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MONITORING OF ANIMAL DIROFILARIOSIS INCIDENCE IN KHARKIV REGION OF UKRAINE

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Monitoring of Animal Dirofilariosis Incidence in Kharkiv Region of Ukraine. Paliy, A. P., Sumakova, N. V., Pavlichenko, O. V., Palii, A. P., Reshetylo, O. I., Kovalenko, L. M., Grebenik, N. P., Bula, L. V. — A total of 38 species of mosquitoes of the Culicidae family of two subfamilies *Anophelinae* and *Culicinae* belonging to 6 genera, were identified on the territory of Kharkiv Region from 2009 to 2019. Dirofilaria larvae were found in 932 insects, which was 4.46 % of the surveyed mosquitoes. The prevalence of the infection of female mosquitoes by dirofilaria was 4.46 ± 0.24 %, while the mean intensity of dirofilariae was 5.4 ± 2.1. Infection of mosquitoes with dirofilariae is currently detected in 11 districts of Kharkiv Region and the City of Kharkiv. The infection rate of mosquitoes of the genus *Aedes* was 3.83 ± 0.63 %, of the genus *Culex* — 0.75 ± 0.34 %, of the genus *Anopheles* — 0.12 ± 0.39 %, whereas females of the genera *Culiseta, Coquillettidia*, and *Uranotaenia* were uninfected. It was found that in Kharkiv Region there were 2 types of canine dirofilariae — *Dirofilaria immitis* (Leidy, 1856) and *Dirofilaria repens* (Railliet & Henry, 1911), with a predominance of *Dirofilaria repens*. For the period from 2009 to 2019, we examined 378 blood samples from dogs (190 males and 188 females) aged from 8 months to 13 years with suspected dirofilariasis. According to the results of the studies, 140 samples were positive, the prevalence of infection by the larvae of *Dirofilaria* spp. in blood samples from dogs with suspected dirofilariasis was 37.03 ± 0.12 %, while infection with *Dirofilaria immitis* was 2.86 ± 0.45 %, and *Dirofilaria repens* — 97.35 ± 0.24 %. The number of positive samples from mongrel dogs was 42 (30 %). It was found that cats are more often amicrofilariaemic than dogs.
Key words: dirofilariasis, dirofilariae, vectors, dogs, blood, mosquitoes.

Introduction

Parasitic diseases of animals bring significant economic losses, and many diseases are socially significant parasitocenoses (Pisarski, 2019; Saichenko et al., 2021). Despite the progress made in studying and solving the problems of parasitic diseases, many issues of their distribution, clinic, pathogenesis, specific prevention and treatment remain relevant (Bogach et al., 2020; Rzayev, 2021; Maurizio et al., 2021).

Dirofilariasis, being a transmissible natural focal zooanthroponosis, is an actual problem of both veterinary and medical parasitology. This disease is common in many countries of the world. The largest number of human cases of dirofilariasis was registered in the Mediterranean countries, Asia (Tarello, 2008; Anvari et al., 2019), including China (Hou et al., 2011), and in the last two decades — in some European countries (Landum et al., 2014; Genchi & Kramer, 2017), America (Dantas-Torres & Otranto, 2013), Brazil (Furtado et al., 2009), Malaysia (Shekhar et al., 1996).

In four provinces of the Republic of Serbia, microfilariae *Dirofilaria repens* (Leidy, 1856), *D. immitis* (Railliet & Henry 1911), and *Acanthocheilonema reconditum* were found in 49.2 %, 7.2 % and 2.1 % of dogs, respectively (Tasić et al., 2008). The prevalence of dirofilariasis among Australian hunting dogs ranges from 12.5 to 21.0 % (Orr et al., 2020). Over the past 10 years, the prevalence of dirofilariasis among dogs has increased in Taiwan (Wu & Fan, 2003). Dirofilariae were found in 118 (20.0 %) police dogs and 10 (8.4 %) military dogs located in Slovakia (Miterpáková et al., 2010). In Poland, foci of *D. repens* infection in dogs were noted in 2009 with a prevalence of 37.5 % (Cielecka et al., 2012). In Austria, more than 50 cases of dirofilariasis among dogs caused by both *D. immitis* and *D. repens* were reported (Fuehrer et al., 2016).

