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Dirofilaria immitis and D. repens in dog and cat: A questionnaire study in Italy

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Abstract: *Dirofilaria immitis* and *D. repens* are vector-borne parasites of dogs and cats, with zoonotic potential, endemic in many parts of Europe, including Italy. Control and prevention of dirofilariosis are essential points to reduce their diffusion in animals and humans and veterinarians are the main subjects involved in this "battle". With the aim to better understand current practice by veterinarians, an online questionnaire on different aspects of *D. immitis* and *D. repens* was sent to companion animal veterinary facilities registered with the Italian Veterinary chamber. The overall response rate was 25%. Approximately 47% had diagnosed at least one case of *Dirofilaria* spp. in dogs in the last year and approximately 6% had diagnosed at least one case of *Dirofilaria* spp. in cats. Of the 662 facilities surveyed, 83.8% used serology to diagnose heartworm infection. For the diagnosis of *D. repens* infection in dogs and cats, a high percentage of facilities relied on an external laboratory. Most recommended beginning prevention of canine heartworm disease in April-May. Topical and injectable moxidectin and oral ivermectin were the two most commonly used preventives for *D. immitis* in dogs. The drug most commonly used for heartworm adulticide treatment in dogs was melarsomine. This study shows that veterinary facilities working in an endemic area for *D. immitis* and *D. repens* are aware of recent developments in adulticide therapy. Results would suggest however that diagnosis is not always carried out according to what is currently recommended by international guidelines and that the timing for starting prevention may not be optimal.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:

all data are exposed in the study

Prof. Marco Genchi DVM PhD
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Parma, Italy

05 November 2018

Dear Editor

Please find attached our manuscript entitled "*Dirofilaria immitis* and *D. repens* in dog and cat: a questionnaire study in Italy" for consideration by Veterinary Parasitology.

This study was carried out to assess the experience and knowledge of veterinarians about the infection of *D. immitis* and *D. repens* in dog and cat. To evaluate this, an electronic questionnaire was sent to all Italian veterinary facilities. The questionnaire was sent to 2795 veterinary facilities. The overall response rate was 25%.

With this study we have obtained information on the distribution, prevention, diagnosis and treatment of these two vector-borne parasites, widespread throughout Europe.

We believe that the strength of this manuscript is the high number of responses and information obtained in an area historically endemic to these parasites. These data may also be useful for other researchers in countries where *Dirofilaria* spp. is spreading.

Yours Sincerely,

Marco Genchi on behalf of the authors

Reviewer Comments:

The authors thank the Editor and have taken note of the annotations.

CoEdiC: L242-244 - correct citation style - see guide to authors

Done

L190 - correct citation style (ESDA, 2018) - also in Introduction section of the manuscript

Done

L272 - reference incomplete - see guide to authors

Done

Highlights

- The questionnaire was sent to 2795 veterinary facilities. The response rate was 25%.
- At least one case of *Dirofilaria* spp. in dogs has been diagnosed in 47% facilities and in 6% for cats.
- To diagnose heartworm infection 83.8% of the facilities used serology
- Prevention of *D. immitis* was begun mainly in April-May.
- Prevention of *D. immitis* in cats was not recommended in 51% of the facilities.

1 ***Dirofilaria immitis* and *D. repens* in dog and cat: a questionnaire study in Italy**

2

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14 **Keywords:** *Dirofilaria immitis*, *Dirofilaria repens*, dog, cat, questionnaire, epidemiology,
15 diagnosis, treatment, Italy

16

17 **Abstract**

18 *Dirofilaria immitis* and *D. repens* are vector-borne parasites of dogs and cats, with zoonotic
19 potential, endemic in many parts of Europe, including Italy. Control and prevention of
20 dirofilariosis are essential points to reduce their diffusion in animals and humans and
21 veterinarians are the main subjects involved in this “battle”. With the aim to better
22 understand current practice by veterinarians, an online questionnaire on different aspects
23 of *D. immitis* and *D. repens* was sent to companion animal veterinary facilities registered
24 with the Italian Veterinary chamber. The overall response rate was 25%. Approximately
25 47% had diagnosed at least one case of *Dirofilaria* spp. in dogs in the last year and
26 approximately 6% had diagnosed at least one case of *Dirofilaria* spp. in cats. Of the 662
27 facilities surveyed, 83.8% used serology to diagnose heartworm infection. For the
28 diagnosis of *D. repens* infection in dogs and cats, a high percentage of facilities relied on
29 an external laboratory. Most recommended beginning prevention of canine heartworm

30 disease in April-May. Topical and injectable moxidectin and oral ivermectin were the two
31 most commonly used preventives for *D. immitis* in dogs. The drug most commonly used
32 for heartworm adulticide treatment in dogs was melarsomine. This study show that
33 veterinary facilities ians working in an endemic area for *D. immitis* and *D. repens* are
34 aware of recent developments in adulticide therapy. Results would suggest however that
35 diagnosis is not always carried out according to what is currently recommended by
36 international guidelines and that the timing for starting prevention may not be optimal.

