

PREVALENCE OF *DIROFILARIA IMMITIS* IN DOGS IN BULGARIA

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ABSTRACT

A study on the prevalence of the nematode *Dirofilaria immitis* in dogs in Bulgaria is presented. 1709 domestic and stray dogs from 10 cities in Bulgaria were studied in veterinary clinic “Sveti Georgi” for the period from 1 January 2017 until 30 June 2019. For this purpose, “IDEX SNAP 4Dx Plus TEST” was used for immunochromatographic detection of Ag in the blood of the patients tested.

Of all 1609 animals tested, 223 were antigen positive and they were also tested by the modified Knott method for the presence of microfilariae. 9 of them were negative for presence of microfilaria and 11 of them had a co-invasion with *Dirofilaria repens*.

The total percentage of positive animals compared to all tested animals was 13.04%. The obtained values show that the highest percentage of infested animals was in the Central Southern Region with 17.8% and the lowest is in the Southwestern Region – 5.5%. The largest number of infested dogs was found in Plovdiv region – 20.2%, and the lowest in Sofia region – 4.8%. The distribution for other cities is as follows: Ruse region – 18.7%, Svishtov region – 18.4%, Burgas region – 16.5%, Lovech region – 15.4%, Asenovgrad region – 14.6%, Gotse Delchev region – 11.3%, Dobrich region – 9.3%, and Vidin region – 7.9%.

Key words: *Dirofilaria immitis*, dogs, Bulgaria, Ag immunochromatographic tests, prevalence.

Introduction

In recent years, more attention is paid to the issues related to so-called vector-borne diseases. One of the helminthiasis belonging to them is Dirofilariosis (Canine heartworm disease), whose causative agent *Dirofilaria immitis* is located in the pulmonary arteries and the right part of the heart (Manov, 2020) and is transmitted by about 70 species of Culicoides mosquitoes, mainly the genera *Culex spp.*, *Aedes spp.*, *Anopheles spp.*, *Culiseta spp.*, and *Coquilletidia spp.* (Cancrini & Kramer 2001; Cancrini et al., 2006). By 2001, the canine heartworm disease was found mainly in Southern European countries such as Spain, Portugal, Italy and France. Greece, Turkey and some eastern countries report several separate studies. Helminthiasis is currently spreading to Eastern, Central and Northern Europe, affecting countries or areas previously considered to be free of this parasite (Morchon et al., 2012).

In the early 90's the canine heartworm disease has not yet been fully studied and there was insufficient data on its prevalence in our country. The first cases were described through 1996 (Kanev et al. 1996). A year later Georgieva et al. (1997) also reported on dirofilariosis with *Dirofilaria immitis* in dogs. At the end of the last century between year 1997 and 1999 Georgieva et al. (2001) studied the prevalence of the heartworm disease in stray and domestic dogs and found a prevalence of 7.4%. For the period between year 2001 and 2007, Kirkova et al. (2007) performed a study on domestic dogs from different parts of the country and 9.19% were positive for presence of microfilariae with *immitis* larvae. Since 2007, there have been several studies showing the following prevalence rates in the following regions: Plovdiv 7.75 and 12.1% (Kirkova et al., 2007; Kostadinov, 2007), Sofia 8.75% (Kostadinov, 2012), Ruse 15.7% (Kostadinov, 2012) and Burgas 12.5% (Kostadinov, 2013). The next information is for the period from 2012 until 2013 where Panayotova–

Pencheva et al. (2016) found 33.33% of infestation after dog autopsies. Pantchev et al. (2015) established 16.2% of infected animals in the Stara Zagora region. One year later, Radev et al. (2016) performed a study on the prevalence of *Dirofilaria spp.* in stray dogs in Sofia and 15% of the dogs tested were found to have *D. immitis* microfilariae. Borisov et al. (2017) found 7.5% infestation rate of dogs in the Sofia region. The latest up-to-date information on the prevalence of the heart parasite in stray dogs in Sofia and the region is provided by Stoyanova et al. (2019) with an established percentage of 31.25% and Manev 11,26% (2020a) and 13.39% (2020b).