In Ukraine, the registration of cases of dirofilariasis has become mandatory since 1975, and this disease has was included in the national epidemiological surveillance system. Until December 31, 2012, 1533 cases were registered, of which 1465 cases were in the last 16 years. Most cases of dirofilariasis were registered in six regions: Kyiv, Donetsk, Zaporizhzhay, Dnepropetrovsk, Kherson and Chernyhiv. In 1997–2002 the highest incidence rate was noted in Kherson Region in the south of the country (9.79 per 100 000 people), and the lowest in Western Ukraine (0.07–1.68 per 100 000 people) (Sałamatin et al., 2013). In Kharkiv Region of Ukraine from 2002 to 2004, 13 cases of human disease with dirofilariasis were registered. In 2011, 10 people fell ill with dirofilariasis in Kharkiv Region, 7 of them in the city of Kharkiv. Thus, in the Kharkiv Region, there is a tendency to an increase in this disease cases among people (Filipcova et al., 2016).

The causative agent of the disease — dirofilaria (literally from the Latin "evil thread") parasitizes canines, less often cats and other carnivores. Today, three types of dirofilaria are distinguished: *Dirofilaria immitis, Dirofilaria repens*, *Acanthocheilonnema grassi* (imaginal stage) (Magnis et al., 2013). The causative agent *D. immitis* causes a cardiac form of the disease, as it parasitizes in the heart cavity, *D. repens* parasitizes in the subcutaneous tissue, causes the cutaneous form of dirofilariasis, which is often found in dogs, cats and people in Ukraine. The imaginal stage of *A. grassi* can also inhabit body cavities, muscles, and subcutaneous tissue (Ionică et al., 2014).

Infection of humans and animals occurs through the bites of blood-sucking mosquitoes of the *Culicidae* family (genera *Anopheles, Ochlerotatus, Aedes, Stegomyia, Culex, Culiseta, Coquillettidia*, etc.) (Bocková et al., 2015; Trari et al., 2017). Mosquitoes are infested by domestic dogs, wild carnivores (wolves, foxes, bears, etc.), and more rarely by cats (Todorovic & McKay, 2020). In a study of insects in Slovakia, *D. repens* was identified in *Anopheles messeae* and *An. maculipennis* mosquitoes and the *Cx. pipiens* complex (Miterpáková et al., 2010). According to the data of the Kharkiv Laboratory Center, the number of mosquitoes of the genus *Aedes* in 2013 compared to 2010 decreased by 0.6% and amounted to 20.5%, of the genus *Anopheles* — by 0.7% and amounted to 17.7%, of the genus *Culex* — by 1.5% and amounted to 18.2% (Filipcova et al., 2016).

An increase in the number of stray animals, their mass migration in nature and in settlements, and the process of urbanization contribute to an increase in the transmission of dirofilariasis from wild carnivores to domestic animals and humans (Diaz, 2015). Since dirofilariasis is a vector-borne disease, its spread and infection rates have undergone a significant transformation under the influence of global climate change (Simón et al., 2010).

Material and methods

The seasonal dynamics of the mosquito population was monitored regularly in the city of Kharkiv and Kharkiv Region. The studies began in the last ten days of April — the first ten days of May after the emergence of the first generation mosquitoes, and then the studies were carried out once a decade until the end of the insect activity season.

We regularly counted and caught mosquitoes during their mass flight (from April to September) in the basements of multi-storey buildings, in the territories of summer cottages, private households, near water bodies, on dogs, people in different parts of the region. Since 2015, light traps have been used to catch mosquitoes. All mosquitoes sitting on the surface were counted in 3–4 places on an area of 0.25–0.5–1 m^2, depending on their density. With a larger mosquito population, there was a smaller survey area. Then the number of insects per 1 m^2 was counted. Counting the number of mosquitoes in the vegetation surrounding the building was carried out by catching them with a net for 10 minutes at 2–3 points. In suburban conditions, to account for

the number of adult mosquitoes, regular surveys were carried out in the same backyards. The modern species names and the taxonomic position of mosquitoes are given according to Reinert (2009) and Wilkerson with co-authors (2015).