37

38 **Introduction**

39 Heartworm disease (HWD; *Dirofilaria immitis*) and subcutaneous dirofilariasis (SCD;
40 *Dirofilaria repens*) are vector-borne diseases of dogs and cats with widespread distribution
41 in Europe (Otranto et al., 2013). They are also both important and emerging agents of
42 vector-borne zoonosis (Simón et al., 2012). Correct diagnosis and prevention of *Dirofilaria*
43 spp. infections are essential to avoid the spread of disease to other animals and to
44 humans. In recent years several guidelines have been published in order to assist
45 veterinary practitioners in the diagnosis, treatment and prevention of HWD and SCD.

46 However, awareness of these diseases on the part of veterinary practitioners can vary
47 among geographical regions. Genchi et al. (2014), reported that approximately 10-12% of
48 practitioners in Europe were aware of HWD and, more interestingly, that there were no
49 statistical differences between veterinarians working in endemic vs. non-endemic areas.

50 The aim of the present study was to evaluate the current practices of veterinary
51 practitioners in an area of Europe endemic for both *D. immitis* and *D. repens* in dogs and
52 cats. Particular attention was paid to prevention (i.e. use of macrocyclic lactones to avoid
53 development of adult parasites), diagnostic methods used to reveal patent infections and
54 treatment for these when diagnosed. Awareness of currently available guidelines was also
55 assessed. Data was gathered through an electronic questionnaire.

56

57 **Materials and methods**

58 Between November 2017 and February 2018, an electronic questionnaire was developed
59 that took into consideration different aspects of *Dirofilaria immitis* and *D. repens* in dogs
60 and cats. In order to encourage participation, the number of questions was limited to 31
61 and the questionnaire was anonymous.

62 The questionnaire was sent by e-mail to all companion animal veterinary facilities
63 (surgeries, clinics, hospitals and public facilities), working in all 110 Italian provinces and
64 registered with the Italian national veterinary association (Federazione nazionale ordini
65 veterinari italiani; FNOVI). The first survey distribution was made through FNOVI and its
66 associated Veterinary Chambers. A second email was sent directly from the institutional
67 email of one of the authors (MG). In those provinces where no replies were received or
68 where less than 10% of veterinary facilities had replied, a reminder was sent out 3 weeks
69 after the second email. Moreover, the questionnaire was also advertised through social
70 media (Facebook).

71 A cover letter was attached to the e-mail, which explained the purpose of the survey,
72 briefly illustrated the two parasites and the diseases they caused. In the case of more than
73 one practitioner working in a facility, it was asked that only one respond for the facility and
74 that replies be based on the facility's practice.

75 The first questions were general, including the province where the facility was located, the
76 number of veterinary practitioners working there and whether the facility was a surgery (no
77 hospitalization), a clinic (hospitalization), a hospital (hospitalization and emergency), a
78 laboratory or a public facility such as a University. The second part focused more
79 specifically on *D. immitis* and *D. repens*. In particular, the number of cases of heartworm
80 disease or subcutaneous dirofilariasis seen in the practice (based on clinical records) and
81 how diagnosis, treatment and prevention were performed in both dogs and cats. In

82 addition, veterinarians were asked if they were aware of the European Society for
83 Dirofilariosis and Angiostrongylosis (ESDA; <https://www.esda.vet>), the European Scientific
84 Council for Companion Animal Parasites (ESCCAP; <https://www.esccap.org>) and/or the
85 American Heartworm Society (AHS; <https://www.heartwormsociety.org>) and of the
86 guidelines they publish and if they had participated in conferences or congresses on these
87 specific parasites. The survey was conducted using the Microsoft Forms program, a new
88 part of Office 365 Education that allows users to quickly and easily create surveys,
89 questionnaires and registrations online.

90

91 **Results**

92 All results (Table 1-10) are consultable on supplementary material

93 The questionnaire was sent to 2795 veterinary facilities. Approximately 165 e-mails
94 bounced back due to incorrect addresses. The overall response rate was 25%. No
95 veterinary facilities from 12 of the 110 Italian provinces responded to the online
96 questionnaire. Among the 662 responders, 79.5% (526/662) were surgeries (no
97 hospitalization), 15.9% (105/662) clinics (hospitalization), 3.2% (21/662) hospitals
98 (hospitalization and emergency), 1.2% (8/662) public facilities (i.e. university), and 0.3%
99 (2/662) external diagnostic laboratories. Approximately 64% of the facilities employed 1-2
100 veterinarians, 25.4% employed 3-5, 7.4% employed from 5 to 10 veterinarians and only
101 2.7% employed 10 or more.

102 Approximately 47% (315) of the 662 facilities had diagnosed at least one case of *Dirofilaria*
103 spp. in the dog in the last year, while 51.8% (342/662) saw no cases. At least one case of
104 *D. immitis* monoinfection (Map 1) had been diagnosed in 32% (223/662) facilities, 3.3%
105 (21/662) had diagnosed at least one case of *D. repens* monoinfection (Map 2), while
106 12.7% (71/662) of the facilities had diagnosed co-infections with both parasites (Map 3).

