

Vector-borne parasitic infections in dogs in Romania: the first online veterinary questionnaire on canine infections with *Dirofilaria immitis*, *D. repens* and *Leishmania infantum*

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Abstract. Canine dirofilariases and canine leishmaniasis (CanL) caused by *Dirofilaria immitis*, *D. repens*, and *Leishmania infantum* are widespread endemic vector-borne diseases in the Mediterranean countries that have changed their patterns of distribution in the past years expanding to central and eastern European regions, including Romania. The aim of the study was to evaluate the veterinary approach of dirofilariases and CanL in Romania, with the challenges present in the diagnosis, treatment, diseases prevalence and epidemiology in the country, through a publicly available online survey, distributed through RoSAVA - The Romanian Small Animal Veterinary Association. Out of 122 answers, grouped by regions, the majority were received from the southern region of Romania (n=58). From the total number of the clinicians that answered, 39.0% had no cases of heartworm diseases and 84.4% had no cases of CanL. Regarding dirofilariases, 58.2% of the practitioners have informed their clients regarding the risks of the cardiac form. Prophylactic measures were applied once a month by 49.0% of the veterinarians. Regarding CanL, only 8.2% of the practitioners considered that they are geographically situated in a region with possible risk of disease transmission and 73.8% were not familiar with ESCCAP guidelines. Prophylactic measures regarding CanL were used by 50% of the veterinarians and 92.3% aimed the control of the vector using repellent substances. This survey gives a comprehensive overview about the current situation regarding diseases caused by *Dirofilaria immitis*, *D. repens*, and *Leishmania infantum* in Romania. The results suggest that diagnosis is not always carried out according to what is currently recommended by the international guidelines.

Keywords: *Dirofilaria immitis*; *Dirofilaria repens*; *Leishmania infantum*; Canine leishmaniasis; Questionnaire; Romania.

Boli parazitare transmise de către specii vector la câinii din România: primul chestionar veterinar online despre bolile produse la câini de către *Dirofilaria immitis*, *D. repens* și *Leishmania infantum*

Rezumat. *Dirofilarioza* și *leishmanioza canină* cauzate de *Dirofilaria immitis*, *D. repens* și *Leishmania infantum* sunt boli endemice în țările mediteraneene transmise de către specii vector de țânțari și de flebotomi. În ultimii ani, aceste specii și-au schimbat distribuția, extinzându-se în regiunile Europei Centrale și de Est, inclusiv România. Scopul studiului a fost evaluarea abordării veterinare a acestor boli în România, cu provocările prezente în diagnosticul, tratamentul, prevalența bolilor și epidemiologia din țară, printr-un sondaj online disponibil public, distribuit prin RoSAVA - Asociația Medicilor Veterinari de Animale de Companie din România. Din 122 de răspunsuri, grupate pe regiuni, majoritatea au fost primite din regiunea de sud a României (n = 58). Din numărul total de clinici care au răspuns, 39,0% nu au avut cazuri de *dirofilarioză* cardiacă și 84,4% nu au avut cazuri de *leishmanioză* canină. În ceea ce privește *dirofilarioza*, 58,2% dintre practicieni și-au informat clienții cu privire la riscurile formei cardiace. Măsurile profilactice au fost aplicate o dată pe lună de 49,0% dintre medicii veterinari. În ceea ce privește *leishmanioza canină*, doar 8,2% dintre practicieni au considerat că sunt situați geografic într-o regiune cu posibil risc de transmitere a bolii și 73,8% nu erau familiarizați cu ghidurile ESCCAP. 50% dintre medicii veterinari au utilizat măsuri profilactice privind *leishmanioza canină* și 92,3% au vizat controlul insectelor vectori folosind substanțe repelente. Acest sondaj oferă o imagine de ansamblu asupra situației actuale cu privire la bolile cauzate de *Dirofilaria immitis*, *D. repens* și *Leishmania infantum* în România. Rezultatele sugerează că diagnosticul nu este întotdeauna realizat în conformitate cu recomandările internaționale actuale.

Cuvinte cheie: *Dirofilaria immitis*, *Dirofilaria repens*, *Leishmania infantum*, *leishmanioză canină*, chestionar, România.

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Introduction

Dirofilariases and *leishmaniasis* are vector-borne zoonoses, prevalent in the Mediterranean countries that affect mainly dogs and other carnivores. In the last few decades, their pattern of distribution and their area of their endemicity has expanded and other non-endemic regions seem to have suitable conditions for the occurrence of these diseases (Genchi et al., 2005; Otranto et al., 2009; Pantchev et al., 2011; Sassnau et al., 2014; Ionică et al., 2015; Fuehrer et al., 2016).

In the Mediterranean region, the human visceral leishmaniasis (VL) and canine leishmaniasis (CanL), both caused by *Leishmania infantum* are regarded as highly

endemic (Maroli et al., 2008). In the last years, *L. infantum* has been reported in parts of Northern, Central, and Eastern Europe, in countries such as Czech Republic, Poland, Ukraine, Hungary, Romania, or Bulgaria, where sporadic cases of human and animal leishmaniasis were described (Mihalca et al., 2019). Recent updates on sand fly seasonality and species diversity in Romania were recorded between 2013 and 2018, confirming the presence of the sand fly vectors in warmer regions of the country (Cazan et al., 2019a; 2019b). The first serological survey on CanL in the country, indicated the presence of a CanL focus, in what appears to be a re-emergence focus of the diseases, with a low but worrying prevalence for a non-endemic region (3.7% seropositivity and 8.7% PCR positivity)

(Dumitrache et al., 2016). In this new context, it is necessary to evaluate the veterinary approach (management, prevention and treatment) of CanL in the country.