Female mosquitoes, 20 individuals each, were placed in laboratory tubes and delivered to the laboratory of veterinary sanitation and parasitology of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine". After species identification, entomological dissection of insects was carried out, the number of egg-laying was determined, and dirofilariae were identified. The preparation of mosquitoes for microscopic examination for microfilariae infection was carried out according to modern methods (Manrique-Saide et al., 2010; Silaghi et al., 2017). Females were immobilized with chloroform. Before dissection, the wings and legs of the mosquito were removed and placed on a glass slide under a stereoscopic microscope. Holding the abdomen with a dissecting needle, the body of a mosquito was dissected with a spear-shaped needle in the middle of the thoracic region. To extract the malpighian glands and intestines, the chitinous cover of the abdomen was torn between segments 2 and 3, counting from the posterior end. Then one needle was stuck into the insect's chest, the other into the end of the abdomen and moved apart in different directions. The preparations prepared from females saturated with blood, after opening, were treated with several drops of a 3.0 % solution of acetic acid. After drying in air, all preparations were fixed for 10–15 minutes, stained with Romanovsky-Giemsa stain for 20–30 minutes and microscoped with a lens magnification × 90–100.

To dissect the thoracic region, the head of the mosquito was cut off, the needle was pressed flat on the insect's chest and carried forward. The preparation was microscoped in saline under a cover glass (×40 objective). The rate of feeding insects was determined by microscopic examination: all females with the presence of blood in the midgut were considered to be feeding on blood.

From clinics in Kharkiv and the region received blood samples from dogs and cats for parasitological studies. A blood test for the presence of dirofilariae was performed by direct microscopy of a drop of fresh blood under a low microscope magnification (×10) — the easiest, most convenient, and fastest method for diagnosing dirofilariasis (Chagas et al., 2020). The mobile larvae of the parasite are noticeable by their active movement between erythrocytes. Concentration research methods (modified Knott method) were also used (Weil & Ramzy, 2007; Chungpivat & Taweethavonsawat, 2008).

Microscopic identification of L1 dirofilaria larvae was performed in a native blood smear and in blood serum (Furtado et al., 2010; Simsek et al., 2011).

Also, for the diagnosis of dirofilariasis, immunostrips were used — immunochromatographic instrumentless test systems for express analysis of invasion. When screening for cardiopulmonary dirofilariasis, antigen tests for adult dirofilaria were used (Trancoso et al., 2020).

Results

The studies were carried out in the period from 2009 to 2019 on the territory of Kharkiv Region of Ukraine. During the study period, the average number of mosquitoes in vegetation ranged from 0.62 to 3.96 per m^2, and by autumn it increased from 0.67 to 5.53 per m^2. Indoors, the number of mosquitoes ranged from 1.22 to 3.71 per m^2. According to the monitoring data on the territory of Kharkiv Region, the main groups of blood-sucking mosquitoes were the genera *Anopheles*, *Aedes*, *Culex*, *Culiseta*, *Coquillettidia* and *Uranotaenia*. The most numerous genus *Aedes* in the region consisted of 23 species. In total, according to our monitoring, 38 species of mosquitoes belonging to 6 geners of two subfamilies *Anophelinae* and *Culicinae* of the *Culicidae* family were found in the studied region (table 1).

We carried out xenomonitoring of the presence of dirophilaria larvae in mosquitoes collected in the districts of Kharkiv Region and the City of Kharkiv during their activity (from April to September). During the whole period we examined 20,876 specimens of blood-sucking mosquitoes of different genera (table 2).

Dirofilaria larvae were found in 932 insects, which was 4.46 % of the surveyed mosquitoes. The prevalence of microfilariae invasion of female mosquitoes was 4.46 ± 0.24 %, while the mean intensity was 5.4 ± 2.1 dirofilariae.

According to the data of previous years, females infected with dirofilaria were identified in the city of Kharkiv, Balakliia, Zmiiv, Chuhuiv districts of Kharkiv Region. In 2009, infected females were detected in Izium and Kharkiv districts, in 2011 — in Krasnokutsk District, in 2012 — in Kolomak District, in 2013 — in Krasnohrad District, in 2014 — in Zolochiv District, in 2016 — in Sakhnovshchyna and Vovchans'k Districts. Infection of mosquitoes with dirofilariae has been detected at the present in 11 districts of the Kharkiv Region and the City of Kharkiv.

