of D. immitis: Anopheles maculipennis, Aedes caspius, Culex modestus, and C. pipiens (Table 1). Out of 4828 examined mosquitoes, 237 (4.9%; 95%±CI 4.9±0.005) were infected with D. immitis microfilariae. The highest mosquito infestation was recorded in the Jomboy district (130/237; 54.8%).

4. Conclusion

In conclusion, this pioneering study offers provides an in-depth insight into the prevalence and transmission dynamics of Dirofilaria immitis in the Samarkand region of Uzbekistan. By adopting a multifaceted approach, we uncovered significant regional variations in infection rates, with Urgut, Jomboy, and Payariq districts emerging as high-risk areas. The interplay of geographical, climatic, and ecological factors, such as the presence of the Zarafshan River, underscores the need for localized, targeted preventive measures.

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