



## Implications of zoonotic and vector-borne parasites to free-roaming cats in central Spain

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### ABSTRACT

Cats are definitive hosts and reservoirs for several parasites, some of which are responsible for serious zoonotic diseases. We conducted a case-control study of data from a trap-neuter-return (TNR) programme (years 2014–2017) designed to examine the prevalence of zoonotic parasites in free-roaming cats living in urban areas of central Spain. In the animal population tested (n = 263), we detected a 29.2% prevalence of endoparasites, including high rates of cestodes (12.9%) and *Toxocara cati* (11.7%). While faecal samples showed no *Toxoplasma gondii* oocysts, the seroprevalence of *T. gondii* infection was 24.2%. Antibodies to *Leishmania infantum* were detected in 4.8% of the animals, though all skin and blood samples analyzed were PCR negative for this parasite. Ectoparasites (ticks and fleas) were found in 4.6% of the cat population, and 10.6% of the cats were detected with *Otodectes cynotis*. Finally, 6.3% and 7.9% cats tested positive for feline leukaemia virus and feline immunodeficiency virus, respectively. Our study provides useful information for animal-welfare and public-health, as the parasites detected can affect native wild animals through predation, competition and disease transmission. Our detection of zoonotic parasites such as *L. infantum*, *T. gondii*, *T. cati*, *Giardia duodenalis* and several ectoparasites prompts an urgent need for health control measures in stray cats.

### 1. Introduction

Cats are definitive hosts to a large number of parasites, some of which cause important zoonoses like the larva *migrans* syndromes (toxocarosis and ancylostomatidosis), toxoplasmosis and giardiasis. Besides intestinal parasites, cats are also reservoirs for other vector-borne zoonotic diseases like *Leishmania infantum* infection in endemic regions (Day, 2011; Pennisi et al., 2015) or, as recently shown, *Bartonella* spp. and *Rickettsia* spp. infections (Case et al., 2006; Day, 2011; Ayllón et al., 2012; Diakou et al., 2017).

Stray animals may have significant impacts on public health due to factors such as a lack of preventive measures (e.g. vaccines, deworming), easy access to intermediate hosts (e.g. rats and birds), and unrestricted entry to public areas such as parks and playgrounds. This means that the presence of free-roaming animals is a major risk for the transmission of zoonotic diseases (Otranto et al., 2017a).

Feline colonies are stable groups of free-roaming cats living outdoors in public or private urban areas with access to sources of food. These colonies can be intentionally nourished by people or maintained

by human waste (Centonze and Levy, 2002).

A healthy female cat is capable of producing several offspring during her lifespan, which leads to the rapid exponential growth of free-roaming cat populations if there are no control interventions. Overpopulation is detrimental to the animals themselves, poses a risk to public and environmental health, and generates numerous inconveniences in urban areas such as excessive noise and car accidents (Baker and Harris, 2007). Many strategies have been developed and implemented for population control of stray and feral cats including ‘trap-neuter-return’ (TNR) and ‘trap and euthanize’ (TE) control programmes (Scott et al., 2002; Schmidt et al., 2009).

Trap-neuter-return interventions consist of trapping the animals, providing them with veterinary care and sterilization, and returning them to the site of capture (Gibson et al., 2002; Levy and Crawford, 2004; Schmidt et al., 2009). The goals of a TNR programme are to reduce or maintain population size, improve the condition and health of cats and lengthen their lifespan. Such initiatives are considered an effective and ethical approach to the population and health control of cat colonies, and are especially effective if other measures are implemented

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in parallel such as the adoption of socialized cats and campaigns to discourage abandonment (Boone, 2015). Controlling cat colonies has a direct impact on public health by diminishing health risks and transmission of zoonotic diseases (Baker and Harris, 2007; Murray et al., 2015). The risks of overpopulation include food and territory conflicts among cats, which promotes the transmission of feline retroviruses through direct contact, scratches or bites (Finkler et al., 2011; Murray et al., 2015).

Trap and euthanize control programmes, besides raising ethical issues, have proven ineffective because of the vacuum effect. This means that if all animals in a given area are wiped out, resource space becomes available, which attracts immigrant or abandoned cats to the same area. Further, the presence of cats is important to ensure an ecological balance, as they are predators of small mammals like birds, rodents and reptiles (Boone, 2015). It is thus essential that veterinarians participate in monitoring feline colonies, and efforts should be made to design and implement appropriate population control methods. Such measures need to consider population biology and economic resources, which is often a limiting factor for TNR programmes (Boone, 2015).

