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A STUDY ON THE INCIDENCE AND DIAGNOSISTICS OF DIROFILARIOSIS (HEARTWORM DISEASE) IN CARNIVORES

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Summary

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During the period 1997–1999, 258 canine blood samples were analysed. The animals were of a various origin: 65 working dogs, 27 shepherd dogs, 55 rural dogs, 40 stray dogs and 71 companion dogs. Microfilariae of *Dirofilaria immitis* were detected in 19 or 7.4% of all studied samples by the method of Knott. The incidence of parasites was the greatest in stray (12.5%) and rural (10.9%) followed by working (7.7%) and shepherd (7.4%) dogs. Dogs kept as companion animals were the least invaded (1.4%).

All 19 microfilaria-positive samples reacted positively with the Pet Check HTWMPT test (Idexx) for detection of *D. immitis* antigens.

The study on 25 blood samples from microfilariaemic dogs, showing doubtful clinical signs of dirofilariosis yielded positive results in 2 animals (8%) with the Pet Check test.

The uncomplete helminthological necropsy of 40 stray dogs, 78 foxes, 45 jackals, 18 wolves, 21 martens, 16 wild cats and 6 badgers revealed mature *D. immitis* organisms in the heart and pulmonary arteries of 5 stray dogs, 4 foxes, 2 jackals and 1 wolf.

The *D. immitis* invasion within the risk groups of stray, working and rural dogs and wild canids creates prerequisites for its transmission through mosquitos to other, less endangered, but susceptible canine populations.

Key words: Dirofilariosis, *Dirofilaria immitis*, dogs, fox, jackal, wolf

INTRODUCTION

Dirofilariosis is a nematodosis in carnivores and more rarely, in men. It is caused by the nematode *Dirofilaria immitis* that is residing primarily in the venous return, right ventricule and the pulmonary outflow tracts. The invasion is identified by detection of microfilariae in blood circulation. There are also standartized immunological tests for detection of *D. immitis* antigens using the ELISA.

The disease is encountered among domestic and wild carnivores in various regions of the world. According to the studies of the American Heartworm Society (1977) dirofilariosis is found out in naturally invaded dogs in 50 states. Doby et al. (1980) and Bima-Blum (1993) observed a tendency towards a greater distribution of the diseases among carnivores in France. In the region of New Caledonia the percentage of invaded dogs is increased up to 60.7% in 1993 compared to 37.7% in 1986. Dirofilariosis is observed in mediterranean countries - Spain (Valladares et al., 1984; Ortega Mora et al., 1991; Anguera-Galiana, 1995), Greece (Himonas and Lazarides, 1973); Papazaharidou et al., 1994), Italy (Marconcini

and Magi, 1991), former Yougoslavia (Milosavijevic and Kulisic, 1989) . According to Olteanu (1996), dirofilariosis is met in all regions of Rumania with maximum extensity of the invasion in the south regions.

In Bulgaria, the disease is not common and not well-known. Kanev (1996) and Georgieva et al., (1997) reported dirofilariosis in various populations of dogs during the last 5-6 years.

The trend towards increase in canine population in the country, the presence of stray dogs in the areas of big cities, the lack of information about the wild carnivores, the favourable climatic conditions for increase in mosquito population necessitated the performance of investigations on the incidence of dirofilariosis in carnivores in order to reveal their role in the epizootology of the disease. Therefore, our studies had the same task.

MATERIALS AND METHODS

During the period 1997-1999, 258 canine blood samples were analysed. The animals were of a various origin: 65 working dogs, 27 shepherd dogs, 55 rural dogs, 40 stray dogs and 71 companion (city) dogs.

The modified method of Knott (Jackson, 1969) was used for detection of *D. immitis* microfilariae. *D. immitis* larvae were differentiated from those of *Dipetalonema reconditum* by the specific morphological features (length, width, shape of the posterior and anterior end and location of the excretory and anal openings).

The samples, positive for *D. immitis* microfilariae and another 25 blood samples of dogs with doubtful clinical signs indicating dirofilariosis were analysed with Pet Check HTWMPT (Idexx) test for detection of *D. immitis* antigen in canine serum by ELISA.

An incomplete helminthological necropsy of 40 stray dogs, 78 foxes, 45 jackals, 18 wolves, 21 martens, 16 wild cats and 6 badgers was performed, studying the heart and the lungs.

RESULTS

The data obtained from the analysis of blood samples by Knott's test are presented in Table 1.

Microfilariae of *D. immitis* were detected in 19 (7.4%) of all 258 samples. The percentages of invaded dogs varied according to the type of population: it was the highest in stray dogs (12.5%) followed by rural (10.9%). The blood samples obtained from 65 working and 27 shepherd dogs gave 5 (7.7%) and 2 (7.4%) positive results respectively. The lowest percentage of positive samples was that in companion dogs – only 1 out of 71 samples

Table 1. Incidence of *Dirofilaria immitis* in various canine populations

Canine populations	Number of blood samples	Number (percentage) of samples positive for *D. immitis* larvae
Working dogs	65	5 (7.7**)
Shepherd dogs	27	2 (7.4**)
Rural dogs	55	6 (10.3**)
Stray dogs	40	5 (12.5**)
Companion dogs	71	1 (1.4**)
Total	258	19 (7.4*)

* % vs the total number; ** % vs the number for the respective population.

