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 show that veterinary facilities ians 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

Cover Letter

Prof. Marco Genchi DVM PhD
Department of Veterinary Sciences,
University of Parma,
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-born 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

Revision Note

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.

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 show that veterinary facilities ians 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.

Introduction

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

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

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

Materials and methods

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

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

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

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

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

Results

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Conclusions

This survey provides an overview of current practices of veterinary facilities working in a geographical area endemic for *D. immitis* and *D. repens*. While they are aware of recent developments, for example, in adulticide therapy, results would suggest 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. It is important to remember, finally, that *Dirofilaria* spp. have zoonotic potential (Simón et al., 2012) and the correct management of these infections will also protect public health.

Acknowledgements

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Figure captions

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

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

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

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

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Introduction

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

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

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

Materials and methods

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

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

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

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

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

Results

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Conclusions

This survey provides an overview of current practices of veterinary facilities working in a geographical area endemic for *D. immitis* and *D. repens*. While they are aware of recent developments, for example, in adulticide therapy, results would suggest 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. It is important to remember, finally, that *Dirofilaria* spp. have zoonotic potential (Simón et al., 2012) and the correct management of these infections will also protect public health.

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Figure captions

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

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

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

Figure

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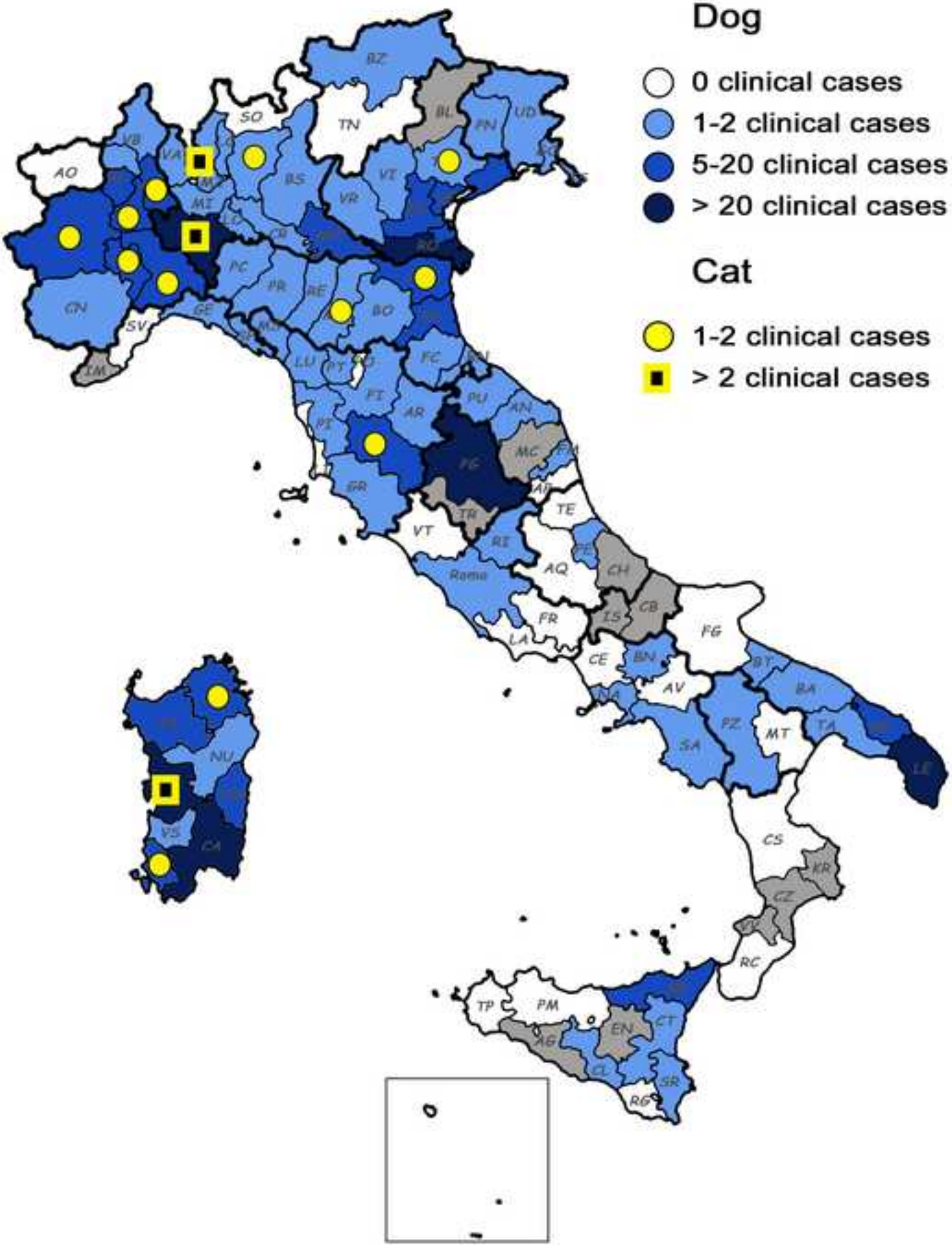