The present study sought to determine the prevalences of endoparasites of zoonotic interest, *L. infantum* and *Toxoplasma gondii* in free-roaming cats living in colonies in the central region of mainland Spain, and to assess the impacts of a TNR programme on these prevalences.

## 2. Material and methods

### 2.1. Study population

From 2014–2017, the Animal Protection Society ALBA in Madrid has been undertaking a health control programme for free-roaming cats living in colonies. So far, a large number of cats have been captured, neutered and returned to their site of capture (Griffin et al., 2016). Apart from the TNR programme, animals were tested for feline retroviruses and other pathogens, dewormed and treated, if needed, to ensure that only healthy animals are returned. Treatment after sterilization surgery consists of a topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel (Broadline<sup>®</sup>, Merial) and a non-steroidal anti-inflammatory drug (meloxicam) plus a broad spectrum antibiotic (amoxicillin/clavulanic acid). All neutered cats are also ear-tipped according to standard recommendations for their subsequent identification (Griffin et al., 2016).

### 2.2. Sedation and sampling

After sedation (with 80 µg of medetomidine/kg plus 5 mg ketamine/kg), each animal was subject to a thorough physical examination by clinical veterinarians to record age, sex, clinical signs and place of capture, and to collect blood and tissue samples. Blood samples were obtained (for whole blood in EDTA or serum) by jugular venipuncture. Other samples collected were ear swabs, ear tip and skin scrapings, if there were skin lesions, and faeces. These last samples were obtained directly from the rectum using a swab or from the cages where the cats were housed. Samples were kept at 4 °C until processing within 24 h at the laboratory.

### 2.3. *Toxoplasma gondii* infection

Antibodies against *T. gondii* were measured in serum using a direct agglutination test (DAT) kit (Toxo-Screen DA; Biomérieux) as described by Desmonts and Remington (1980). We considered an antibody titre of 1:40 to indicate a cat had been exposed to *T. gondii* (Desmonts and Remington, 1980).

### 2.4. *Leishmania* infection

#### 2.4.1. Serological diagnosis

For serological tests, specific antibodies to *L. infantum* were detected using an indirect immunofluorescence antibody test (IFAT) against in-house cultured promastigotes. This test for anti-*Leishmania*-specific immunoglobulin G (IgG) antibodies was performed as described previously using a cut-off  $\geq 1:100$  to define seropositivity (Ayllon et al., 2008).

#### 2.4.2. Molecular diagnosis

The QIAamp<sup>®</sup> DNA Micro Kit (50) (QIAGEN<sup>®</sup>) was used to obtain DNA from blood (100 µl) and ear skin samples according to the manufacturer's instructions. Extracted DNA was eluted in sterilized water (70 µl) and stored at –20 °C until use. A 5 µl aliquot of eluted DNA was used for each polymerase chain reaction (PCR) for *Leishmania* detection and species identification.

*Leishmania* DNA detection was performed by two PCR methods targeting internal transcribed spacers 1 and 2 (ITS-1 and ITS-2) using the primer pairs LITSR (5'-CTGGATCATTTCCGATG-3')/L5.8S (5'-TGATACCACTTATCGCACTT-3') and L5.8SR (5'-AAGTGGGATAAGTGTA-3')/LITSV (5'-ACACTCAGGTCTGTAAAC-3') as described by Kuhls et al. (2005). The PCR amplification product size was 280–330 bp.

### 2.5. Enteric parasites

Faeces samples were tested for oocysts, cysts, eggs and larvae of enteric parasites using the modified FLOTAC method plus merthiolate-iodine-formalin staining and Baermann-Wetzel methods followed by examination under a light microscope (Thienpont et al., 1979; Cringoli et al., 2010).

In addition, we used a qualitative immunochromatographic (ICT) commercial strip assay for the rapid simultaneous detection of *Cryptosporidium* and/or *Giardia* (Stick Crypto-Giardia<sup>®</sup>; Operon, Zaragoza, Spain) on all stool samples. Tests were conducted at room temperature according to the manufacturer's instructions.

### 2.6. Ectoparasites

Ear swabs were examined under the microscope to determine the presence of ectoparasites such as *Otodectes cynotis* in secretions.