In Romania, dirofilariases (caused by *Dirofilaria immitis* and *D. repens*) have been reported in dogs and other carnivores, in the southern, south-eastern, eastern and western regions of the country (Ciocan et al., 2013; Ionică et al., 2015; Ionică et al., 2017; Ciucă et al., 2016), as well as in humans (Neghină et al., 2011; Popescu et al., 2012; Lupșe et al., 2014).

When comparing the prospective epidemiological studies and questionnaire-based studies, there are some differences between the two types of approaches. It is almost impossible for the former to comprise the real distribution data of one disease, while through the latter a number of cases closer to reality for both dirofilariases and CanL could be accounted, as many of them represent unpublished clinic-based data.

The aim of the present survey was to evaluate the distribution of dirofilariases and leishmaniasis in Romania through a questionnaire. The present questionnaire-based study aimed also to bring new lights into the diagnosis methods, the challenges present in the dirofilariases and leishmaniasis diagnosis and treatment, diseases prevalence, and epidemiology in Romania.

Materials and methods

An open-access online survey was developed and distributed to private practices, veterinary clinicians and rural practices from Romania. All questions referred to dirofilariases caused by *Dirofilaria immitis*, *Dirofilaria repens* and leishmaniasis caused by *Leishmania infantum*. The survey was distributed online from October 2017 until December 2018, via email and social media by RoSAVA (The Romanian Small Animal Veterinary Association). The survey was conducted using EUSurvey and included 38 questions. The first part included general questions about the clinics' location (city, county) and its size (number of patients/year),

while the second and third part has consisted of specific questions about dirofilariases and leishmaniasis. The ethical approval was deemed unnecessary according to national regulations and a written consent to participate was obtained from all veterinarians who answered the questionnaire.

For the purpose of this study, the counties were grouped in 4 groups, according to their geographic position as follows: intra-Carpathian (Alba, Bistrița-Năsăud, Brașov, Cluj, Harghita, Hunedoara, Maramureș, Mureș, Sălaj, Sibiu), eastern (Bacău, Galați, Neamț, Suceava, Vaslui), western (Arad, Bihor, Timiș) and southern (Argeș, Brăila, București, Buzău, Călărași, Constanța, Dâmbovița, Dolj, Giurgiu, Gorj, Ilfov, Teleorman, Tulcea, Vâlcea).

Frequency, prevalence and its 95% confidence interval (CI) were calculated for each of the three pathogenic agents included in the questionnaire. Chi-square test and multiple regression analysis was performed to find out if there are significant differences among *D. immitis* (number of cases and presence/absence) and *D. repens* cases by number of cases, type of cases, region, area, size of clinic, screening and prophylaxis. The significance level was set at a value of $p \leq 0.05$. The statistical analysis was performed using EpiInfo software.

The correlation between the *D. immitis/D. repens/L. infantum* number of cases and region of the country, veterinary clinic size, area (urban, rural), screening performing for subclinical cases detection and specific prophylaxis applied were determined using Spearman's rank correlation test and multiple regression analysis. The strength of correlation was established based on absolute value of $r(s)$ coefficient, as follows: 0.00-0.19 "very weak" correlation; 0.20-0.39 "weak" correlation; 0.40-0.59 "moderate" correlation; 0.60-0.79 "strong" correlation; 0.80-1.0 "very strong" correlation. The analysis was performed with MedCalc® software version 18.11.3.

The distribution maps for all three species, based on the questionnaire's answers were generated using QGIS 3.4 software (www.qgis.org).

Results

The survey received 122 answers from 32 counties out of 41. The answers were from Bucharest (22.13%), Cluj and Timiș (9.84% each), Brașov (8.20%), Ilfov (7.38%), Bihor (4.10%), Dâmbovița and Dolj (3.28% each), Bacău, Bistrița-Năsăud, Constanța, Maramureș, and Sălaj (2.46% each), Buzău, Mureș, Sibiu, Suceava, and Tulcea (1.64% each), Alba, Arad, Argeș, Brăila, Călărași, Galați, Giurgiu, Gorj, Harghita, Hunedoara, Neamț, Tulcea, Vâlcea, and Vaslui (0.82% each). Grouped by regions, the response received were distributed as follows: intra-Carpathian (n=38), southern (n=58), eastern (n=8), and western (n=18).

Dirofilaria immitis

Out of 122 practices, 39.0% had no cases of heartworm in the past year, 20.0% had between 1 and 5 cases, 15.0% had 5-10 cases, 8.0% had 10-20 cases, and 16.0% had over 20 cases the previous year. The prevalence of practices that diagnosed *D. immitis* was 93.1% in the southern region, 72.2% in the western region, 25.0% in the eastern region and in the intra-Carpathian region 13.2% (table 1). All counties with positive cases of *D. immitis* are shown in figure 1. There was a strong correlation between the number of *D. immitis* cases and the region ($r = 0.735$; $p < 0.001$) (figure 2).