Table 1. Species composition of mosquitoes of the Culicidae family on the territory of the Kharkiv Region (2009–2019)

Genus	Subgenus	Species
Anopheles Meigen, 1818	*Anopheles* Meigen, 1818	*Anopheles (Anopheles) plumbeus* Stephens, 1828 *Anopheles (Anopheles) claviger* Meigen, 1804 *Anopheles (Anopheles) maculipennis* Meigen, 1818 *Anopheles (Anopheles) messeae* Falleroni, 1926 *Anopheles (Anopheles) atroparvus* Van Thiel, 1927 *Anopheles (Anopheles) hyrcanus* Pallas, 1771
Aedes Meigen, 1818	*Ochlerotatus* Lynch Arribalzaga, 1891	*Aedes (Ochlerotatus) diantaeus* Howard, Dyar et Knab, 1913 *Aedes (Ochlerotatus) intrudens* Dyar, 1919 *Aedes (Ochlerotatus) caspius* Pallas, 1771 *Aedes (Ochlerotatus) dorsalis* Meigen, 1830 *Aedes (Ochlerotatus) pulcritarsis* Rondani, 1872 *Aedes (Ochlerotatus) cantans* Meigen, 1818 *Aedes (Ochlerotatus) riparius* Dyar et Knab, 1907 *Aedes (Ochlerotatus) behningi* Martini, 1926 *Aedes (Ochlerotatus) excrucians* Walker, 1856 *Aedes (Ochlerotatus) annulipes* Meigen, 1830 *Aedes (Ochlerotatus) flavescens* Muller, 1764 *Aedes (Ochlerotatus) cyprius* Ludlow, 1919 *Aedes (Ochlerotatus) communis* De Geer, 1776 *Aedes (Ochlerotatus) sticticus* Meigen, 1838 *Aedes (Ochlerotatus) cataphylla* Dyar, 1916 *Aedes (Ochlerotatus) leucomelas* Meigen, 1804 *Aedes (Ochlerotatus) detritus* Haliday, 1833 *Aedes (Ochlerotatus) punctor* Kirby in Richardson, 1837 *Aedes (Ochlerotatus) pullatus* Coquillett, 1904
	Aedimorphus Theobald, 1903	*Aedes (Aedimorphus) vexans vexans* Meigen, 1830
	Aedes Meigen, 1818	*Aedes (Aedes) cinereus* Meigen, 1818 *Aedes (Aedes) rossicus* Dolbeshkin, Goritzkaja et Mitrofanova, 1930
	Finlaya Theobald, 1903	*Aedes (Finlaya) geniculatus* Olivier, 1791
Culiseta Felt, 1904	*Culiseta* Felt, 1904	*Culiseta (Culiseta) alaskaensis alaskaensis* Ludlow, 1906 *Culiseta (Culiseta) annulata* Schrank, 1776
	Culicella Felt, 1904	*Culiseta (Culicella) morsitans* Theobald, 1901
Culex Linnaeus, 1758	*Culex* Linnaeus, 1758	*Culex (Culex) territans* Walker, 1856
		Culex (Culex) pipiens Linnaeus, 1758 *Culex theileri* Theobald, 1903
	Barraudius Edwards, 1921	*Culex (Barraudius) modestus* Ficalbi, 1890
Coquillettidia Dyar, 1905	*Coquillettidia* Dyar, 1905	*Coquillettidia (Coquillettidia) richiardii* Ficalbi, 1889
Uranotaenia Lynch Arribálzaga, 1891	*Pseudoficalbia* Theobald, 1912	*Uranotaenia unguiculata unguiculata* Edwards, 1913

In the salivary glands of female mosquitoes, dirofilariae in the amount of 2 to 11 specimens were detected starting from May 24 to September 28, and in some years (2013, 2015) — until October 18 in the amount of 2 to 5 dirofilariae. In blood clots in the intestine, the largest number of dirofilariae (15–33) was detected from July to September.

The morphological characteristics of dirofilariae in a blood clot from a mosquito intestine revealed that *D. repens* was 300–360 μm long, 5.8–8.0 μm wide, without sheath. The dorsal part of the parasite was rectangular in shape, enlightened, the caudal part was wide, long, the tail end was bent in the form of an umbrella handle. In *D. immitis*, the body length was 262–349 μm, and the width was 4.8–7.0 μm. The dorsal part was dark-colored in the form of a cone, the caudal part was thin and straight. According to our data, *D. immitis* larvae were detected in mosquitoes for the first time in 2013. It was revealed that 75 % of females produced at least 2 ovipositions, that is, they fed on blood at least once. Different level of infestation of female mosquitoes with species of *Dirofilaria* spp. larvae was revealed (table 3).