Whole skin was examined to check for skin lesions and ectoparasites. Any parasites found were stored in ethanol 70° until their identification under the microscope using identification keys (Krämer and Mencke, 2001; Bowman, 2002).

### 2.7. Retrovirus infection

All cats were tested for feline leukaemia virus (FeLV) antigen and antibodies to feline immunodeficiency virus (FIV) using a commercial ELISA kit (PetChek<sup>®</sup> FIV/FeLV; IDEXX Laboratories) (Tonelli, 1991).

### 2.8. Ethics

The study was carried out in accordance with Spanish legislation guidelines and with the International Guiding Principles for Biomedical Research Involving Animals issued by the Council for International Medical Science Organizations.

### 2.9. Statistical analysis

Association between all the variables examined were identified by the Chi-square test (SPSS 17.0). Significance was set at  $p < 0.05$ .

**Table 1**  
Distribution of cats population related with infectious diseases analyzed and epidemiological variables (sex, age, capture area and year).

Epidemiological variables		% (n/N)					
		<i>T. gondii</i> IgG ≥ 1:80	<i>L. infantum</i> IgG ≥ 1:100	Intestinal parasites	FELV – Positive	FIV- Positive	% (n)
Sex	Male	21.8 (28/128)	8.8 (8/91)	31.5 (66/209)	7.7 (20/261)	11.5 (30/261)	41.3 (261)
	Female	25.5 (58/228)	2.5 (4/158)	27.2 (68/250)	5.4 (20/371)	5.4 (20/371)	58.7 (371)
<i>p-value</i>		0.450	0.026	0.304	0.24	0.005	
Age	≤ 1yr	11.9 (8/67)	0 (0/33)	32.1 (18/56)	1.4 (1/72)	4.2 (3/72)	11.4 (72)
	> 1yr	27 (78/289) *	5.6 (12/216)	28.8 (116/403)	6.6 (37/560)	8.4 (47/560)	88.6 (560)
<i>p-value</i>		0.009	0.165	0.604	0.079	0.211	
Capture area	Madrid	19.7 (47/239)	2.4 (4/164)	25.5 (77/302)	5 (22/443)	6.5 (29/443)	70.1 (443)
	Toledo	36.1 (22/61) *	14 (6/43) *	33.3 (26/78)	6.1 (6/99)	9.1 (9/99)	15.7 (99)
	Guadalajara	26.9 (14/52)	5.3 (2/38)	32.9 (23/70)	12.3 (10/81)	14.8 (12/81)	12.8 (81)
	Cuenca	75 (3/4) **	0 (0/4)	22.2 (2/9)	22.2 (2/9) †	0 (0/9)	1.4 (9)
<i>p-value</i>		0.004	0.019	0.389	0.017	0.06	
Year	2014	21.9 (37/169)	4.8 (8/167)	30.8 (53/172)	8.5 (17/172)	11.6 (20/172) †	27.2 (172)
	2015	45.5 (15/33) †	–	28.1 (25/89)	6.2 (10/161)	8.7 (14/161)	25.5 (161)
	2016	23 (3/13)	–	29.2 (38/130)	2.9 (4/138)	2.9 (4/138)	21.8 (138)
	2017	21.9 (31/141)	4.8 (4/82)	26.5 (18/68)	5.6 (9/161)	7.4 (12/161)	25.5 (161)
<i>p-value</i>		0.004	0.975	0.915	0.087	0.041	
Total		24.2 (86/356)	4.8 (12/249)	29.2 (134/459)	6.3 (40/632)	7.9 (50/632)	632

\* Epidemiological variables with differences statistically significant ( $p < 0.05$ ).  
\*\* Not included in statistical analysis.