Table 1. Prevalence and frequency of *D. immitis* cases depending on region, environment and practice size

	Frequency	Prevalence	95% CI	χ^2	p	OR ^c
Region						
Eastern (n=8)	2 ^a	25.0	3.2-65.1	66.7827	0.0000	5.10*** (2.8-9.4)
Intra Carpathian (n=38)	5 ^b	13.2	4.4-28.1			
South (n=58)	54	93.1	83.3-98.1			
West (n=18)	13	72.2	46.5-90.3			
Environment						
Urban (n=97)	61	62.9	52.5-72.5	0.5837	0.44	0.74 (0.16-3.52)
Rural (n=25)	13	52.0	31.3-72.2			
Practice size						
1-1000 (n=41)	15	36.6	22.1-53.1	17.8343	0.0001	3.86** (1.32-11.30)
1000-5000 (n=67)	46	68.7	56.2-79.4			
>5000 (n=14)	13	92.9	68.5-98.7			

^a Only 2 practices, one from Bacău and one from Galați had diagnosed *Dirofilaria* spp. in the past year;

^b From Brasov 3 out of 4 practices had only imported cases;

^c Logistic regression model (variable prophylaxis was excluded from model): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

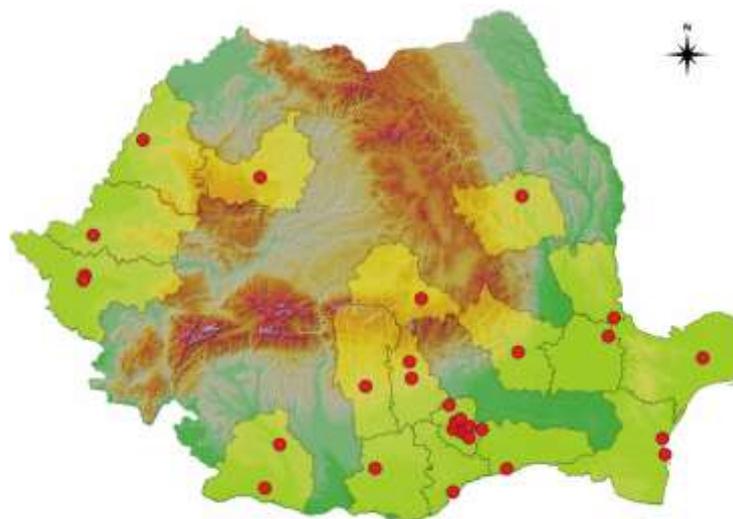


Figure 1. Positive cases of canine dirofilariasis caused by *D. immitis* (cardiac dirofilariasis)