Material and methods

For the period from 01 January 2017 until 30 June 2019, 1709 domestic and stray dogs from 10 cities in Bulgaria were studied in the veterinary clinic "Sveti Georgi", Sofia: Sofia – 475 dogs, Plovdiv – 247 dogs, Lovech – 104 dogs, Svishtov – 38 dogs, Ruse – 208 dogs, Gotse Delchev – 53 dogs, Asenovgrad – 192 dogs, Vidin – 88 dogs, Dobrich – 43 dogs, and Burgas – 261 dogs.

Depending on the listed cities, the territory of Bulgaria was divided into six parts:

- *Northwestern region (NWR)* – Northwestern Bulgaria (Vidin) – 88 animals;
- *Central Northern Region (CNR)* – Central Northern Bulgaria (Lovech and Svishtov) – 142 animals
- *Northeastern region (NER)* – Northeastern Bulgaria (Dobrich and Ruse) – 251 animals;
- *Southwestern region (SWR)* – Southwestern Bulgaria (Gotse Delchev and Sofia) – 528 animals;
- *Central Southern Region (CSR)* – Central Southern Bulgaria (Asenovgrad and Plovdiv) – 439 animals;
- *Southeastern region (SER)* – Southeastern Bulgaria (Burgas) – 261 animals.

The number of domestic animals was 1081 and the number of the stray animals was 628. 908 of them were male and 801 were female. The age of the animals ranged from 6 months to 15 years old and the animals were divided into three age groups: Group I – from 6 months to 2 years; Group II from 2.1 years to 7 years; Group III – over 7.1 years. Most of the dogs were tested for prophylactic purposes and the rest showed a pronounced cardiac and pulmonary clinic expressed by heart murmur, fatigue, shortness of breath, dry cough, refusal of physical activity, cyanotic mucous membranes, epistaxis, jaundice, ascites, lethargy, etc. In the majority of the dogs, regular prophylaxis against the disease has not been performed.

The tests were performed using "IDEX SNAP 4Dx Plus TEST" with a sensitivity of 99.0% and a specificity of 99.3% to the antigens of *Dirofilaria immitis* (www.idex.com). The test includes the detection of Ag secreted by the nematode *Dirofilaria immitis* and Ab secreted by the vector-borne bacteria *Anaplasma phagocytophilum*, *Anaplasma platys*, *Ehrlichia canis* и *Borrelia burgdorferi*. In the study, all animals were tested with whole blood obtained in a tube with EDTA anticoagulant. Antigen-positive dogs were subjected to a test for the presence of microfilariae using the modified Knott method (Kanchev, et al. 2016), for the presence of L1 larvae in the blood.

Results

Of all 1609 animals tested, 223 were antigen positive and they were also tested by the modified Knott method for the presence of microfilariae. 9 of them were amicrophylaric and 11 of them had a co-invasion with *Dirofilaria repens*. Anigen.

The distribution of the studied dogs according to the regions and the respective cities is presented in Fig. 1.



Figure 1: Territorial distribution of the tested dogs by regions and cities.

The ratio between the dogs with positive results and their total number in percentage is presented in Table 1.

Table 1: The ratio between the dogs with positive results and their total number by region in percentage.

| Region/City | Number of animal tested | Ag positive dogs (number) | Percentage (%) |
|----------------------------------|-------------------------|---------------------------|----------------|
| NORTHWESTERN BULGARIA | 88 | 7 | 7.9 |
| 1. Vidin | 88 | 7 | 7.9 |
| CENTRAL NORTHERN BULGARIA | 142 | 23 | 16.2 |
| 1. Lovech | 104 | 16 | 15.4 |
| 2. Svishtov | 38 | 7 | 18.4 |
| NORTHEASTERN BULGARIA | 251 | 43 | 17.1 |
| 1. Ruse | 208 | 39 | 18.7 |
| 2. Dobrich | 43 | 4 | 9.3 |
| SOUTHWESTERN BULGARIA | 528 | 29 | 5.5 |
| 1. Sofia | 475 | 23 | 4.8 |
| 2. Gotse Delchev | 53 | 6 | 11.3 |
| CENTRAL SOUTHERN BULGARIA | 439 | 78 | 17.8 |
| 1. Plovdiv | 247 | 50 | 20.2 |
| 2. Asenovgrad | 192 | 28 | 14.6 |
| SOUTHEASTERN BULGARIA | 261 | 43 | 16.5 |
| 1. Burgas | 261 | 43 | 16.5 |
| Total: | 1709 | 223 | 13.04% |

The obtained values show that the highest percentage of infested animals is in the Central Southern Region with 17.8% and the lowest in the Southwest Region with 5.5%.