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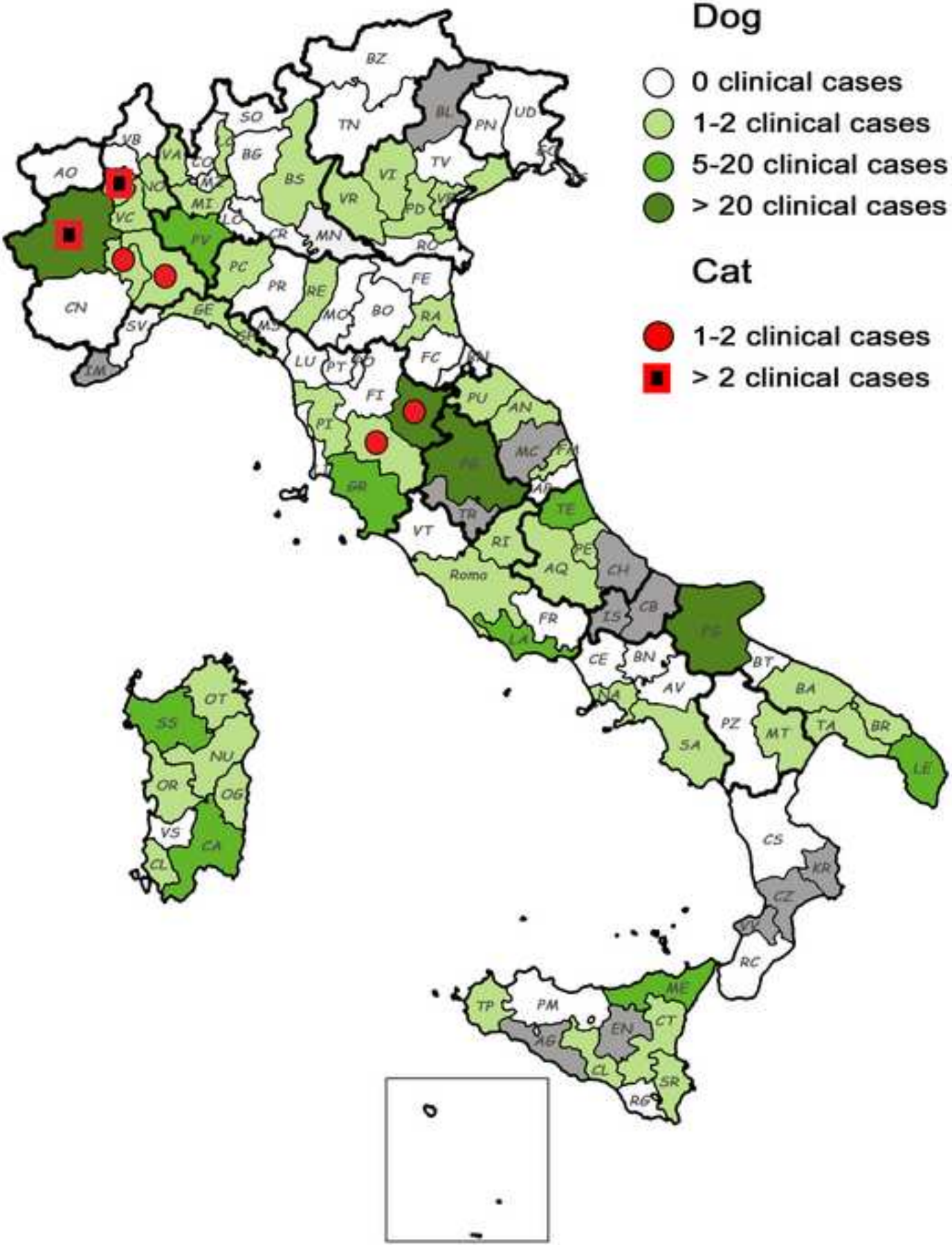