Table 2. Results of a study of female mosquitoes for infection with dirofilaria larvae

N	Year	Number of investigated, ex.	Number of infected microfilariae, ex.	Prevalence of the infection microfilariae, %	The mean intensity of infection microfilariae, ex.
1	2009	1506	80	5.31	5.5 ± 2.5
2	2010	1520	91	5.98	4.0 ± 1.0
3	2011	1560	79	5.06	5.0 ± 2.0
4	2012	1585	81	5.11	5.5 ± 2.5
5	2013	1900	96	5.04	5.5 ± 2.5
6	2014	1350	87	6.44	4.0 ± 2.0
7	2015	1690	81	4.79	7.0 ± 3.0
8	2016	2195	85	3.87	4.0 ± 1.0
9	2017	2750	88	3.20	5.5 ± 2.5
10	2018	2850	93	3.26	5.5 ± 2.5
11	2019	1850	71	3.84	3.5 ± 1.5
Total		20876	932	4.46	5.4 ± 2.1

Table 3. Infection of mosquitoes with dirofilaria larvae

Year	Number of investigated, ex.	Infection by larvae of *Dirofilaria* spp. female mosquito			
		Number of infected, ex.	Genus *Aedes*, %	Genus *Culex*, %	Genus *Anopheles*, %
2009	1506	80	4.51	0.8	0
2010	1520	91	5.10	0.92	0
2011	1560	79	3.84	1.22	0.13
2012	1585	81	4.42	0.69	0
2013	1900	96	4.37	0.68	0
2014	1350	87	5.48	0.96	0
2015	1690	81	3.96	0.82	0
2016	2195	85	2.78	0.68	0.41
2017	2750	88	2.50	0.47	0.22
2018	2850	93	2.10	0.52	0.28
2019	1850	71	3.10	0.48	0.27
Total	20876	932	3.83 ± 0.63	0.75 ± 0.34	0.12 ± 0.39

It was found that females of the genus *Aedes* were the most infected. Thus, the infection of mosquitoes of the genus *Aedes* was 3.83 ± 0.63 %, of the genus *Culex* — 0.75 ± 0.34 %, of the genus *Anopheles* — 0.12 ± 0.39 %, while in females of the genera *Culiseta* and *Mansonia* (*Uranotaenia*) dirofilariae were not found.

For the period from 2009 to 2019 we examined 378 blood samples from dogs (190 males and 188 females) aged from 8 months to 13 years with suspected dirofilariasis (table 4).

It was found that in Kharkiv Region there were 2 species of canine dirofilariae — *D. immitis* and *D. repens*, with a predominance of *D. repens*. According to the results of the blood sample studies, dirofilariasis was detected in 140 (37.04 %) samples out of 378 samples with a prevalence of infection 37.03 ± 0.12. Moreover, two samples were amikrofilariemic, but the test system detected antigen to *D. immitis*. In two samples, dirofilariae were detected both by the direct blood test method and by the concentration method, and the antigen to *D. immitis* was detected by the test system. In 136 samples (35.99 %), dirofilariae were detected by both methods, but antigen to *D. immitis* was not detected. In 238 cases (62.96 %), the diagnosis of dirofilariasis was not confirmed. The number of positive samples from mongrel dogs was 30 % (42). The prevalence of infection by larvae of *Dirofilaria* spp. in blood samples from dogs with suspected dirofilariasis was 37.03 ± 0.12 %, while infection with *D. immitis* was 2.86 ± 0.45 %, and *D. repens* — 97.35 ± 0.24 %.

Table 4. The number of blood samples tested for infection with *Dirofilaria* spp. larvae

Year	Number of samples analyzed	Number of positive samples	Prevalence of infection	*D. immitis*		*D. repens*	
				positive	%	positive	%
2009	32	7	21.8	0	0	7	100
2010	28	11	39.3	0	0	11	100
2011	43	16	37.2	0	0	16	100
2012	35	15	42.9	0	0	15	100
2013	41	14	34.1	0	0	14	100
2014	40	18	45.0	2	11.1	16	88.9
2015	39	12	30.8	1	8.3	11	91.7
2016	25	11	44.0	0	0	11	100
2017	29	12	41.4	0	0	12	100
2018	37	14	37.8	1	7.1	13	92.9
2019	29	10	34.5	0	0	10	100
Total	378	140	37.03 ± 0.12	4	2.86 ± 0.45	136	97.35 ± 0.24

We noted a difference in the character of movement in the erythrocytic layer of larvae of *D. immitis* and *D. repens*. Thus, *D. immitis* dirofilariae are distinguished by directional wave-like movement along the body axis, while *D. repens* move chaotically, mainly in one place.