**3. Results**

The results of our study are provided in Table 1 and Fig. 1. The distribution of the 632 cats examined by province was: 443 in Madrid, 99 in Toledo, 81 in Guadalajara and 9 in Cuenca; 261 cats (41.3%) were males and 371 (58.7%) females. The cats were classified by age into 72

(11.4%) younger than or having 1 year old and 560 (88.6%) older than 1 year. Clinical signs detected in the physical exam were skin lesions (alopecia, pustules, and wounds due to bites) in 21 cats, ocular lesions (conjunctivitis, keratitis and uveitis) in 18, gingivostomatitis in 12, pale mucous membranes in three and lameness in one of the forelegs in a further three cats. Other clinical signs (e.g., neurological signs) could

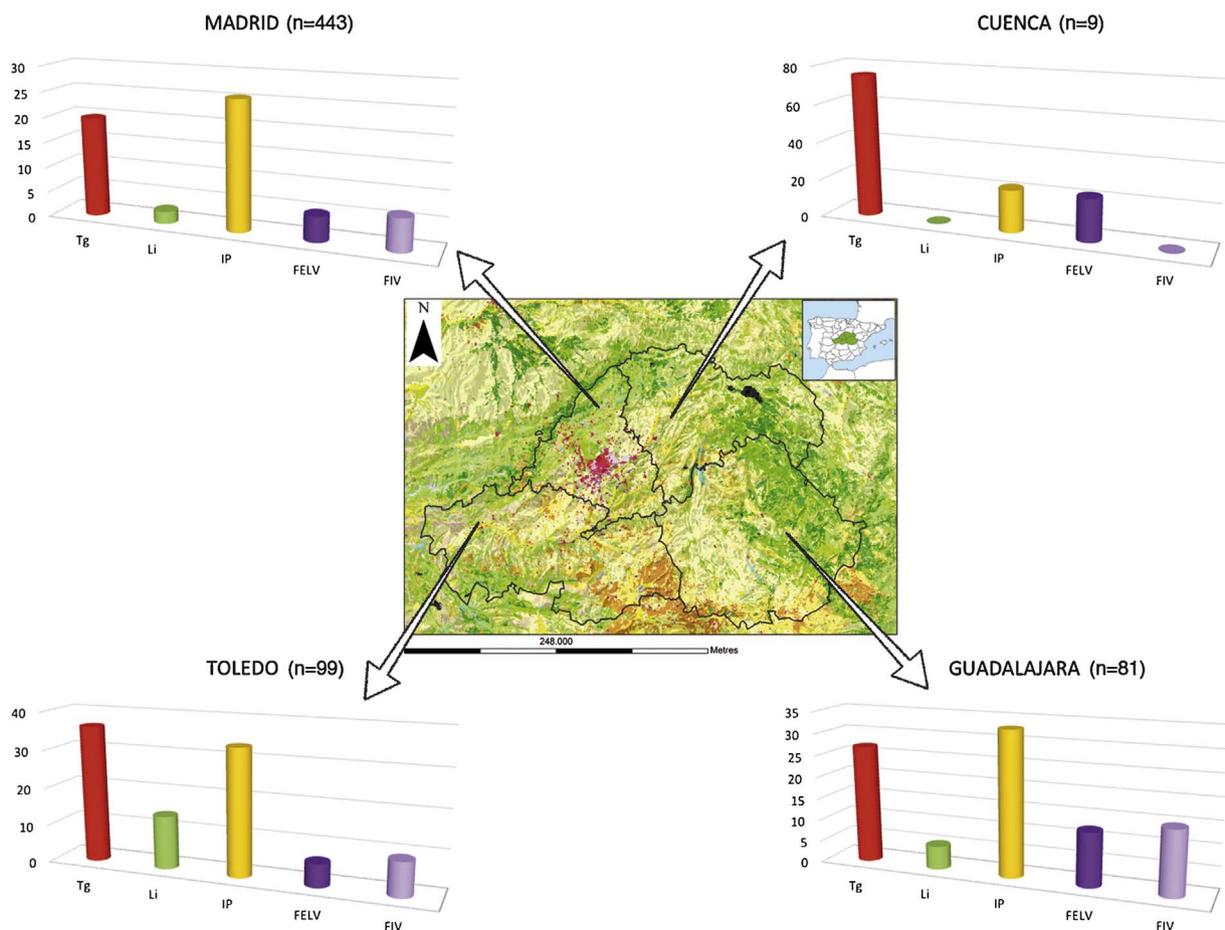


Fig. 1. Prevalence for the infectious diseases studied by geographical region.

not be assessed as the cats were sedated before examination.