The table shows that the highest percentage of diseased animals was found in Plovdiv – 20.2%, and the lowest in Sofia – 4.8%. The overall percentage of animals with positive results to all tested animals was 13.04%.

The percentage distribution of positive animals according to their habitat is presented in the following Table 2.

Table 2: Distribution of dogs with positive test results by their habitat.

| Habitat | Number of animals tested | Number of positive animals | % ratio |
|----------|--------------------------|----------------------------|---------|
| Domestic | 1081 | 59 | 5.4% |
| Stray | 628 | 164 | 26.1% |

The results obtained show a significantly higher incidence of the disease, nearly five times, in stray dogs than in domestic dogs.

The sex distribution of the dogs with positive results by region is presented in Table 3.

Table 3: Distribution of dogs with positive test results by sex.

| Region | Total number of animals tested | Number of male animals tested | Number of female animals tested | Total number of positive animals | Number of positive male animals | Number of positive female animals |
|--------|--------------------------------|-------------------------------|---------------------------------|----------------------------------|---------------------------------|-----------------------------------|
| NWR | 88 | 49 | 39 | 7 | 5 | 2 |
| CNR | 142 | 71 | 71 | 23 | 12 | 11 |
| NER | 251 | 127 | 124 | 43 | 22 | 21 |
| SWR | 528 | 274 | 254 | 29 | 18 | 11 |
| CSR | 439 | 242 | 197 | 78 | 46 | 32 |
| SER | 261 | 145 | 116 | 43 | 23 | 20 |
| Total: | 1709 | 908 | 801 | 223 | 126 | 97 |

When comparing the number of positive male and female animals to the total number of tested male and female dogs, respectively, a higher percentage was found in male animals with 13.9% and 12.1%. The following Table 4 shows the number of animals with positive results by region according to the respective age groups. The three age groups are: animals from 6 months to 2 years old, from 2 to 7 years old and animals over 7 years old.

Table 4: Number of dogs with positive test results by region according to the age groups.

| Region | Total number of animals tested | Total number of positive animals | Age between 6 m. – 2 y. (number of positive animals) | Age between 2.1 y. – 7 y. (number of positive animals) | Age above 7 y. (number of positive animals) |
|--------|--------------------------------|----------------------------------|--|--|---|
| 1. NWR | 88 | 7 | 9(0) | 59(5) | 20(2) |
| 2. CNR | 142 | 23 | 28(2) | 85(16) | 29(5) |
| 3. NER | 251 | 43 | 43(2) | 136(33) | 72(8) |
| 4. SWR | 528 | 29 | 88(2) | 271(20) | 169(7) |
| 5. CSR | 439 | 78 | 79(6) | 250(56) | 110(16) |
| 6. SER | 261 | 43 | 29(4) | 173(28) | 59(11) |
| Total: | 1709 | 223 | 276(16) | 974(158) | 459(49) |

The percentage between the number of positive animals in the individual groups to the number of all tested animals in the same groups shows that we have the highest percentage of infested dogs in the second group with 16.2%, followed by the third group with 10.7%, and the lowest percentage in the first group with 5.8%. The youngest diagnosed animal was an 8-month-old Labrador and the oldest was a 13-year-old mixed-breed domestic animal.