107 When taking into consideration the geographical distribution of canine heartworm infection,
108 no cases were diagnosed in 21 provinces, 1-5 cases were diagnosed in 53 provinces, 5-20
109 in 18 provinces and over 20 cases were reported from 6 provinces (Map 1).

110 No cases of sub-cutaneous dirofilariosis (*D. repens*) were diagnosed in 44 provinces, 42
111 provinces reported 1-5 cases, 8 provinces reported 5-20 cases and 4 provinces indicated
112 over 20 cases (Map 2).

113 *D. immitis* infection in cats had been diagnosed in 4.8% (32/662) facilities in the last year,
114 no cases were diagnosed in 95 provinces, 1-2 cases were diagnosed in 12 provinces and
115 over 2 cases were diagnosed in 3 provinces (Map 1). In particular: 1-2 cases, in 4.2%
116 (28/662) facilities, over 2 cases in 0.6% (4/662) facilities and 0 cases in 95.2% (630/662)
117 facilities.

118 *D. repens* infection in cats had been diagnosed in 1.1% (7/662) facilities in 6 different
119 provinces in the last year; 1-2 cases were diagnosed in 4 provinces, over 2 cases were
120 diagnosed in 2 provinces and 0 cases in 655 provinces (Map 2).

121 Of the 662 facilities surveyed, 555 (83.8%) responded that they use serology to diagnose
122 heartworm infection in dogs. Twenty-four percent indicated that they use serology alone,
123 while the majority of the remaining responders combined serology with a number of other
124 diagnostic tools including fresh blood smear (45.9%, 304/662), external laboratory (23.1%,
125 153/662), ultrasound examination (18.9%, 125/662), Knott test (17.5%, 116/662), and
126 thoracic radiology (9.4%, 62/662). Approximately 8% responded that they rely solely on an
127 external laboratory for diagnosis.

128 For the diagnosis of *D. repens* infection in dogs, 54.7% of the facilities responded that they
129 rely on an external laboratory. Approximately 33% only use an external lab, while the rest
130 combine this with other diagnostic tools, including fresh blood smear (36.6%, 242/662),
131 skin biopsy (26.1%, 173/662), Knott test (23.4%, 155/662), and ultrasound examination
132 (5.4%, 36/662).

133 The most frequently used technique for the diagnosis of heartworm infections in the cat
134 was serology, either alone (26.9%) or in combination with fresh blood smears (22.7%,
135 150/662), ultrasound examination (16.2%, 107/662), Knott test (9.2%, 61/662), and
136 radiographic examination (8.0%, 53/662). Interestingly, 27.4% of the facilities surveyed
137 rely solely on an external laboratory for diagnosis.

138 For the diagnosis of *D. repens* infections in the cat, most facilities rely on an external
139 laboratory (46.8%), followed by skin biopsy (11.6%), and fresh blood smear (9.6%).

140 When asked when they begin prevention of canine heartworm disease, most facilities
141 responded April-May (54.8%), followed by February-March (28.5%), while 10.3% gave
142 preventives all year. Only 3.2% did not prescribe prevention.

143 Topical and injectable moxidectin and oral ivermectin were the two most commonly used
144 preventives for *D. immitis* in dogs, with 40% and 28% respectively, followed by milbemycin
145 (22%) and selamectin (10%). Most of the facilities used only one drug (47%), 38% used 2
146 drugs, 12% used 3 drugs and 3% used 4 drugs.

147 Prevention of heartworm infection in cats was not carried out in 50.8% (336/662) of the
148 facilities surveyed. When a preventive was prescribed, selamectin was the most widely
149 used drug 29.0% (192/662), followed by oral ivermectin 15.3% (101/662), milbemycin
150 10.4% (69/662), and moxidectin 7.7% (51/662).

151 The drug most widely used for heartworm adulticide treatment in dog was melarsomine
152 (35.2%), followed by ivermectin in association with doxycycline (29.0%). Of the 662
153 responding practitioners, 56.8% had attended a conference on *Dirofilaria* spp., 29.9%
154 knew of the guidelines from the American Heartworm Society (AHS), 23.9% those
155 European Society of Dirofilariosis and Angiostrongylosis (ESDA) and 27.8% those
156 European Scientific Counsel Companion Animal Parasites (ESCAAP).

157

158 **Discussion**

159 The results of the present study offer an up-dated evaluation of the current practices in the
160 diagnosis, prevention and treatment of *Dirofilaria* spp. infection in dogs and cats in Italy,
161 considered an endemic country for both parasites. Interestingly, the number of
162 participants, compared to other similar studies (14.8%, Halos et al., 2013; 9.1%, Genchi et
163 al., 2014) was quite high, with 25% questionnaires completed.

164 As the epidemiological maps show, these two filarial parasites are distributed throughout
165 the country. *D. immitis* is more greatly distributed in the northern and central regions,
166 historically endemic areas. *D. repens* seems uniformly distributed along the entire
167 peninsula.