Table 2. Results from the analysis of canine blood samples with the Pet Check test

Type of samples	Number of blood samples	Number (percentage) of positive samples	Number (percentage) of negative samples
Positive for *D. immitis* microfilariae by the Knott's test*	19	19 (100)	-
Negative for *D. immitis* microfilariae by the Knott's test*	25	2 (8)	23 (92)

* modification of the Knott's test (Jackson, 1969).

Table 3. Results of helminthological necropsies

Carnivore species	Number of animals	Number (percentage) of positive animals
Stray dogs	40	5 (12%)
Foxes	78	4 (5.2%)
Jackals	45	2 (4.4%)
Wolves	18	1 (5.5%)
Martens	21	-
Wild cats	16	-
Badgers	6	-

was positive for *D. immitis* microfilariae.

The results obtained using the Pet Check HTWMPF test are shown in Table 2. All nineteen positive samples reacted positively with the Pet Check test as well. The study of 25 samples, negative according to the Knott's test, gave 2 positive results (8%) with the Pet Check test. The condition of one of both positevely reacted dogs deteriorated and it died after 20 days. At necropsy, sexually mature dirofilariae were present in its heart.

The results of the partial helminthological necropsy are presented in Table 3. Mature *D. immitis* forms were present in the heart and pulmonary arteries in 5 stray dogs, 4 foxes, 2 jackals and 1 wolf. No parasites were encountered in martens, wild cats and badgers.

DISCUSSION

The results of our study showed that dirofilariosis is a common nematodosis in the various canine populations in the country. The differences in percentages of affected animals could be explained by their mode of living. The outdoor dogs (stray and rural dogs) are the most infested probably because they are exposed to contact with mosquitos, vectors of the invasion. This suggestion is confirmed by the very low percentage of invasion in indoor companion dogs (1.4%), that are the least at risk to invasion.

The data also suggest that the climatic conditions in the country are favourable for the development of mosquitos and for

D. immitis larvae, parasitizing in invaded insects.

In similar studies performed in various regions of Bulgaria in 1991–1996, Kanev et al. (1996) observed *D. immitis* microfilariae in 14 (7.3%) out of 192 stray dogs, in 2 (6.5%) out of 31 rural dogs and 2 (1.7%) out of 118 companion dogs. Georgieva et al. (1997) reported positive results in 3 (2.5%) out of 120 working dogs. The comparison of those data with the present ones is indicative for a trend towards increase in the percentage of invaded individuals in the most exposed canine populations.

Ortega-Mora et al. (1991) found out that in Spain, *D. immitis* microfilariae in 9.7% of rural dogs and 4.5% of working dogs by the method of Knott. They also observed the lowest percentage of invasion in companion dogs (2.1%). According to Prieto et al. (1996), *D. immitis* is encountered in 40% of dogs in Pavia, Italy and in 0.4% in Sicily. In Romania, Olteanu (1996) reported that the maximum extensity of invasion in dogs was 35% in south regions.

The incidence of dirofilariosis in former Yougoslavia (Milosavijevic and Kulisic, 1989) and Greece (Himonas and Lazarides, 1973; Papaharidou et al., 1994) has an epizootological importance. Under favourable climatical conditions, the disease could be spread by mosquitos. Other favourable factors are the long-term residence of mature forms in dogs and their transporation to long distances.

The detection of *D. immitis* after the necropsies of stray dogs, foxes, jackals and wolves showed persuasively that those carnivore species could play the role of a natural reservoir of invasion of mosquitos. This is confirmed by the data of El-Azazy et al. (1984) who established the epizootological role of wild carnivores as reservoir of dirofilariosis in Egypt. Our results are however different from those of Kanev et al. (1996) that have not detected mature dirofilariae after the necropsies of stray dogs and wild canids. Dirofilariosis in wolves and foxes are reported by Olteanu (1996) in Romania.

The positive results obtained by the method of Knott in our studies were 100% confirmed by the Pet Check test for detection of *D. immitis* antigens in canine blood sera. The two positive results given by this test in dogs with clinical signs of dirofilariosis, but negative according to the method of Knott indicated that the Pet Check test could be used for detection of occult invasions.

Guerero et al. (1989) reported hidden invasions in 6.3% of dogs in big cities using a test for detection of *D. immitis* antigens. In endemic regions in North Italy, the authors reported a occult invasion rate of 18.4% in studied dogs.

The invasion with *D. immitis* among the risk groups of stray, working and rural dogs and wild canids creates prerequisites for its transmission through mosquitos to other, less endangered, but susceptible canine populations. It is a hazard as a source of invasion in humans as well. According to Prieto et al. (1996), in several regions in South Europe, the percentage of seropositive men correlates with that of *D. immitis* positive carnivores.

In our studies, the diagnostical properties of Knott's test - precision, rapidity and easy performance, have been confirmed. It could be successfully used for routine diagnostics and for performance of epizootological studies. At the same time, our data showed that immunodiagnostical tests for detection of *D. immitis* antigens could be used for differential diagnosis and when occult infections in microfilaraemic dogs were present.

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