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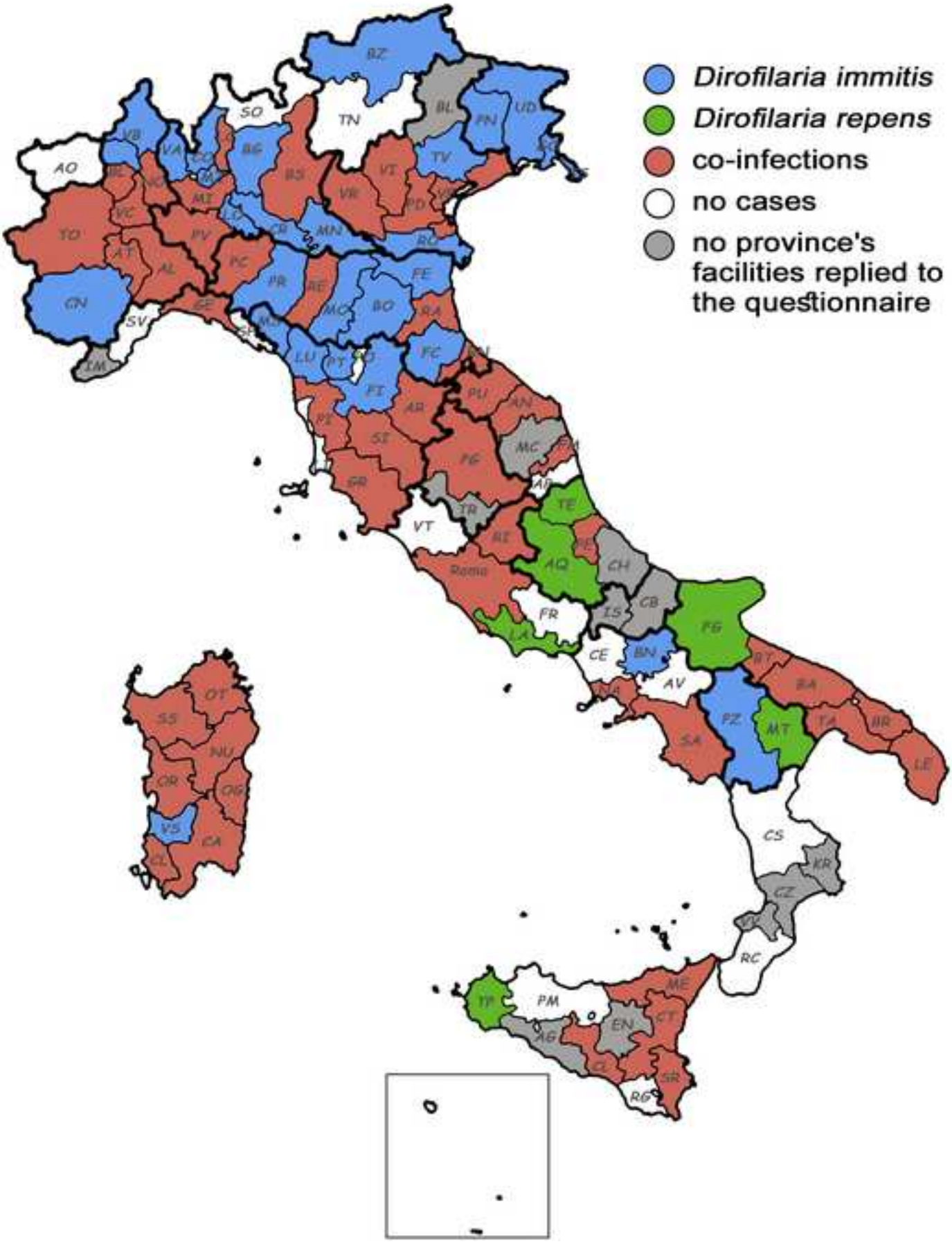

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Declarations of interest: none

All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.