Faeces samples were obtained from 459 cats (many had no faeces present in the rectum at the moment of sampling). Intestinal parasites were detected in 29.2% (134/459) of the cats: Cestoda (13.1%) (*Taenidae* [8.3%] and *Dipylidium caninum* [4.6%]), *Toxocara cati* (11.7%), *Giardia duodenalis* (5%), *Cystoisospora* spp. (2.5%), *Toxascaris leonina* (2.5%), *Aelurostrongylus abstrusus* (1.3%) and *Cryptosporidium* spp. (0.4%). Although the seroprevalence of *T. gondii* (Ig G cut-off  $\geq 1:80$ ) was 24.2% (86/356), the presence of *T. gondii* oocysts was not observed in any of the faeces samples analyzed.

Anti-*L. infantum* antibodies (Ig G cut-off  $\geq 1:100$ ) were observed in 4.8% (12/249) of the feline population examined, while PCR of ear skin and blood samples were negative in all cases. Only two cats testing seropositive for *L. infantum* infection showed clinical signs compatible with feline leishmaniosis, yet PCR was negative.

Ectoparasite infestations were detected in 13.6% of the cats (86/632): *O. cynotis* (n = 67), *Ctenocephalides felis* (n = 17) *Spilopsyllus cuniculi* (n = 6), *Rhipicephalus sanguineus* sensu lato (n = 5), *Felicola subrostratus* (n = 3), *Nosopsyllus fasciatus* (n = 1), and *Notoedres cati* (n = 1), and 14 cats with mixed infestations.

Feline retroviruses (FeLV-FIV) were detected in 13.3% (84/632) cats. The prevalence of FeLV antigen was 6.3% (40/632) and of FIV antibody was 7.9% (50/632); six of these cats were positive for both retroviruses.

Of the epidemiological variables analyzed, sex was associated with *L. infantum* infection ( $p = 0.026$ ) and FIV ( $p = 0.005$ ) with higher prevalences of both in males than females (Table 1). Age was only significantly related to *T. gondii* infection, as cats older than 1 year showed a higher seroprevalence ( $p = 0.009$ ). Locality was related to a broad spectrum of infectious diseases (Table 1). However, for some capture sites, sample sizes were too small for adequate statistical power (e.g. for the Cuenca province we only had data from nine cats). No significant differences in yearly rates of infectious and parasite diseases were observed during the course of our study (2014–2017) with the exception of *T. gondii* infection which showed a higher seroprevalence in 2015 ( $p = 0.004$ ).

#### 4. Discussion

In this 4-year study, we assessed the impact of a controlled TNR programme on the clinical status of stray cats and on the prevalence of zoonotic infections and diseases shown by these free-roaming cat populations. In an ongoing project starting in 2004, we have been addressing the implications of stray cat and dog populations for public health, the environment and even for other domestic or wild animals (Miró et al., 2004, 2014). The present study assessed the health status of cat colonies in central Spain. Our aim has been to further understand the risks posed by these animals for the health of humans and domestic animals, so that effective healthcare and control measures can be designed. Most of the cats captured were females (58.5%). This is likely due to behavioural differences, as female cats tend to roam less than males and are easier to trap. The sterilization of females to reduce their offspring is crucial to manage overpopulation in these colonies. However, the sterilization of males is also useful to avoid their roaming to colonies of non-neutered females and productive mating. The majority of the animals examined in this study were clinically healthy. This is in agreement with the findings of other authors reporting fewer than 10% of stray cats showing a poor clinical condition or needing veterinary care (Wenstrup and Dowidchuk, 1999).

The presence of zoonotic endoparasites in stray cats is a serious public health risk. Children may be especially at risk as these animals have access to public areas such as parks and playgrounds, and children are also more susceptible than adults to infectious diseases (Dado et al., 2012; Kleine et al., 2017). A further concern is that many parasites, such as the eggs of *Toxocara*, are highly resistant and may survive for long periods in the environment (Overgaauw and van Knapen, 2013).