168 Risk of infection has likely decreased over the years due to preventive treatment against
169 *D. immitis* and *D. repens* (Brianti et al., 2018). If the risk of infection is due to the presence
170 of infected dogs, then the wide-spread use of preventives will decrease the risk. In our
171 study, only 3.2% responding facilities did not recommend prevention against *D. immitis*
172 and 19% did not recommend prevention for *D. repens* in the dog. However, these two
173 parasitic diseases are currently widening their geographical distribution in both Italy and in
174 Europe, including into areas that were considered non-endemic until recently (Genchi et
175 al., 2005; Ciucă et al., 2016; Fuehrer et al., 2016). This is likely due to the movement of
176 infected animals and to climate changes that allow a longer survival and a greater number
177 of parasitic cycles of the vectors and consequently of their ability to transmit pathogens
178 (Genchi et al., 2009; Genchi et al., 2011; Sassnau et al., 2014).

179 Correct diagnosis of patent infection is very important. According to AHS 2018 and ESDA
180 2018 guidelines a correct diagnosis of *D. immitis* in dogs should include both a Knott test
181 and serological testing. Thoracic radiology and cardiac ultrasound are also recommended
182 to evaluate severity of disease. However, even though 57% of responding practitioners
183 reported having participated in a conference on *Dirofilaria* and 27% had consulted the AHS
184 and/or ESDA guidelines, data from the questionnaire indicate that 56.5% of the facilities

185 use either a fresh blood smear or serology to diagnosis infection and only 1.8% perform a
186 diagnosis as reported in the guidelines. Furthermore, approximately 25% of the
187 responding facilities used the fresh blood smear only to diagnose both *D. immitis* and *D.*
188 *repens*. It must be emphasized that this test, even if very suggestive, does not allow to
189 identify the different species of circulating microfilariae in the blood and its sensitivity is
190 extremely poor (ESDA, 2018).

191 Similar considerations can be made regarding *D. repens*. In fact, the prevalence of *D.*
192 *repens* in the different provinces may be underestimated, due to the very low incidence of
193 clinical signs induced by this parasite (Albanese et al., 2013) and the difficulty in diagnosis
194 (Genchi and Kramer, 2017). It is thus likely that dogs that have moved to endemic areas of
195 southern Italy become infected with *D. repens* and return to northern regions having no
196 apparent clinical signs and acting as donors of microfilariae to local mosquito populations.
197 Moreover, the annual screening that is recommended for the prevention of heartworm
198 infection is mainly done by serology, which does not allow for the diagnosis of *D. repens*.

199 If we compare *Dirofilaria* spp. distribution with timing of prevention, the survey suggests
200 that responding practitioners may not take into account the geographical location of their
201 veterinary facilities or the current effects of climate change that may have prolonged the
202 transmission season. However, facilities that did recommend prevention for all 12 months
203 were evenly distributed throughout the peninsula, suggesting that this practice is becoming
204 common.

205 Even though melarsomine is still widely used for adulticide treatment of *D. immitis*
206 infections, our data suggest that the combination of monthly macrocyclic lactones along
207 with doxycycline, which targets the bacterial endosymbiont *Wolbachia* (Bazzocchi et al.,
208 2008; Kramer et al., 2018) is currently being used by many veterinary facilities.

209 Our data shows that the diagnostic methods for *D. immitis* in the cat are extremely varied,
210 including fresh blood smear, serology or a combination of both. It should be noted that at

211 the time of the survey the only serological test available was the *D. immitis* antigen test.
212 The cat is identified as susceptible but a resistant host. However, studies have shown that
213 there is a prevalence of 9-18% in cats in endemic areas in relation to prevalence in dogs
214 (Venco et al., 2011). It is particularly concerning that over half of the practices surveyed
215 did not recommend prevention in the cat, given that the disease can be fatal and that there
216 is no current registered adulticide for use in cats. There are many possible reasons why
217 prevention is not recommended. Cost may be a factor, even though most products for
218 prevention in cats (as in dogs) are wide-spectrum, making them financially advantageous.
219 Availability would not seem to influence the decision whether to do prevention or not as
220 products are readily available throughout Italy. The product most often used according to
221 survey results is a topical formulation and quite easy to administer. The authors would
222 suggest that many facilities still do not consider the disease in cats as a priority. More
223 effort must be put into convincing practitioners of the importance of preventing this
224 infection in their feline patients.

225 All 32 cases of *D. immitis* in the cat were identified in the northern regions and in Sardinia,
226 with the exception of a practice in the province of Siena (central Italy) (Map 1). This is
227 likely due to a greater awareness on the part of practitioners, given that these regions are
228 historically endemic for *D. immitis* (Genchi et al., 2007), and thus they are more inclined to
229 screen for this parasite in the cat.

230 There are several limits to the present study. Practitioners who were interested in the
231 subject were likely and more motivated to participate than others, which may have led to
232 some bias in responses. Furthermore, even though the cover letter specifically asked that
233 the responding practitioner reply based on the facilities practices and not on her or his
234 own, there is no way to know if this was indeed the case. It is not known what test(s) is
235 used when veterinarians rely on external laboratories for diagnosis. Indeed, the survey did
236 not specify whether serology is carried out in-house or by an external laboratory. It was

237 also not asked when practitioners recommend finishing preventive treatment and this is an
238 important gap in the data due to the warmer winters that are currently present in Europe
239 and the associated risk of a prolonged transmission season. Surveys of this type also have
240 inherent limits, including the effect of non-responders (in this study 75%). However, it has
241 been reported that increasing response rates (for example through reminders) usually has
242 only a modest influence on the conclusions of a survey (Perneger et al., 2005).
243 Retrospective studies are also flawed if record keeping is not accurate within the facility. In
244 the present study, this effect was likely minimal for those questions regarding drugs for
245 prevention and adulticide treatment, given the stringent laws governing the sale of
246 veterinary pharmaceuticals.