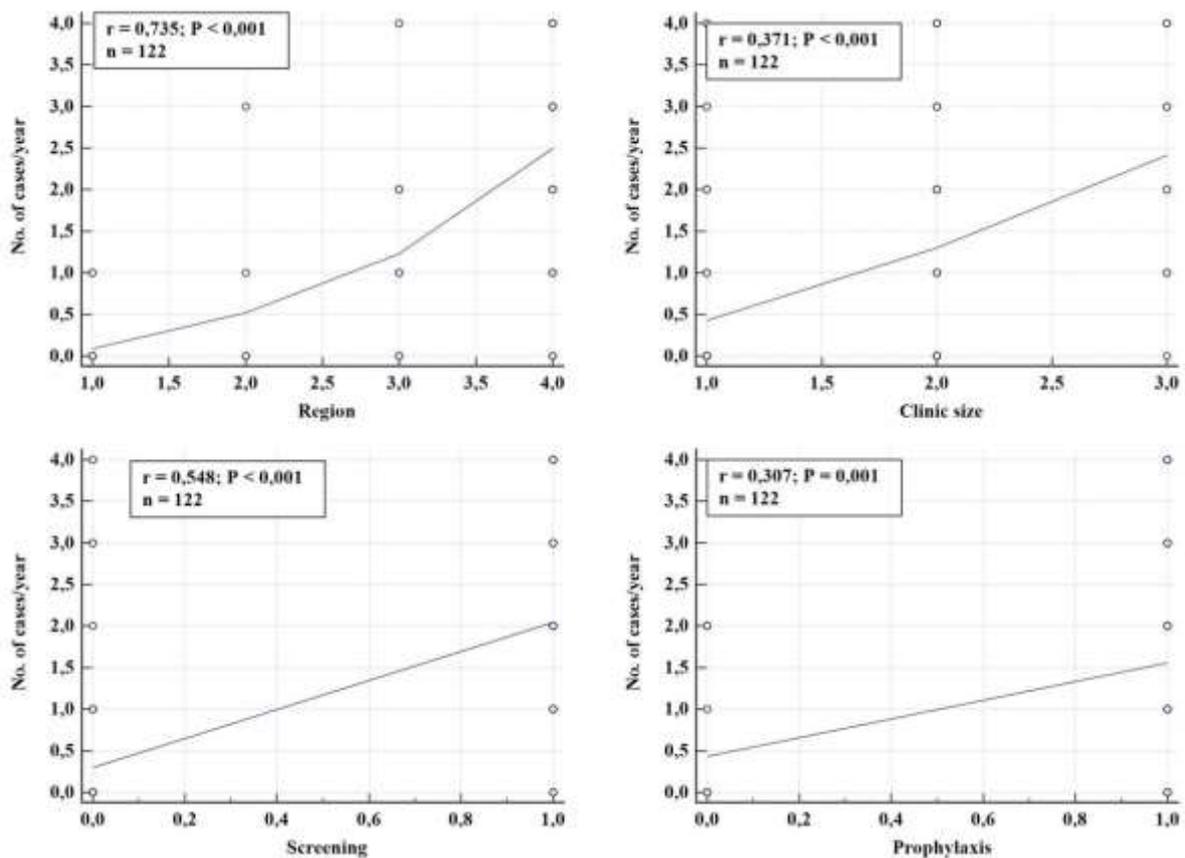


Figure 2. Correlation data between the number of *D. immitis* cases and region, clinic size, screening and prophylaxis (Spearman's rank correlation test)

In the light of pet owner's awareness regarding dirofilariasis only 58.2% of practitioners have informed their clients regarding the risks posed by the canine heartworm. Regarding the awareness of the international guidelines of heartworm disease in dogs, 42.0% of the veterinarians who answered the questionnaire, are familiar with the European Scientific Council Companion Animal Parasites (ESCCAP) guidelines, 38.0% declared that they are familiar with the American Heartworm Society (AHS) guidelines, 28.0% are familiar with both guidelines, while 49.0% are not familiar with either of them.

The diagnosis of heartworm infection was based upon several methods, among which, the most frequent were the rapid tests (93.2%), followed by smear examination (74.3%), clinical exam (45.9%), echocardiography (39.2%), Knott's test (32.4%) and molecular methods (10.8%). Screening methods were applied by 54.91% (n=67) of practitioners (table 2). Prophylactic measures were applied

once a month by 49.0% of the veterinarians and milbemycin was recommended by 89.2% of the practitioners as prophylactic drug (table 2).

Dirofilaria repens

Autochthonous cases of *D. repens* were reported by 19.7% (n=24) of the responders. All practices that reported this pathogen were located in the southern (27.6%) and western (44.4%,) regions of the country (figure 3). From 19.7% of the practitioners which had positive cases of *D. repens*, only 79.2% of them considered themselves in a highly risk zone. The diagnosis was most often based upon the examination of the blood smear (79.2%). For treatment, the practitioners who diagnosed *D. repens* have chosen the surgery (41.7%), and the therapy with melarsomine (29.2%). In the light of pet owner's awareness regarding dirofilariasis only 38.5% of practitioners have informed their clients regarding the risks of subcutaneous dirofilariasis.

Table 2. Regression analysis for the evaluation of significant differences among *D. immitis* cases by region, area, number of cases in the clinic, size of clinic, screening and prophylaxis

	Frequency	Prevalence	95% CI	χ^2	p	OR ^c
Region						
Eastern (n=8)	2 ^a	25.0	3.2-65.1	66.7827	0.0000	5.10*** (2.8-9.4)
IC (n=38)	5 ^b	13.2	4.4-28.1			
South (n=58)	54	93.1	83.3-98.1			
West (n=18)	13	72.2	46.5-90.3			
Area						
Urban (n=97)	61	62.9	52.5-72.5	0.5837	0.44	0.74 (0.16-3.52)
Rural (n=25)	13	52.0	31.3-72.2			
Size of the clinic						
1-1000 (n=41)	15	36.6	22.1-53.1	17.8343	0.0001	3.86** (1.32-11.30)
1000-5000 (n=67)	46	68.7	56.2-79.4			
>5000 (n=14)	13	92.9	68.5-98.7			
Screening						
Yes (n=67)	57	85.1	74.3-92.6	34.8989	<0.00001	10.50*** (2.60-42.34)
No (n=55)	17	30.9	19.1-44.8			
Profilaxy						
Yes (n=102)	69	67.6	57.7-76.6	11.0193	0.0009	-
No (n=20)	5	25.0	8.7-49.1			

^a Bacău and Galați Counties; ^b 3 imported cases only; Brașov and Cluj Counties (n=1);

^c Logistic regression model (variable prophylaxis was excluded from model): * p < 0.05; ** p < 0.01; *** p < 0.001.