The prevalence of endoparasites found in the present study was 29.2%, which is similar to reported rates emerging from our prior studies in the same geographical region (23–26.5%) and to the prevalence of 31.1% observed in cat colonies in Lisbon, Portugal (Duarte et al., 2010). Other reported prevalences of intestinal parasites have ranged from 8.6% in Australia (McGlade et al., 2003) to 83.3% in Belgium (Vanparijs et al., 1991). Among the zoonotic intestinal parasites, *T. cati* was the most prevalent detected in 11.7% of the present cats. According to other studies conducted in Madrid where the prevalence in stray cats (7.7%) (Miró et al., 2014) and domestic cats with regular outdoor access (9.5%) (Giannelli et al., 2017) while in the Northeast of Spain (Mallorca Island and mid-Ebro Valley), the prevalence of this parasite in stray cats can be as high as 35–55.2% (Calvete et al., 1998; Millán and Casanova, 2009). In this study, we detected a prevalence of cestodes that ranged from 4.6% for *D. caninum* to 8.3% for *Taenidae* while in domestic cats with regular outdoor access the prevalence was lower (0.5%) for both genus (Giannelli et al., 2017). A similar study conducted in European countries (Austria, Belgium, France, Hungary, Italy, Romania and Spain) returned prevalences of 5% and 0.1–1% for *Taenia* spp. and *D. caninum*, respectively (Beugnet et al., 2014). *Giardia duodenalis* is another important potential zoonotic parasite detected here in 5% of samples, compared to previously reported prevalence of 2.4–4% for Madrid, Spain (Giannelli et al., 2017; Miró et al., 2014). However, it should be noted that prevalence estimates of *G. duodenalis* vary substantially according to the technique used. A prevalence of 0.9% using coprologic methods versus 6.8% using ELISA has been described for the detection of coproantigens (Becker et al., 2012; Epe et al., 2010). Further work is needed to determine the genotype of *G. duodenalis* and assess its zoonotic potential and possible implications for public health. In effect, there are eight different assemblages (A–H) showing different host specificities; being assemblages A and B found in humans and primates, livestock, companion animals and some wildlife species and assemblage F have been primarily found in cats (Feng and Xiao, 2011). *Cryptosporidium* spp. was only detected in one faecal sample using the ITC technique. This result should be supported by a molecular diagnosis to confirm the genotype. Nevertheless, our detection of *Cryptosporidium* spp. in stray cats may be considered a potential zoonotic risk, albeit limited due to its low prevalence.

Remarkably, we were unable to detect *T. gondii* oocysts in any of the faeces samples analyzed. An inability to detect oocysts of *T. gondii* in seropositive cats has been reported by others (Miró et al., 2004; Dubey et al., 2006; Montoya-Matute et al., 2007). Oocysts can be observed in fewer than 1% of faeces samples (Childs and Seegar, 1986; Pena et al., 2006). A likely explanation for this is that cats only excrete oocysts after primo-infection and only over a short period of time (between 1 and 2 weeks) (Dubey, 1994). The detection of 24.2% of seropositive cats could determine that these animals are excreting oocysts in the environment at a given time, representing a risk to public health and to other animals living in the same area. Our seroprevalence of *T. gondii* infection is similar to reported rates for stray cats in central Spain, though a higher prevalence has been reported for cats in rural areas of this region (Miró et al., 2004, 2014). This is not unexpected as cats living in urban areas are constantly fed by humans and have easy access to human waste and less contact with intermediate hosts. These results are in agreement with observations by Sukthama et al. (2003), who attributed the low prevalence of *T. gondii* detected in Bangkok to the easy access of cats to waste from houses and restaurants.

In our study, sex was not a determining factor for *T. gondii* infection though significantly different prevalences have been observed between young and adult cats in other studies (Gauss et al., 2003; Miró et al., 2004).

Although dogs are considered the main reservoir of *L. infantum*, recent studies have shown that lagomorphs can also be competent reservoirs and may play a role in the sylvatic cycle in urban areas, such as Madrid metropolitan area, where an outbreak of human leishmaniosis occurred (Molina et al., 2012; Moreno et al., 2014). This highlights the

importance of sero-epidemiological studies in stray animals to detect new potential reservoirs of infection. In the present study, we detected a seroprevalence of *L. infantum* infection in stray cats of 4.8%. This figure is in line with seroprevalences of this parasite reported for central Spain (3.2–9.3%) (Ayllon et al., 2008; Ayllón et al., 2012; Miró et al., 2014; Moreno et al., 2014) and other countries in the Mediterranean basin (Pennisi et al., 2015). Nevertheless, in endemic areas with high seroprevalences of *L. infantum* in dogs, a higher seroprevalence in cats was also observed (Martín-Sánchez et al., 2007; Sherry et al., 2011; Otranto et al., 2017b). In the present study, no significant correlation was found between *L. infantum* infection and age or clinical status, as reported in similar studies (Martín-Sánchez et al., 2007; Miró et al., 2014). However, we did note a higher seroprevalence in males cats although, apparently, *L. infantum* infection is not related to sex in dogs or cats (Martín-Sánchez et al., 2007; Miró et al., 2014).