247 **Conclusions**

248 This survey provides an overview of current practices of veterinary facilities working in a
249 geographical area endemic for *D. immitis* and *D. repens*. While they are aware of recent
250 developments, for example, in adulticide therapy, results would suggest that diagnosis is
251 not always carried out according to what is currently recommended by international
252 guidelines and that the timing for starting prevention may not be optimal. It is important to
253 remember, finally, that *Dirofilaria* spp. have zoonotic potential (Simón et al., 2012) and the
254 correct management of these infections will also protect public health.

255

256

257 **Acknowledgements**

258 This work would not be possible without the willingness of the Veterinarians who agreed to
259 participate in the study, FNOVI (Federazione Nazionale Ordini Veterinari Italiani) and
260 Veterinary Chambers, who are sincerely thanked.

261

262

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359 **Figure captions**

360

361 Map 1: Distribution of *Dirofilaria immitis* in dogs and cats. In grey: no province's facilities
362 replied to the questionnaire.

363

364 Map 2: Distribution of *Dirofilaria repens* in dogs and cats. In grey: no province's facilities
365 replied to the questionnaire.

366

367 Map 3: Distribution of *Dirofilaria immitis*, *D. repens* and co-infections

1 ***Dirofilaria immitis* and *D. repens* in dog and cat: a questionnaire study in Italy**

2

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14 **Keywords:** *Dirofilaria immitis*, *Dirofilaria repens*, dog, cat, questionnaire, epidemiology,
15 diagnosis, treatment, Italy

16

17 **Abstract**

18 *Dirofilaria immitis* and *D. repens* are vector-borne parasites of dogs and cats, with zoonotic
19 potential, endemic in many parts of Europe, including Italy. Control and prevention of
20 dirofilariosis are essential points to reduce their diffusion in animals and humans and
21 veterinarians are the main subjects involved in this “battle”. With the aim to better
22 understand current practice by veterinarians, an online questionnaire on different aspects
23 of *D. immitis* and *D. repens* was sent to companion animal veterinary facilities registered
24 with the Italian Veterinary chamber. The overall response rate was 25%. Approximately
25 47% had diagnosed at least one case of *Dirofilaria* spp. in dogs in the last year and
26 approximately 6% had diagnosed at least one case of *Dirofilaria* spp. in cats. Of the 662
27 facilities surveyed, 83.8% used serology to diagnose heartworm infection. For the
28 diagnosis of *D. repens* infection in dogs and cats, a high percentage of facilities relied on
29 an external laboratory. Most recommended beginning prevention of canine heartworm

30 disease in April-May. Topical and injectable moxidectin and oral ivermectin were the two
31 most commonly used preventives for *D. immitis* in dogs. The drug most commonly used
32 for heartworm adulticide treatment in dogs was melarsomine. This study show that
33 veterinary facilities ians working in an endemic area for *D. immitis* and *D. repens* are
34 aware of recent developments in adulticide therapy. Results would suggest however that
35 diagnosis is not always carried out according to what is currently recommended by
36 international guidelines and that the timing for starting prevention may not be optimal.

37

38 **Introduction**

39 Heartworm disease (HWD; *Dirofilaria immitis*) and subcutaneous dirofilariasis (SCD;
40 *Dirofilaria repens*) are vector-borne diseases of dogs and cats with widespread distribution
41 in Europe (Otranto et al., 2013). They are also both important and emerging agents of
42 vector-borne zoonosis (Simón et al., 2012). Correct diagnosis and prevention of *Dirofilaria*
43 spp. infections are essential to avoid the spread of disease to other animals and to
44 humans. In recent years several guidelines have been published in order to assist
45 veterinary practitioners in the diagnosis, treatment and prevention of HWD and SCD.

46 However, awareness of these diseases on the part of veterinary practitioners can vary
47 among geographical regions. Genchi et al. (2014), reported that approximately 10-12% of
48 practitioners in Europe were aware of HWD and, more interestingly, that there were no
49 statistical differences between veterinarians working in endemic vs. non-endemic areas.

50 The aim of the present study was to evaluate the current practices of veterinary
51 practitioners in an area of Europe endemic for both *D. immitis* and *D. repens* in dogs and
52 cats. Particular attention was paid to prevention (i.e. use of macrocyclic lactones to avoid
53 development of adult parasites), diagnostic methods used to reveal patent infections and
54 treatment for these when diagnosed. Awareness of currently available guidelines was also
55 assessed. Data was gathered through an electronic questionnaire.

56

57 **Materials and methods**

58 Between November 2017 and February 2018, an electronic questionnaire was developed
59 that took into consideration different aspects of *Dirofilaria immitis* and *D. repens* in dogs
60 and cats. In order to encourage participation, the number of questions was limited to 31
61 and the questionnaire was anonymous.