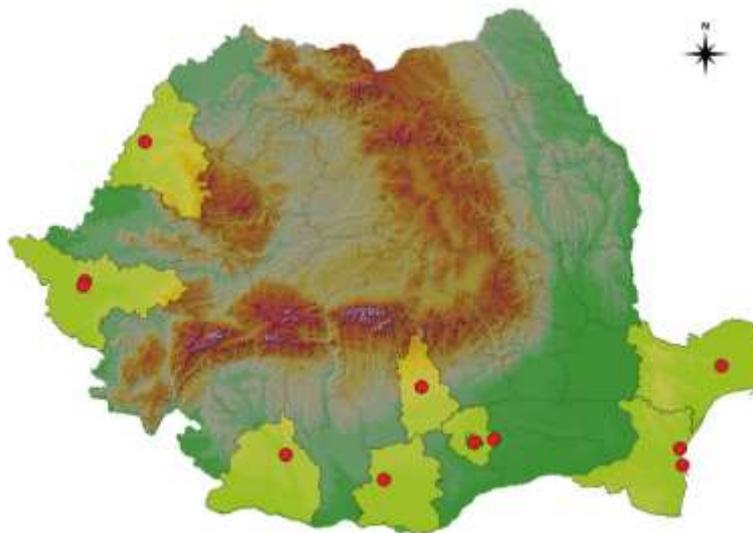


Figure 3. Positive cases of canine dirofilariasis caused by *D. repens* (subcutaneous dirofilariasis)

Leishmania infantum

Out of 122 practices, 84.4% (103/122) had no cases of CanL in the past year. The practices with CanL cases in the past year were located in the southern counties of Romania (7/19 cases in București, 1/19 case in Constanța, 1/19 case in Giurgiu, and 1/19 case in Brăila), western region (4/19 cases in Timiș and 1/19

case in Bihor), intra-Carpathian (2/19 cases in Cluj and 1/19 case in Maramureș), and eastern region (1/19 case in Galați) (figure 4).

The CanL cases were diagnosed with a frequency of 2/41 and a prevalence of 4.9% in practices with more than 1000 patients/year, 14/67 (20.9%) in practices with the total number of patients situated between 1000 and

5000/year, and 3/14 (21.4%) in practices with more than 5000 patients/year, with no statistical significance (table 3). No information was provided if the cases were autochthonous or imported. From the total practitioners that

answered the questionnaire, 10/122 (8.2%) considered that they are geographically situated in a region with possible risk of CanL transmission.

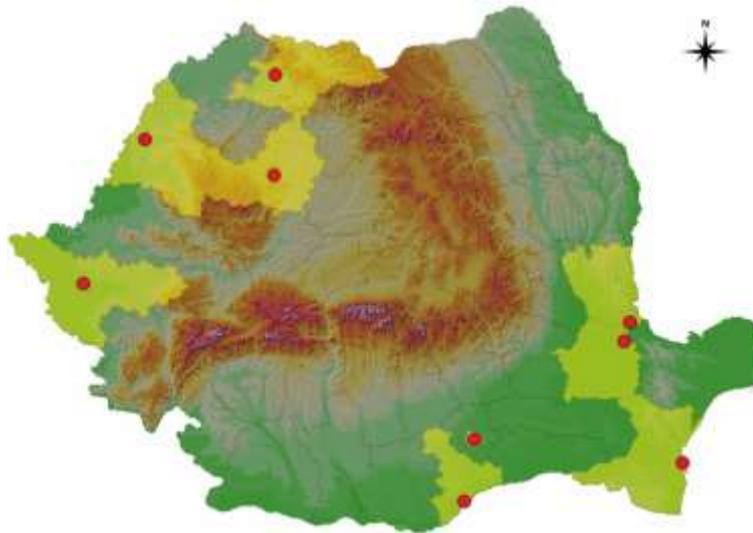


Figure 4. Positive cases of canine leishmaniasis caused by *L. infantum*

Table 3. Prevalence and frequency of *L. infantum* cases depending on region, and practice size in Romania

Total answers (n=122)	Frequency	Prevalence	95% CI	χ^2	p	OR
CanL cases	19	15.6	9.6-23.2			
Clinical diagnosis	9	47.4	27.3-68.3	23.52	<0.0001	
Skin biopsy	3	15.8	5.5-37.6			
Rapid test	15	79.0	56.7-91.5			
Serological methods	5	26.3	11.8-48.8			
Molecular methods	3	15.8	5.5-37.6			
Region						
Eastern (n=8)	1	12.5	0.3-52.7	3.92	0.27	1.72 (0.71-4.15)
Intra-Carpathian (n=38)	3	7.9	1.7-21.4			
Southern (n=58)	10	17.2	8.6-29.4			
Western (n=18)	5	27.8	9.7-53.5			
Practice category						
<1000 (n=41)	2	4.9	0.6-16.5	5.38	0.07	1.98 (0.67-5.99)
1000-5000 (n=67)	14	20.9	11.9-32.6			
>5000 (n=14)	3	21.4	4.7-50.8			
Screening						
Da (n=17)	4	23.5	6.8-49.9	0.38	0.54	0.17 (0.01-2.04)
Nu (n=105)	15	14.3	8.2-22.5			
Profilaxy						
Yes (n=13)	10	76.9	46.2-95.0	36.59	>0.0001	92.08 (9.82-863.86)
No (n=109)	9	8.3	3.8-15.1			
Risk zone						
No (n=66)	9	13.6	6.4-24.3	1.78	0.41	1.03 (0.51-2.07)
Yes (n=10)	3	30	6.7-65.2			
I don't know (n=46)	7	15.2	6.3-28.9			

The diagnosis was based upon several methods (table 4), among which, the most frequent were the rapid tests (78.9%). Screening methods were applied by 13.9% (17/122) of the practitioners (table 5). All practitioners that applied screening methods used the rapid test (100%). Prophylactic measures were used by 50% (13/26) of the veterinarians (table 5). The control of the vector using repellent substances was done by 92.3% (12/13) of the veterinarians, followed by 30.8% (4/13) of veterinarians that used insecticides, and 7.7% (1/13) that used the preventive vaccination of the dogs. The most commonly used drugs for the treatment of

CanL were the off-label allopurinol, in 63.15% (12/19), amphotericin B 42.1% (8/19), and meglumine antimoniate 36.84% (7/19) (table 5).

