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BACKGROUND. *Dirofilaria repens* and *Dirofilaria immitis* are nematodes which are mainly parasites of carnivores, but can be found accidentally in humans as well. Most of the infected dogs are latent carrier of the parasite so final *Dirofilaria* infection is confirmed in most cases accidentally. Increasing migration of infected animals, vector spread, global warming and changes in human activity are leading to an increase in the number of infected animals and humans.

AIM

The aim was to determine the microfilaria presence including the nematode species, and antibody presence which will show the exposure of dogs to *Dirofilaria sp.* nematodes and therewith reveal the potential reservoirs of infection for susceptible hosts.

MATERIAL AND METHODS

This study was conducted on 72 blood samples. The presence of microfilaria (mf) and mf number/ml of blood was done by modified Knott's test and their measurements (body length) revealed species, while additional information about a host's contact with this nematode i.e. antibody presence, was revealed using “in-house” developed indirect immunofluorescence antibody test (IFAT). IFAT was performed by exposing the *D. repens* microfilaria antigen to sera diluted 1/20 and 1/40 in PBS (30 min/37°C), and secondary fluorescein-labeled antibodies.

The test results were read with a fluorescent microscope "Olympus Cx40", analyzed and statistically analyzed.

RESULTS

Knott's test and species identification. 53 blood samples were examined by the modified Knott's test method and total of 18 positive samples were found. Based on the length of the microfilariae, all samples contained *D. repens* (Table 1). During IFAT antigen preparation no formalin was used and *D. repens* microfilariae were measured and compared with microfilariae during standard implementation of Knott's test (Table 1, *D. repens**).

	Mean value (µm)	Min (µm)	Max (µm)	Standard deviation of the sample (µm)
<i>D. repens</i>	345,6764228	292,7	384,1	17,03304927
<i>D. immitis</i>	292,1487805	274,6	306,4	8,565603363
<i>D. repens</i> *	384,57	339,4	415,1	22,72706

Table 1. Microfilaria measurement results

Mf/ml. For each microfilaremic dog a number of mf/ml was determined by counting and when some samples did not contain enough blood, the No of mf/ml was calculated up to 1ml. The results for each sample are visible in Table 4.

sample group	IFAT		samples group	Knott's test negative	IFAT (positive/negative)	place of animal residence	
	positive	negative				outside (positive/negative)	indoor (positive/negative)
Slavonski Brod	9 (69,2%)	4 (30,8%)	Slavonski Brod	13	9/4	7/3	1/2
Archived serum samples from the Department of Parasitology	9 (47,4%)	10 (53,6%)					
Military police	26 (83,9%)	5 (16,1%)	Military police	14	11/3	11/3	0
Total	44 (69,8%)	19 (30,2%)					

Table 2. IFAT results of canine sera/plasma samples

The presence of antibodies against microfilariae. Positive samples showed a yellow-green fluorescence of the membrane at the borderline dilution 1/40 (Figure 1, lower part) while negative samples showed red fluorescence (Figure 1, upper part). Out of the 63, 19 samples tested by IFAT method were negative and 44 positive (Table 2). According to information about the animal residence, the Knott's test findings were compared with IFAT. Unfortunately it was not possible to compare all methods with all samples due to the unavailability of anamnestic data and samples (Table 3, Table 4).

CONCLUSIONS

- the percentage of infected animals living outdoors is higher
- non-toxic distilled water can be used instead of toxic formalin to prepare the Knott's test, but then the microfilaria are longer
- *D. repens* exposure can be proved by IFAT
- the Knott's test and IFAT are two methods that add up each other and could be used in the routine diagnosis of dirofilariosis
- due to the proven high exposure of dogs to *D. repens*, seropositive dogs should be monitored clinically and treated in case of microfilaria presence in the blood to prevent the possible vector infection and consequently other susceptible hosts including humans.

Table 3. Comparative results of IFAT, Knott's test and animal residence data

SAMPLES	KNOTT'S test	NUMBER OF MF/ML	IFAT (1:40)
sample 1	+	216,66 mf/ml	++
sample 2	-		++
sample 3	+	2133,33 mf/ml	++
sample 4	+	1200 mf/ml	++
sample 5	+	1466,66 mf/ml	++
sample 6	+	12,5 mf/ml	++
sample 7	+	50 mf/ml	++
sample 8	+	366,66 mf/ml	++
sample 9	+	4,65 mf/ml	++
sample 10	-		++
sample 11	+	2433,33 mf/ml	--
sample 12	+	1430 mf/ml	++
sample 13	+	1433,33 mf/ml	++
sample 14	+	7,69 mf/ml	++
sample 15	-		++
sample 16	+	3083,33 mf/ml	--
sample 17	+	40 mf/ml	++
sample 18	+	2,5 mf/ml	++
sample 19	+	633,33 mf/ml	++
sample 20	-		++
sample 21	+	16 mf/ml	++
sample 22	-		--
sample 23	-		++
sample 24	-		++
sample 25	-		++
sample 26	-		--
sample 27	-		++
sample 28	-		++
sample 29	-		++

Table 4. Results of the Knotts test and IFAT in military dogs.

Figure 1. Picture above– negative results of IF; picture down– positive results of IFAT