Only 53 cats showed lesions compatible with feline leishmaniasis, and of these only two cats were seropositive for *L. infantum* infection, although both were PCR negative in skin and blood samples. As previously reported, only a small number of infected cats will develop disease due to their distinct immune response. It seems that an effective Th1 response occurs in the majority of infected cats, and this may be responsible for the spontaneous cure of skin lesions some time after infection. Seroconversion is followed by the resolution of skin lesions and the chance of detecting the parasite by PCR methods is much reduced (Pennisi et al., 2015; Day, 2016; Soares et al., 2016).

Several disease agents of humans and animals are transmitted by arthropod ectoparasites. In this study, fleas were the most prevalent ectoparasite detected, as observed in other studies (Bond et al., 2007; Salant et al., 2014). However, *R. sanguineus* s.l. was also detected. These results highlight a potential risk for the spread of vector-borne infections of zoonotic relevance (e.g. *Bartonella* spp., *D. caninum*, *Anaplasma phagocytophilum* or *Francisella tularensis*) (Salant et al., 2014; Lefkaditis et al., 2015; Otranto et al., 2015). Another important ectoparasite found in the present study was *O. cynotis* (10.6%), which causes external otitis in cats, although its prevalence was lower than reported in studies conducted in Italy (55.1%) (Perego et al., 2014) and Greece (25.5%) (Sotiraki et al., 2001).

A high prevalence of parasitic diseases is expected in colonies of stray cats, suggesting a risk to humans and other animals. During the course of our study, animals were dewormed and further treated. Unfortunately, in general these colonies are not subject to routine effective deworming. To improve this situation, one approach could be the routine addition of antiparasitic drugs products to the food ingested by cat colonies with the hope that most would ingest a sufficient amount (Beugnet et al., 2014).

Retroviruses (FeLV and FIV) also may cause serious illness in cat colonies. Even though feline retroviruses are not transmitted to humans, their control is crucial because infected cats become infectious for other cats and immunocompromised animals are more susceptible to other infectious diseases caused by protozoans, fungi, bacteria or viruses (Mancianti, 2004). We detected a 6.3% prevalence of FeLV and 7.9% prevalence of FIV infection, which reflects the importance of this type of intervention as these prevalences are reduced over those described earlier for the same region (9.2–16.4%) (Arjona et al., 2000; Miró et al., 2014). In our control programme, animals testing positive for FeLV or FIV are euthanized to reduce their prevalence and clinical impacts on the cats. However, despite this reduced prevalence of viral infections, euthanasia is controversial and some authors recommend its reconsideration. According to the American Association of Feline Practitioners (AAFP) and the Advisory Board on Cat Diseases (ABCD), an isolated positive result does not imply the need for euthanasia since up to 30% of FeLV positive cats can have a transient infection. Moreover, kittens under 6 months of age can test positive without being infected due to the transfer of colostral antibodies from infected queens (Addie et al., 2000; Little et al., 2011). The ideal approach would be to isolate positive animals for subsequent retesting, but limited resources

is a common obstacle when working with this type of population. Other authors suggest only testing animals showing a poor clinical status or belonging to colonies where the prevalence of retroviruses is high (Lloret, 2015).

So far, there is no clear evidence of effective control of cat colonies by implementing TNR programmes due to factors such as immigration or abandoning of unsterilized cats. Overcoming these obstacles can be quite challenging, and an integrated action by veterinarians and public organizations is needed to obtain positive results. Encouraging the sterilization or spaying of owned outdoor cats is essential to reduce feline colonies. Under regional legislation, this is now compulsory in the Madrid Community (Law 4/2016 of July 22, Protection of Companion Animals of the Community of Madrid.) (Natoli et al., 2006; Boone, 2015; Gunther et al., 2016; Murray et al., 2015).

## 5. Conclusions

The findings of this study highlight the beneficial impacts of a TNR programme on colonies of free-roaming cats, as prevalences of important zoonotic diseases may be drastically reduced. Our findings also reflect an urgent need for health control measures in these cat populations to reduce the risk of infection transmission to other animals or humans. Because of the close relationship between people and animals, a “One World, One Health” approach is key to preventing and controlling zoonotic diseases in both animal and human populations.

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