Discussion

Dirofilaria immitis

The majority of the answers of the present study came from clinics that were located in the southern region of Romania (54.0%) which is also the region with the largest percentages of clinics that diagnosed cardiac dirofilariasis (93.1%).

Table 4. Prevalence and frequency of combination of used methods in CanL diagnosis in Romania

	Frequency	Prevalence	95% CI
Clinical + Rapid test	4	21.1	8.5-43.3
Clinical + Skin biopsy	1	5.7	0.9-24.6
Rapid test + Serological methods	3	15.8	5.5-37.6
Clinical + rapid test + serological + molecular methods	1	5.7	0.9-24.6
Clinical + rapid test+molecular methods	1	5.7	0.9-24.6
Clinical + skin biopsy + serological + molecular methods	1	5.7	0.9-24.6
Total	11	57.9	36.8-76.9

Table 5. Prevalence and frequency of screening, prophylaxis and treatment of *L. infantum* cases in Romania

	Frequency	Prevalence	95% CI
Screening			
Rapid tests	17	13.9	8.3-21.4
Serological methods	3	2.5	0.5-7.0
Molecular methods	3	2.5	0.5-7.0
Screening frequency			
Once in 6 months	4	3.3	1.3-8.1
Once per year	8	6.6	2.9-12.5
Once at two years	2	1.6	0.5-5.8
Once in the lifetime	2	1.6	0.5-5.8
Another protocol	1	0.8	0.1-4.5
Prophylaxis			
Insecticides	4	3.3	0.9-8.2
Repellents	12	9.8	5.2-16.6
Vaccination	1	0.8	0.0-4.5
Treatment (15/19)			
Yes	15	79.0	56.7-91.5
No	4	21.1	8.5-43.3
Allopurinol	12	80.0	54.8-93.0
Amphotericin B	8	53.3	30.1-75.2
Meglumine antimoniate	7	46.7	24.8-69.9
Combination of substances			
Allopurinol + Meglumine antimoniate	4	26.7	10.9-52.0
Allopurinol + Meglumine antimoniate + Amphotericin B	2	13.3	3.7-37.9
Allopurinol + Amphotericin B	4	26.7	10.9-52.0
Total combination of substances	10	66.7	41.7-84.8
Allopurinol only	2	13.3	3.7-37.9
Amphotericin B only	2	13.3	3.7-37.9
Meglumine antimoniate only	1	6.7	1.2-29.8

Other studies performed in southern Romania showed a prevalence of *D. immitis* of 60% in Galați county, 15.9% in Tulcea county, 13.7% in Teleorman, or 7.8% in Dolj (Ciocan et al., 2013; Ionică et al., 2015; Ciucă et al., 2016).

Even if AHS and ESDA (European Society of Dirofilariosis and Angiostrongylosis) guidelines recommend the combination of serological tests and Knott test for a thorough diagnosis (AHS, 2018; ESDA, 2018), only 27% of the practitioners used both of these tests for diagnosis. On the other hand, given that almost half of the practitioners who answered are not aware of the guidelines, the results are not surprising. Interestingly, the practices which used frequent prophylaxis (once per month or once every 3 months) also had over 20 cases of *D. immitis* in the past year. Similar results were obtained in another study where the prevalence in the past year was compared to the prevalence in the last 5 years. There, results showed an increasing number of cases, despite veterinarians' prescriptions of prophylactic treatments (Genchi et al., 2014). One explanation for this might be the incompliance of pet owners to prophylactic treatment, because in the countries where pet owner compliance is good the number of cases is decreasing (Genchi et al., 2014).

As screening is concerned, from the same category of clinicians that were unaware of the guidelines, most of them applied screening once a year (23.3%) or once every 6 months (11.7%). Nevertheless, there was a large proportion that did not apply screening methods at all (45.1%).

Given these results, a huge amount of concern arises on how the lack of screening and prophylaxis might influence the prevalence of *D. immitis* in Romania, in the current context of climatic changes and increased traveling that may alone increase the spread of the disease (Sassnau et al., 2014; Genchi et al., 2009; Otranto et al., 2009). With deficits of screening and prophylaxis, as well as of delivering information to the pet owners regarding the risks of the disease, one question yet arises on how do those clinicians manage a heartworm disease case.

The approach preferred by the responders in the treatment of *D. immitis* was the use of milbemycin, followed by the use of other macrocyclic lactones. Only 25.7% aimed for specific adulticide treatment with melarsomine. However, these results did not reveal the protocol used in the treatment of heartworm disease in dogs. The importance of the approach resides in the efficacy of treatment: for example, the long-term use of macrocyclic lactones is capable of killing adult parasites, whereas a single or a short-term use leads to killing just the circulating microfilariae (McCall et al., 2001).