62 The questionnaire was sent by e-mail to all companion animal veterinary facilities
63 (surgeries, clinics, hospitals and public facilities), working in all 110 Italian provinces and
64 registered with the Italian national veterinary association (Federazione nazionale ordini
65 veterinari italiani; FNOVI). The first survey distribution was made through FNOVI and its
66 associated Veterinary Chambers. A second email was sent directly from the institutional
67 email of one of the authors (MG). In those provinces where no replies were received or
68 where less than 10% of veterinary facilities had replied, a reminder was sent out 3 weeks
69 after the second email. Moreover, the questionnaire was also advertised through social
70 media (Facebook).

71 A cover letter was attached to the e-mail, which explained the purpose of the survey,
72 briefly illustrated the two parasites and the diseases they caused. In the case of more than
73 one practitioner working in a facility, it was asked that only one respond for the facility and
74 that replies be based on the facility's practice.

75 The first questions were general, including the province where the facility was located, the
76 number of veterinary practitioners working there and whether the facility was a surgery (no
77 hospitalization), a clinic (hospitalization), a hospital (hospitalization and emergency), a
78 laboratory or a public facility such as a University. The second part focused more
79 specifically on *D. immitis* and *D. repens*. In particular, the number of cases of heartworm
80 disease or subcutaneous dirofilariasis seen in the practice (based on clinical records) and
81 how diagnosis, treatment and prevention were performed in both dogs and cats. In

82 addition, veterinarians were asked if they were aware of the European Society for
83 Dirofilariosis and Angiostrongylosis (ESDA; <https://www.esda.vet>), the European Scientific
84 Council for Companion Animal Parasites (ESCCAP; <https://www.esccap.org>) and/or the
85 American Heartworm Society (AHS; <https://www.heartwormsociety.org>) and of the
86 guidelines they publish and if they had participated in conferences or congresses on these
87 specific parasites. The survey was conducted using the Microsoft Forms program, a new
88 part of Office 365 Education that allows users to quickly and easily create surveys,
89 questionnaires and registrations online.

90

91 **Results**

92 All results (Table 1-10) are consultable on supplementary material

93 The questionnaire was sent to 2795 veterinary facilities. Approximately 165 e-mails
94 bounced back due to incorrect addresses. The overall response rate was 25%. No
95 veterinary facilities from 12 of the 110 Italian provinces responded to the online
96 questionnaire. Among the 662 responders, 79.5% (526/662) were surgeries (no
97 hospitalization), 15.9% (105/662) clinics (hospitalization), 3.2% (21/662) hospitals
98 (hospitalization and emergency), 1.2% (8/662) public facilities (i.e. university), and 0.3%
99 (2/662) external diagnostic laboratories. Approximately 64% of the facilities employed 1-2
100 veterinarians, 25.4% employed 3-5, 7.4% employed from 5 to 10 veterinarians and only
101 2.7% employed 10 or more.

102 Approximately 47% (315) of the 662 facilities had diagnosed at least one case of *Dirofilaria*
103 spp. in the dog in the last year, while 51.8% (342/662) saw no cases. At least one case of
104 *D. immitis* monoinfection (Map 1) had been diagnosed in 32% (223/662) facilities, 3.3%
105 (21/662) had diagnosed at least one case of *D. repens* monoinfection (Map 2), while
106 12.7% (71/662) of the facilities had diagnosed co-infections with both parasites (Map 3).

107 When taking into consideration the geographical distribution of canine heartworm infection,
108 no cases were diagnosed in 21 provinces, 1-5 cases were diagnosed in 53 provinces, 5-20
109 in 18 provinces and over 20 cases were reported from 6 provinces (Map 1).

110 No cases of sub-cutaneous dirofilariosis (*D. repens*) were diagnosed in 44 provinces, 42
111 provinces reported 1-5 cases, 8 provinces reported 5-20 cases and 4 provinces indicated
112 over 20 cases (Map 2).

113 *D. immitis* infection in cats had been diagnosed in 4.8% (32/662) facilities in the last year,
114 no cases were diagnosed in 95 provinces, 1-2 cases were diagnosed in 12 provinces and
115 over 2 cases were diagnosed in 3 provinces (Map 1). In particular: 1-2 cases, in 4.2%
116 (28/662) facilities, over 2 cases in 0.6% (4/662) facilities and 0 cases in 95.2% (630/662)
117 facilities.

118 *D. repens* infection in cats had been diagnosed in 1.1% (7/662) facilities in 6 different
119 provinces in the last year; 1-2 cases were diagnosed in 4 provinces, over 2 cases were
120 diagnosed in 2 provinces and 0 cases in 655 provinces (Map 2).

121 Of the 662 facilities surveyed, 555 (83.8%) responded that they use serology to diagnose
122 heartworm infection in dogs. Twenty-four percent indicated that they use serology alone,
123 while the majority of the remaining responders combined serology with a number of other
124 diagnostic tools including fresh blood smear (45.9%, 304/662), external laboratory (23.1%,
125 153/662), ultrasound examination (18.9%, 125/662), Knott test (17.5%, 116/662), and
126 thoracic radiology (9.4%, 62/662). Approximately 8% responded that they rely solely on an
127 external laboratory for diagnosis.