According to the anamnesis, it was found that the most frequently positive result was found in blood samples of dogs aged 4 to 8 years. The maximum number of positive samples was obtained in dogs aged 5 years (27), and the minimum — in dogs aged from 8 months to 2 years and from 11 to 13 years (1) (fig. 1).

A positive test was detected in a puppy of the Alabai breed at the age of 8 months, the puppy was from a local dog kennel. A positive test was found in a mongrel dog at the age of 12 years and in a Dachshund at the age of 13 years. According to the results obtained, it can be stated that the breed has no effect on the possibility of infection with dirophilariosis.

Infection with dirofilaria larvae was practically independent of sex: the prevalence in males (71) was 50.7 %, and in females (69) — 49.3 %, while the mean intensity was 354.5 ± 183.1 (from 8 to 450) larvae in 1 ml of blood.

Until 2019, we did not study blood from cats for dirofilariasis. In July 2019, 25 blood samples of cats aged from 2 to 5 years were tested for dirofilariosis. Animals were admitted to the animal shelter after being caught on the streets of Kharkiv with signs of skin

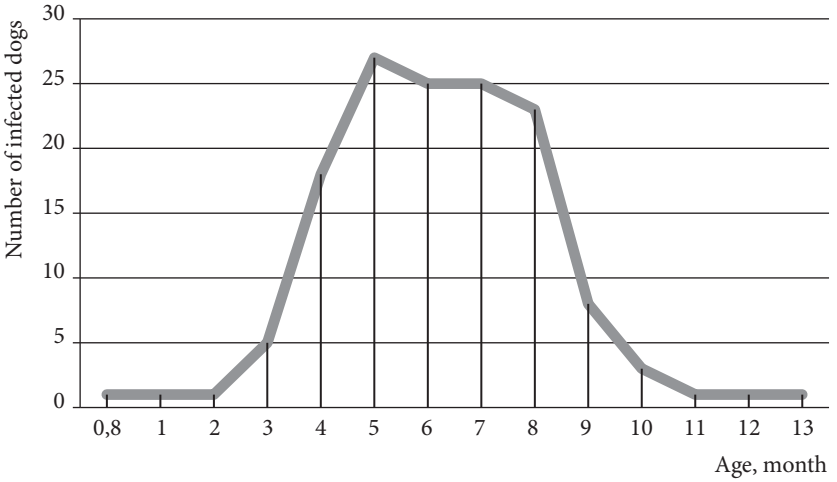

Fig. 1. Infection of dogs with dirofilariae depending on age.

lesions (15) and pulmonary syndrome (10). The modified Knott method gave a positive result in 17 animals, the antigen test was positive in 14 animals, but this included those with a negative Knott method. According to Knott method, dirofilariae were present in the blood of 68 % of cats with suspected dirofilariasis. The antibody test was positive in 20 animals. That is, 80 % of the examined animals were infected, and 56 % were sick, based on the antigen test.

Also, during the year, a study was conducted of 8 blood samples from cats, received from clinics in the city of Kharkiv, with suspected dirofilariasis. The native smear test was negative, the modified Knott method was positive in 2 animals, and antigen testing was positive in one animal. That is, infection was registered in 25 % of the examined animals, and 12.5 % turned out to be sick based on the antigen test.

Infection by dirofilaria larvae is practically independent of sex; the prevalence of infection in male cats (16) was 48.5 %, in female cats (17) — 51.5 %. In this case, the mean intensity averaged 44.3 ± 22.9 (from 1 to 90) larvae in 1 ml of blood.

The minimum number of positive samples was determined in cats aged from 2 to 4 years and the maximum — at the age of 5 years (16). Among stray animals with suspected dirofilariasis, the infection rate was 68 %, among domestic animals — 25 %.

The results showed that cats were more often amycrofilariemic than dogs. Infection with dirofilariae in cats is less likely to cause dirofilariasis, but there are also two forms of *D. immitis* and *D. repens*.

Discussion

The quality of the resulting livestock products, their safety, as well as the health of animals and humans directly depends on the epizootic and epidemiological situation in the region and the level of contamination of environmental objects with pathogens of certain diseases (Shkromada et al., 2019; Paliy et al., 2019). Recently, more and more attention has begun to be paid to ectoparasites and the determination of their role in the occurrence of outbreaks of infectious and invasive diseases (Paliy et al., 2018 b, 2021). Arthropod vector pathogen surveillance is now an important tool for surveillance programs across Europe (Rudolf et al., 2014).