The general impression about the results of this survey is that veterinarians in Romania tend to oversee the implications of the disease, in spite of having diagnosed positive cases in their practices. At the roots of this problem probably lays the lack of informed decisions based on international guidelines, lack of publicity and campaign regarding the zoonotic potential of this disease, as well as the implications regarding the lengthy and risky treatment.

Dirofilaria repens

The number of responders that diagnosed *D. repens* in the past year in Romania was 19.7%, lower than that of *D. immitis* (60.7%). The veterinarians that had positive cases had their practices located in the western and southern regions, the same regions that had also the most diagnosed cases of *D. immitis*. However, in one other study that used both serological and molecular methods for diagnosis, the prevalence of subcutaneous dirofilariosis was 6.9%, slightly higher than of heartworm disease (6.1%), while in most cases the parasites incriminated were found in coinfections (23.9%) (Ionică et al., 2015). On the other hand, the methods used for the diagnosis of subcutaneous dirofilariosis were in 15.8% of cases based solely on the examination fresh blood smear, which even though suggestive, have a very low sensitivity and give frequently false negative results. One of the reasons for a low prevalence of *D. repens* could be that the subcutaneous dirofilariosis can go undetected, under regular clinical examination. Also, in practices where only microscopy is used, the

microfilariae of *D. repens* may be confused with those of *D. immitis*. The statistical analysis found no correlation between the frequency of prophylaxis and the number of cases.

Leishmania infantum

Based on the given answers, *L. infantum* was detected in Western Romania (27.8%), followed by Southern Romania (17.2%), Eastern Romania (12.5%) and intra-Carpathian region of the country (7.9%). These values differ from the available data on CanL in Romania, where the only performed CanL survey revealed 3.7% seropositivity and 8.7% PCR positivity in dogs in the south-central region of Romania (Dumitrache et al., 2016). These differences can be explained by a series of factors: (i) the questionnaire has its limits, some questions were not answered, other missing data; (ii) most of the Romanian Veterinary Faculties run specialized clinics inside their departments where CanL is usually diagnosed, so the prevalence value per region might not reflect reality, as the origin of the cases could be other region than the one where the diagnosis is given, and as the clinics receive cases from the entire country.

If we add that only 26.2% of the veterinarians who answered the questionnaire were aware of the ESCCAP guidelines, while 73.8% were not familiar with them, to inform the practitioners and veterinarians about the public health impact and the possible adaptation of *L. infantum* in local sand fly vectors is a major need in Romania.

The most frequent diagnosis method used by the questionnaire participants was the use of a rapid test (78.9%). The value is much higher than the 37% of the vets from Greece, Italy, Portugal, Spain and southern France that reported the routine use of the same tests (Bourdeau et al., 2014).

The given answers of the participants to the online questionnaire do not exclude, though, a different distribution of the diseases produced by *D. immitis*, *D. repens* and *L. infantum* in Romania, as the study has its limitations (e.g. non-homogeneous data collection, the inability to calculate the response rate, as the total

number of recipients was not known due the online survey distribution, via email and social media).

Conclusions

This survey gives a comprehensive overview about the current situation regarding canine vector-borne diseases caused by *D. immitis*, *D. repens* and *L. infantum* in Romania. The general perception is that there is no methodological approach to screening and prevention of *Dirofilaria* spp. cases as there are practices where those methods are lacking in spite of the positive diagnosed cases.

Regarding CanL, the survey offers data of the clinical management of the disease in Romania. The survey suggests the need for standardized guidelines in CanL diagnosis and treatment, especially in the non-endemic countries like Romania, with increasing number of local CanL cases.

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References

- AHS. American Heartworm Society. Canine Heartworm Guidelines. American Heartworm Society. 2018. <https://www.heartwormsociety.org/images/pdf/2018-AHS-Canine-Guidelines.pdf>. Accessed 20 May 2019.
- Bourdeau P., Saridomichelakis M.N., Oliveira A., Oliva G., Kotnik T., Gálvez R., Foglia Manzillo V., Koutinas A.F., Pereira da Fonseca I., Miró G. 2014. Management of canine leishmaniosis in endemic SW European regions: a questionnaire-based multinational survey. *Parasit. Vectors* 7:110.
- Cazan C.D., Păstrav I.R., Györke A., Oguz G., Alten B., Mihalca A.D. 2019a. Seasonal dynamics of a population of *Phlebotomus (Larrousius) perfilewii* Parrot, 1930 (Diptera: Psychodidae) in North-Eastern Romania. *Parasitol. Res.* DOI: 10.1007/s00436-019-06296-9.
- Cazan C.D., Păstrav I.R., Ionică A.M., Oguz G., Erisoz Kasap O., Dvorak V., Halada P., Dumitrache M.O., Volf P., Alten B., Mihalca A.D. 2019b. Updates on the distribution and diversity of sand flies