128 For the diagnosis of *D. repens* infection in dogs, 54.7% of the facilities responded that they
129 rely on an external laboratory. Approximately 33% only use an external lab, while the rest
130 combine this with other diagnostic tools, including fresh blood smear (36.6%, 242/662),
131 skin biopsy (26.1%, 173/662), Knott test (23.4%, 155/662), and ultrasound examination
132 (5.4%, 36/662).

133 The most frequently used technique for the diagnosis of heartworm infections in the cat
134 was serology, either alone (26.9%) or in combination with fresh blood smears (22.7%,
135 150/662), ultrasound examination (16.2%, 107/662), Knott test (9.2%, 61/662), and
136 radiographic examination (8.0%, 53/662). Interestingly, 27.4% of the facilities surveyed
137 rely solely on an external laboratory for diagnosis.

138 For the diagnosis of *D. repens* infections in the cat, most facilities rely on an external
139 laboratory (46.8%), followed by skin biopsy (11.6%), and fresh blood smear (9.6%).

140 When asked when they begin prevention of canine heartworm disease, most facilities
141 responded April-May (54.8%), followed by February-March (28.5%), while 10.3% gave
142 preventives all year. Only 3.2% did not prescribe prevention.

143 Topical and injectable moxidectin and oral ivermectin were the two most commonly used
144 preventives for *D. immitis* in dogs, with 40% and 28% respectively, followed by milbemycin
145 (22%) and selamectin (10%). Most of the facilities used only one drug (47%), 38% used 2
146 drugs, 12% used 3 drugs and 3% used 4 drugs.

147 Prevention of heartworm infection in cats was not carried out in 50.8% (336/662) of the
148 facilities surveyed. When a preventive was prescribed, selamectin was the most widely
149 used drug 29.0% (192/662), followed by oral ivermectin 15.3% (101/662), milbemycin
150 10.4% (69/662), and moxidectin 7.7% (51/662).

151 The drug most widely used for heartworm adulticide treatment in dog was melarsomine
152 (35.2%), followed by ivermectin in association with doxycycline (29.0%). Of the 662
153 responding practitioners, 56.8% had attended a conference on *Dirofilaria* spp., 29.9%
154 knew of the guidelines from the American Heartworm Society (AHS), 23.9% those
155 European Society of Dirofilariosis and Angiostrongylosis (ESDA) and 27.8% those
156 European Scientific Counsel Companion Animal Parasites (ESCAAP).

157

158 **Discussion**

159 The results of the present study offer an up-dated evaluation of the current practices in the
160 diagnosis, prevention and treatment of *Dirofilaria* spp. infection in dogs and cats in Italy,
161 considered an endemic country for both parasites. Interestingly, the number of
162 participants, compared to other similar studies (14.8%, Halos et al., 2013; 9.1%, Genchi et
163 al., 2014) was quite high, with 25% questionnaires completed.

164 As the epidemiological maps show, these two filarial parasites are distributed throughout
165 the country. *D. immitis* is more greatly distributed in the northern and central regions,
166 historically endemic areas. *D. repens* seems uniformly distributed along the entire
167 peninsula.

168 Risk of infection has likely decreased over the years due to preventive treatment against
169 *D. immitis* and *D. repens* (Brianti et al., 2018). If the risk of infection is due to the presence
170 of infected dogs, then the wide-spread use of preventives will decrease the risk. In our
171 study, only 3.2% responding facilities did not recommend prevention against *D. immitis*
172 and 19% did not recommend prevention for *D. repens* in the dog. However, these two
173 parasitic diseases are currently widening their geographical distribution in both Italy and in
174 Europe, including into areas that were considered non-endemic until recently (Genchi et
175 al., 2005; Ciucă et al., 2016; Fuehrer et al., 2016). This is likely due to the movement of
176 infected animals and to climate changes that allow a longer survival and a greater number
177 of parasitic cycles of the vectors and consequently of their ability to transmit pathogens
178 (Genchi et al., 2009; Genchi et al., 2011; Sassnau et al., 2014).

179 Correct diagnosis of patent infection is very important. According to AHS 2018 and ESDA
180 2018 guidelines a correct diagnosis of *D. immitis* in dogs should include both a Knott test
181 and serological testing. Thoracic radiology and cardiac ultrasound are also recommended
182 to evaluate severity of disease. However, even though 57% of responding practitioners
183 reported having participated in a conference on *Dirofilaria* and 27% had consulted the AHS
184 and/or ESDA guidelines, data from the questionnaire indicate that 56.5% of the facilities

185 use either a fresh blood smear or serology to diagnosis infection and only 1.8% perform a
186 diagnosis as reported in the guidelines. Furthermore, approximately 25% of the
187 responding facilities used the fresh blood smear only to diagnose both *D. immitis* and *D.*
188 *repens*. It must be emphasized that this test, even if very suggestive, does not allow to
189 identify the different species of circulating microfilariae in the blood and its sensitivity is
190 extremely poor (ESDA, 2018).

