Supplementary information

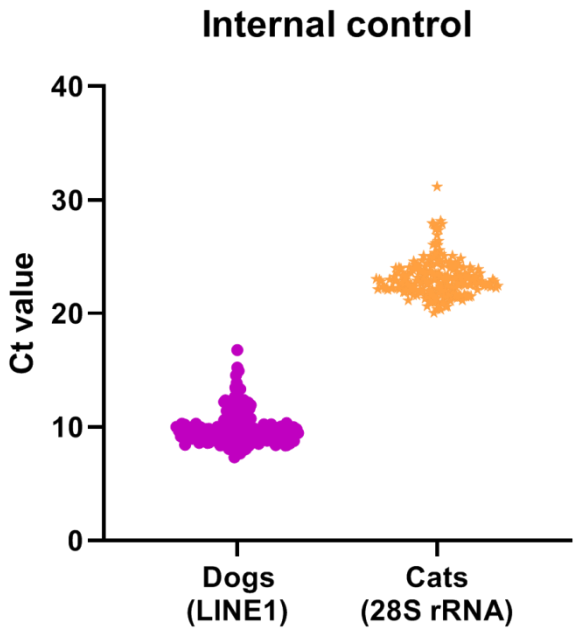

Fig. S1 Comparison of Ct values for internal extraction/amplification controls in Real-Time PCR, using primers targeting LINE1 for dogs and 28S rRNA for cats.

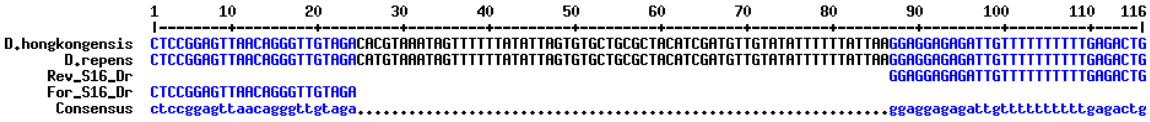

Fig. S2 The specificity of the primers targeted to a fragment of the *Dirofilaria repens 16S rRNA* gene was aligned with homologous sequences from *Dirofilaria* sp. 'hongkongensis'. The GenBank accession numbers of the sequences used in the analysis are: *D. repens*: NC_029975.1; *Dirofilaria* sp. 'hongkongensis': NC_031365.1.

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Table S1 Detailed distribution of canine and feline blood samples across regions, categorized by sex, age, and weight*

*NA, non-analyzed

Origin	Dogs/cats	Sex	Age	Weight	Total number
Lviv	Dogs	Males: 5 Females: 3	<3 years: 1 3-10 years: 4 ≥10 years: 3 Unknown: 3	<10: 2 10-25: 2 ≥25: 4	11
Berdychiv	Dogs	Males: 9 Females: 20	<3 years: 11 3-10 years: 16 ≥10 years: 2	<10: 0 10-25: 21 ≥25: 8	29
Zvenyhorodka	Dogs	Males: 22 Females: 48	<3 years: 32 3-10 years: 33 ≥10 years: 5	<10: 3 10-25: 64 ≥25: 3	70
Sumy	Dogs	Males: 5 Females: 12	<3 years: 0 3-10 years: 11 ≥10 years: 6	<10: 5 10-25: 8 ≥25: 4	17
	Cats	Males: 17 Females: 80	<3 years: 52 3-10 years: 37 ≥10 years: 7 Unknown: 1	NA	97
Kharkiv	Dogs	Males: 16 Females: 77	<3 years: 53 3-10 years: 38 ≥10 years: 2 Unknown: 13	<10: 33 10-25: 52 ≥25: 8	106
	Cats	Males: 26 Females: 90	<3 years: 79 3-10 years: 37 ≥10 years: 0 Unknown: 11	NA	127

Table S2 Detailed distribution of infection patterns in dogs and cats across examined regions*

*DR, *Dirofilaria repens*; DI, *Dirofilaria immitis*; AR, *Acanthocheilonema reconditum*.

Origin	Dogs/cats	DR	DI	AR	DR+DI	DR+AR	DI+AR	DR+DI+AR	Total number
Lviv	Dogs	1	-	-	-	-	-	-	1
Berdychiv	Dogs	10	-	-	-	-	-	-	10
Zvenyhorodka	Dogs	23	2	4	1	4	-	1	35
Sumy	Dogs	-	-	-	1	2	-	-	3
	Cats	3		-	-	-	-	-	3
Kharkiv	Dogs	8	5	-	4	-	-	-	17
	Cats	3	2	-	-	-	-	-	5

Table S3 Chi-square test results for differences in infection prevalence by region, sex, weight, and age in dogs*

* $\chi 2$ - Chi-square; df - degrees of freedom; *p* - statistical significance

Berdychiv					
Lviv	$\chi 2 = 2.579$ df = 1 *p* = 0.108				
Kharkiv	$\chi 2 = 4.842$ df =1 *p* = 0.027	$\chi 2 = 0.369$ df = 1 *p* = 0.543			
Sumy	$\chi 2 = 2.498$ df =1 *p* = 0.027	$\chi 2 = 0.399$ df = 1 *p* = 0.5275	$\chi 2 = 0.027$ df =1 *p* = 0.867		
Zvenyhorodka	$\chi 2 = 1.991$ df =1 *p* = 0.158	$\chi 2 = 6.443$ df = 1 *p* = 0.011	$\chi 2 = 23.360$ df =1 *p* = 0.0001	$\chi 2 = 5.820$ df =1 *p* = 0.015	
	Berdychiv	**Lviv**	**Kharkiv**	**Sumy**	**Zvenyhorodka**

Table S4 Chi-square test results for regional differences in infection prevalence*

* $\chi 2$ - Chi-square; df - degrees of freedom; *p* - statistical significance

	χ^2	df	*p*
Males vs females	5.913	1	0.015
<10 kg vs 10–25 kg	7.704	1	0.006
<10 kg vs ≥25 kg	6.360	1	0.012
10–25 kg vs ≥25 kg	0.14	1	0.709
<3 years vs 3-10 years	5.805	1	0.016
<3 years vs ≥10 years	0.326	1	0.568
<3 years vs 3-10 years	0.598	1	0.440