The fauna of blood-sucking mosquitoes in Kharkiv Region of Ukraine, according to our collection, includes 38 species of mosquitoes that belong to 6 genera. According to other studies, 30 species of blood-sucking mosquitoes of three genera were found on the territory of the studied region: *Anopheles* (*An. maculipennis*, *An. messeae*); *Aedes* (*Ae. cataphylla*, *Ae. leucomelas*, *Ae. caspius dorsalis*, *Ae. excrucians*, *Ae. vexans*); *Culex* (*C. punctatus*, *C. obsoletus*) (Gazzavi-Rogozina, 2015). Perhaps this is due to the fact that they were collected mainly in urban areas.

The researchers note that the species composition and number of mosquitoes of the genus *Aedes* are changing in Kharkiv region. In recent years, the percentage of *Aedes geniculatus* mosquitoes in the number of mosquitoes of this genus has been increasing. In addition, in some districts of the region (Zmiiv, Derhachi, Chuhuiv) and the city of Kharkiv, mosquitoes *Mansonia richiardii* are more and more often recorded (Bodnia & Potapova, 2016). According to the data of the Kharkiv Regional Laboratory Center, the population rates of bloodsucking mosquitoes (of the *Aedes* and *Culex* genera) of utility rooms and basements are 12.6 % against 12.5 % in 2014. According to our data, the population of basements with bloodsucking mosquitoes averaged 13.2 %, that is, slightly higher

According to observations (Filipcova et al., 2016), 563 specimens were examined for the presence of dirofilaria nematodes in Kharkiv Region malaria mosquitoes, while dirofilariae were not identified. In the study of 463 specimens non-malaria mosquitoes in 3 specimens revealed dirofilaria nematodes (0.6 %). A year later, according to laboratory autopsy data, the incidence of Anopheles mosquitoes with nematode dirofilariae was 0.8–0.9 % (Bodnia

et al., 2016). In Belarus, out of 467 female mosquitoes collected in the Brest and Minsk regions, *Dirofilaria* spp. were detected in two pools (5.56 %) (Șuleșco et al., 2016).

According to the obtained results obtained, the infection of mosquitoes of the genus *Aedes* was 3.83 ±0.6 3 %, of the genus *Culex* — 0.75 ± 0.34 %, of the genus *Anopheles* — 0.12 ± 0.39 %, and in females of the genus *Culiseta* (Felt, 1904), *Coquillettidia* (Dyar, 1905), and *Uranotaenia* (Lynch Arribálzaga, 1891) dirofilariae were not found. It was revealed that females of the genus *Aedes* were the most infected. Perhaps the difference in results is due to the fact that the material was collected in different places and at different terms.

Today dirofilariasis of dogs is widespread in the world. In Lithuania, in a study of 2280 blood samples, 61 (2.7 %) were positive for the presence of *D. repens*, while the infection rate was significantly higher in dogs from shelters (19.0 %; 19/100) than in domestic dogs (1.9 %; 42/2180) (Sabūnas et al., 2019). In studies in Poland, dirofilariae belonging to the species *D. repens* were found in blood samples from dogs from Warsaw and 18 districts of the Mazovian Voivodeship. The average prevalence of this species in the province was 25.8 % with an average intensity of 9 dirofilariae. The highest prevalence, reaching 52.9 %, was found in the Radom Region, and the lowest prevalence, at 4.2 %, was found in the Grójec Region (Demiaszkiewicz et al., 2014). Our results are consistent with other researchers (Simón et al., 2012), who also indicate the distribution of two main species of dirofilaria — *D. immitis* and *D. repens*.

According to existing data, in Kharkiv Region, dirofilariae were found in 10.7 % of the surveyed dogs, while in some settlements the percentage of affected dogs reached 19 % and more (Bodnia et al., 2016). According to our data, the prevalence of infection by the larvae of *Dirofilaria* spp. in dogs with suspected dirofilariasis was 37.03 ± 0.12 %. Infection with *D. immitis* was 2.86 ± 0.45 %, and *D. repens* — 97.35 ± 0.24 %. A large number of dogs infected with *D. repens*, in our opinion, is a constant source of infection for dipterans, and as a result, people. In cats with suspected dirofilariasis, infection with dirofilariae was 66.7 %. The infection with *D. immitis* was 54.6 ± 0.45 %, and *D. repens* — 46.4 ± 0.24 %. Dirofilariae in the blood of dogs and cats were found year-round from January to December, but in June and July there were a bit more such cases. A study of the seasonal dynamics of *D. immitis* infection in stray and indoor dogs showed that the proportion of infected dogs in spring and summer was higher than in colder seasons (autumn and winter) (Khedri et al., 2014).