191 Similar considerations can be made regarding *D. repens*. In fact, the prevalence of *D.*
192 *repens* in the different provinces may be underestimated, due to the very low incidence of
193 clinical signs induced by this parasite (Albanese et al., 2013) and the difficulty in diagnosis
194 (Genchi and Kramer, 2017). It is thus likely that dogs that have moved to endemic areas of
195 southern Italy become infected with *D. repens* and return to northern regions having no
196 apparent clinical signs and acting as donors of microfilariae to local mosquito populations.
197 Moreover, the annual screening that is recommended for the prevention of heartworm
198 infection is mainly done by serology, which does not allow for the diagnosis of *D. repens*.

199 If we compare *Dirofilaria* spp. distribution with timing of prevention, the survey suggests
200 that responding practitioners may not take into account the geographical location of their
201 veterinary facilities or the current effects of climate change that may have prolonged the
202 transmission season. However, facilities that did recommend prevention for all 12 months
203 were evenly distributed throughout the peninsula, suggesting that this practice is becoming
204 common.

205 Even though melarsomine is still widely used for adulticide treatment of *D. immitis*
206 infections, our data suggest that the combination of monthly macrocyclic lactones along
207 with doxycycline, which targets the bacterial endosymbiont *Wolbachia* (Bazzocchi et al.,
208 2008; Kramer et al., 2018) is currently being used by many veterinary facilities.

209 Our data shows that the diagnostic methods for *D. immitis* in the cat are extremely varied,
210 including fresh blood smear, serology or a combination of both. It should be noted that at

211 the time of the survey the only serological test available was the *D. immitis* antigen test.
212 The cat is identified as susceptible but a resistant host. However, studies have shown that
213 there is a prevalence of 9-18% in cats in endemic areas in relation to prevalence in dogs
214 (Venco et al., 2011). It is particularly concerning that over half of the practices surveyed
215 did not recommend prevention in the cat, given that the disease can be fatal and that there
216 is no current registered adulticide for use in cats. There are many possible reasons why
217 prevention is not recommended. Cost may be a factor, even though most products for
218 prevention in cats (as in dogs) are wide-spectrum, making them financially advantageous.
219 Availability would not seem to influence the decision whether to do prevention or not as
220 products are readily available throughout Italy. The product most often used according to
221 survey results is a topical formulation and quite easy to administer. The authors would
222 suggest that many facilities still do not consider the disease in cats as a priority. More
223 effort must be put into convincing practitioners of the importance of preventing this
224 infection in their feline patients.

225 All 32 cases of *D. immitis* in the cat were identified in the northern regions and in Sardinia,
226 with the exception of a practice in the province of Siena (central Italy) (Map 1). This is
227 likely due to a greater awareness on the part of practitioners, given that these regions are
228 historically endemic for *D. immitis* (Genchi et al., 2007), and thus they are more inclined to
229 screen for this parasite in the cat.

230 There are several limits to the present study. Practitioners who were interested in the
231 subject were likely and more motivated to participate than others, which may have led to
232 some bias in responses. Furthermore, even though the cover letter specifically asked that
233 the responding practitioner reply based on the facilities practices and not on her or his
234 own, there is no way to know if this was indeed the case. It is not known what test(s) is
235 used when veterinarians rely on external laboratories for diagnosis. Indeed, the survey did
236 not specify whether serology is carried out in-house or by an external laboratory. It was

237 also not asked when practitioners recommend finishing preventive treatment and this is an
238 important gap in the data due to the warmer winters that are currently present in Europe
239 and the associated risk of a prolonged transmission season. Surveys of this type also have
240 inherent limits, including the effect of non-responders (in this study 75%). However, it has
241 been reported that increasing response rates (for example through reminders) usually has
242 only a modest influence on the conclusions of a survey (Perneger et al., 2005).
243 Retrospective studies are also flawed if record keeping is not accurate within the facility. In
244 the present study, this effect was likely minimal for those questions regarding drugs for
245 prevention and adulticide treatment, given the stringent laws governing the sale of
246 veterinary pharmaceuticals.

247 **Conclusions**

248 This survey provides an overview of current practices of veterinary facilities working in a
249 geographical area endemic for *D. immitis* and *D. repens*. While they are aware of recent
250 developments, for example, in adulticide therapy, results would suggest that diagnosis is
251 not always carried out according to what is currently recommended by international
252 guidelines and that the timing for starting prevention may not be optimal. It is important to
253 remember, finally, that *Dirofilaria* spp. have zoonotic potential (Simón et al., 2012) and the
254 correct management of these infections will also protect public health.

255

256

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359 **Figure captions**

360

361 Map 1: Distribution of *Dirofilaria immitis* in dogs and cats. In grey: no province's facilities
362 replied to the questionnaire.

363

364 Map 2: Distribution of *Dirofilaria repens* in dogs and cats. In grey: no province's facilities
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367 Map 3: Distribution of *Dirofilaria immitis*, *D. repens* and co-infections

368

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*Conflict of Interest

Declarations of interest: none

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