Discussion

This study provides up-to-date information regarding the prevalence of the heartworm disease in domestic and stray dogs throughout the country. The total percentage we received among all tested animals proves the increasing prevalence of helminthiasis in our country compared to previous studies, which show a prevalence of 7.4% (Georgieva et al., 2001; Kirkova et al., 2007). These results confirm the opinion of Genchi et al. (2009) and Morchon et al. (2012) taking into account the increasing invasion of the disease across the continent. The results of the prevalence in the individual cities and regions show both a percentage decrease and an increase compared to previous studies. There is a clear trend towards a decrease in dogs with positive results in the region of Sofia compared to previous studies – 8.75% (Kostadinov et al., 2012), 33.33% (Panayotova–Pencheva et al., 2016) and 7.5% (Borisov et al., 2017). On the other hand, there is a percentage increase compared to previous studies in regions such as Plovdiv with past results of 7.75% (Kirkova et al., 2007) and 12.1% (Kostadinov, 2007), Ruse – 15.7% (Kostadinov, 2012) and Burgas – 12.5% (Kostadinov, 2013). The lower percentage of infested dogs in Sofia region can be justified by the fact that owners are receiving more information from veterinarians in various health facilities in the city. This is a disease that is receiving increasing attention in the capital city of Sofia. This fact is perhaps determined by the specific localization of the imaginal forms, as well as the common intermediate hosts, which are well known to the population. The smaller number of stray animals determines the lower percentages in the capital because they are the main reservoir of the disease.

The tendency of parasitosis increase in our country is determined by a number of biotic and abiotic factors such as climatic conditions for vector development, presence and species composition of intermediate hosts, dynamic cross-border transport, increased dog population, degree of infestation, preventive activities, etc. Urban sprawl has led to the formation of the so-called “Heat islands,” such as buildings and parks that retain heat during the day creating microenvironments that can potentially support development of mosquito vectors during colder months, thus lengthening the transmission season (Morchon et al., 2012; Nelson, 2016). The “Heat islands” in urban areas increase the risk of infection, even during the winter months. This means that the probability of infection is never equal to zero (Nelson, 2016).

There is a clear high percentage of animals with positive results in the “domestic” and “stray” categories in favor of the latter. The main factors determining the high infestation of stray dogs on the one hand are the lack of preventive activities for them, on the other hand that they often inhabit parks and gardens, which are biotope sites of intermediate hosts, and on the third hand, the constant risk of contact with vectors. The results obtained show a higher percentage in stray dogs compared to the results of Radev et al. (2016) and lower percentage than the results of Stoyanova et al. (2019). The value difference may be due to the different number of animals studied. The study confirms the theory that the stray dogs remain the main reservoir and spreader of the disease.

The percentage sex distribution largely confirms the results established so far to have very close values in relation to the number of positive male and female dogs (Razi Jalali et al., 2010;

Anvari et al., 2019). The obtained values show that at this stage it is not possible to talk about possible sexual susceptibility on the part of the host. No mechanism has been established or proven for the preimaginal or imaginal forms of *Dirofilaria immitis* that shows greater affinity or easier and faster development in either sex.

The results obtained on the basis of age distribution show the highest percentage in the second group followed by the third. These values are a result of the possibility of prolonged exposure of the definitive hosts to the disease vectors. In this case, as in the case of sexual ratio, we cannot speak of any age predisposition. Purely statistical fact is that the older a dog is, the more likely it is to be infested with microfilariae of the *immitis* species.

The percentage increase in the positive cases of dogs in Bulgaria should signal on the one hand dog owners, and on the other hand all veterinary professionals, that necessary measures to reduce the prevalence should be taken. As a zoonotic, it is desirable for human physicians to pay attention to the helminthiasis. Although individual cases of people with established invasion of the *Dirofilaria immitis* species have been reported so far Tada et al., (1979), Takeuchi et al., (1981), Theis et al., (2001); Kim et al., (2002), we cannot be sure to what extent and severity this disease can affect the human population in the future.

Conclusion

The largest number of dogs with positive results on the territory of Bulgaria was found in the Central Southern Region and the smallest number in the Southwestern Region.

The percentage of stray dogs with positive results is almost five times higher than the percentage of domestic dogs with positive results.

The percentage sex comparison in dogs with positive results is relatively 1:1.

Most animals with positive results are found in the age range from two to seven years of age.

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