- (Diptera: Psychodidae) in Romania. *Parasit. Vectors* 12:247.
- Ciocan R., Mederle N., Jacsó O., Táncoz B., Fok E. 2013. Autochthonous Cases of *Dirofilaria* in Dogs from Timiș County (Western Part) Romania. *Global Journal of Medical Research Veterinary Science and Veterinary Medicine* 13(2):29-34, ISSN: 2249-4618.
- Ciucă L., Musella V., Miron L.D., Maurelli M.P., Cringoli G., Bosco A., Rinaldi L. 2016. Geographic distribution of canine heartworm (*Dirofilaria immitis*) infection in stray dogs of eastern Romania. *Geospatial Health* 11:499.
- Dumitrache M.O., Nachum-Biala Y., Gilad M., Mircean V., Cazan C.D., Mihalca A.D., Baneth G. 2016. The quest for canine leishmaniasis in Romania: the presence of an autochthonous focus with subclinical infections in an area where disease occurred. *Parasit. Vectors* 9:297.
- ESDA. 2018. Guidelines for clinical management of canine heartworm disease. European Society of Dirofilariosis and Angiostrongylosis. <http://esda.vet/wpcontent/uploads/2017/11/guidelines-for-clinical-management-of-canine-heartwormdisease.pdf>. Accessed 20 May 2019.
- Fuehrer H.P., Auer H., Leschnik M., Silbermayr K., Duscher G., Joachim A. 2016. *Dirofilaria* in Humans, Dogs, and Vectors in Austria (1978-2014) – From Imported Pathogens to the Endemicity of *Dirofilaria repens*. *PLoS Negl. Trop. Dis.* 10(5):e0004547.
- Genchi C., Rinaldi L., Cascone C., Mortarino M., Cringoli G. 2005. Is heartworm disease really spreading in Europe? *Vet. Parasitol.* 133:137-148.
- Genchi C., Rinaldi L., Mortarino M., Genchi M., Cringoli G. 2009. Climate and *Dirofilaria* infection in Europe. *Vet. Parasitol.* 163:286-292.
- Genchi C., Bowman D., Drake J. 2014. Canine heartworm disease (*Dirofilaria immitis*) in Western Europe: survey of veterinary awareness and perceptions. *Parasit. Vectors* 7:206.
- Ionică A.M., Matei I.A., Mircean V., Dumitrache M.O., D'Amico G., Györke A., Pantchev N., Annoscia G., Albrechtová K., Otranto D., Modrý D., Mihalca A.D. 2015. Current surveys on the prevalence and distribution of *Dirofilaria* spp. and *Acanthocheilonema reconditum* infections in dogs in Romania. *Parasitol Res.* 114:975-982.
- Ionică A.M., Matei I.A., D'Amico G., Ababii J., Daskalaki A.A., Sándor A.D., Enache D.V., Gherman C.M., Mihalca A.D. 2017. Filarioid infections in wild carnivores: a multispecies survey in Romania. *Parasite Vector* 10(1):332.
- Lupșe M., Mircean V., Cavasi A., Mihalca A.D. 2014. Recurrent subcutaneous human Dirofilariasis due to *Dirofilaria repens* after surgical removal of the worm and anthelmintic treatment. *Parasite Vector* 7(1):P3.
- Maroli M., Rossi L., Baldelli R., Capelli G., Ferroglio E., Genchi C., Gramiccia M., Mortarino M., Pietrobelli M., Gradoni L. 2008. The northward spread of leishmaniasis in Italy: evidence from retrospective and ongoing studies on the canine reservoir and phlebotomine vectors. *Trop. Med. Int. Hlth.* 13:256-264.
- McCall J.W., Guerrero J., Roberts R.E., Supakorndej N., Mansour A.E., Dzimiński M.T., McCall S.D. 2001. Further evidence of clinical prophylactic, retro-active (reach-back) and adulticidal activity of monthly administrations of ivermectin (Heartgard Plus™) in dogs experimentally infected with heartworms. In *Recent Advances in Heartworm Disease Symposium '01*. American Heartworm Society 198-200.
- Mihalca A.D., Cazan C.D., Sulesco T., Dumitrache M.O. 2019. A historical review on vector distribution and epidemiology of human and animal leishmanioses in Eastern Europe. *Res. Vet. Sci.* 123:185-191.
- Neghină R., Neghină A.M., Marincu I., Iacobiciu I. 2011. Epidemiology and history of human parasitic diseases in Romania. *Parasitol. Res.* 108:1333-1346.
- Otranto D., Capelli G., Genchi C. 2009. Changing distribution patterns of canine vector borne diseases in Italy: leishmaniosis vs. dirofilariosis. *Parasit. Vectors* 2(1):S2. DOI:10.1186/1756-3305-2-S1-S2.
- Pantchev N., Etzold M., Dauschies A., Dyachenko V. 2011. Diagnosis of imported canine filarial infections in Germany 2008-2010. *Parasitol. Res.* 105:S63-S74.
- Popescu I., Tudose I., Racz P., Muntea B., Giurcaneanu C., Poppert S. 2012. *Human Dirofilaria repens* Infection in Romania: A Case Report. *Case Rep. Infect. Dis.* Article ID 472976, 4 pages.
- Sassnau R., Dauschies A., Lendner M., Genchi C. 2014. Climate suitability for the transmission of *Dirofilaria immitis* and *D. repens* in Germany. *Vet. Parasitol.* 205:239-245.