It was found that dirofilariasis affects dogs of all age groups, in the age range from 8 months and up to 13 years. There was no statistically significant relationship between the prevalence of *D. immitis* with age, sex, breed, and use of antiparasitic drugs (Anvari et al., 2019). The prevalence of *D. immitis* infection in stray dogs over 5 years old (53.8 %) was higher than in other age groups, while in domestic dogs the infection rate was higher at the age of 3-5 years (27.3 %) (Khedri et al., 2014). There is evidence that the likelihood of infection was significantly higher in older dogs and dogs in an outdoor shelter compared to younger dogs and those kept in an indoor shelter. There were no significant differences in infection between sexes and between purebred and crossbred dogs under the same rearing conditions (Hou et al., 2011). These results also exist in humane medicine, where there was no difference in positivity for infection with dirofilariasis between men and women (Furtado et al., 2009).

Predictive models have recently forecast that current summer temperatures are accelerating incubation of dirofilariae. Global warming, predicted by the Intergovernmental Panel on Climate Change, suggests that warm summers suitable for transmission of dirofilariae in Europe will become common in the coming decades, and if the actual trend of increasing temperatures continues, dirofilariasis invasion should spread to previously free areas (Genchi et al., 2011) in a northern direction (Jokelainen et al., 2016; Pietikäinen et al., 2017). The constant increase in the incidence of *D. repens* human dirofilariasis in Ukraine is indicated. Despite efforts in the field of medicine, infections have become more frequent, and the territory of the spread of the disease has expanded to cover the whole of Ukraine (Sałamatin et al., 2013).

An analysis of the seasonality of invasion in Kharkiv Region showed that dirofilariae are detected year-round, and the peak is in June and July, the month is also marked by an increased dirofilariaemia in the summer months, its decrease in the fall and an insignificant number of larvae in winter. In our opinion, the variability of indicators largely depends on the climatic and geographic characteristics of the region under study, the size of the studied sample of animals, and various diagnostic techniques that were used by the authors to make a diagnosis.

The fight against parasitic diseases of animals is a priority task for specialists in veterinary medicine, and its success directly depends on a comprehensive solution to this problem (Paliy et al., 2018 a; Boyko et al., 2020; Bogach et al., 2020).

Conclusions

The average number of mosquitoes (2009–2019) in vegetation ranges from 0.62 to 3.96 per m^2, and by autumn it increases from 0.67 to 5.53 per m^2. Indoors, the number of mosquitoes ranged from 1.22 to 3.71 per m^2.

On the territory of Kharkiv Region of Ukraine, 38 species of blood-sucking mosquitoes were found, which belong to 6 genera and 38 species. The prevalence of infection by dirofilariae of female mosquitoes is 4.46 ± 0.24 % at a mean intensity is 5.4 ± 2.1 microfilariae. It has been established that the most infected are female mosquitoes of the genus *Aedes*. Infection of mosquitoes of the genus *Aedes* is 3.83 ± 0.63 %, of the genus *Culex* — 0.75 ± 0.34 %, of the genus *Anopheles* — 0.12 ± 0.39 %, in females of the genus *Culiseta, Coquillettidia* and *Uranotaenia* microfilariae were not found.

It was found that in Kharkiv Region there were 2 species of canine dirofilariae *D. immitis* and *D. repens*, with a predominance of the parasite *D. repens*. The species *D. immitis* on the territory of Kharkiv Region was identified by us for the first time in 2014.

Dirofilariae in the blood of dogs and cats were found year-round from January to December, but in June and July a little more often. In the blood of dogs, dirofilariae were detected in 140 out of 378 samples with a prevalence of infection 37.03 ± 0.12 with a mean intensity of 354.5 ± 183.1 (from 8 to 450) larvae in 1 ml of blood. Cats are more likely to be amycrofilariemic than dogs. Infection with dirofilariae in cats less often leads to their disease with dirofilariasis, but there are also two causative agents of the disease (*D. immitis, D. repens*).

The boundaries of the spread of dirofilariasis among vectors and susceptible hosts in Kharkiv Region are expanding annually, at present they cover 11 districts of the region.

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