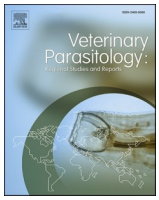
Veterinary Parasitology: Regional Studies and Reports 53 (2024) 101052

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr



Short Communication

Seroprevalence of zoonotic pathogens in stray cats in an urban area of northeast Spain

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ARTICLE INFO

Keywords:
Bartonella henselae
Leishmania infantum
Rickettsia felis
Rickettsia typhi
Seroprevalence
Stray cats
Zoonosis
Toxoplasma gondii

ABSTRACT

The feline population is extensive in urban areas worldwide, comprising stray and domestic cats. Cats, acting as reservoirs, can transmit various zoonotic organisms to humans, which can cause significant public health issues. We evaluated the seroprevalence of zoonotic pathogens in stray cats in an urban area of northeast Spain (the city of Zaragoza) to assess potential risks to human health.

A total of 88 sampled cats (52 females and 36 males) underwent antibody evaluation using the indirect immunofluorescence technique. Seroprevalence rates were determined for IgG antibodies to *Bartonella henselae* (36.3%), *Toxoplasma gondii* (31.8%), *Rickettsia felis* (14.7%), *Rickettsia typhi* (9%), and *Leishmania infantum* (10.2%). Our results confirmed the presence in stray cats of antibodies against all those pathogens, indicating that they all circulate in the feline population in Zaragoza. Male cats exhibited a higher predisposition to *T. gondii*, whereas females showed an increased likelihood of contracting *B. henselae*. This difference may be attributed to distinct behaviors according to sex.

Our findings underscore the importance of maintaining and intensifying surveillance coupled with preventive measures against zoonotic pathogens in cats. They highlight the need for comprehensive control strategies designed to mitigate public health risks associated with feline populations.

1. Introduction

Epidemiological studies have detected *Toxoplasma gondii, Bartonella henselae, Rickettsia typhi, R. felis*, and *Leishmania infantum* in the human population in several regions of Spain. For example, the seroprevalence of *T. gondii* in our country ranges from 35% in the general population to 23% in pregnant women (Calero-Bernal et al., 2023). The seroprevalence of *B. henselae* has been found to range between 3% and 25% (García-García et al., 2005; Pons et al., 2008). Seroprevalences for rickettsiosis vary:18% for *R. typhi* (Santibáñez et al., 2009) and 4 to 6% for *R. felis* (Angelakis et al., 2016). Regarding L. *infantum*, seroprevalence studies among the general population and blood donors have shown values between 1 and 8%, depending on the region (Pérez-Cutillas et al., 2015; Aliaga et al., 2019).

These diseases often go unnoticed, as they may not present symptoms or only subtle symptoms common to other infections (Rabinowitz et al., 2007; Bañuls et al., 2011; Mada et al., 2023). The current increase in cases of zoonotic diseases can be ascribed to multiple factors, such as globalization, climate change, new dietary habits, and bacterial resistance to antibiotics (Rahman et al., 2020). However, a factor often overlooked by humans is our continual contact with household pets, such as cats. It is therefore crucial to ascertain the extent to which such pets may act as disease reservoirs.

The only hosts capable of producing *T. gondii* oocysts are felines, particularly domestic cats (Brennan et al., 2020). Seroprevalence in cats varies greatly depending on the area: in Spain, the seroprevalence of *T. gondii* in cats ranges between 3 and 84% (Gauss et al., 2003; Miró et al., 2004; Millán et al., 2009a; Millán et al., 2009b; Planas, 2019; Villanueva-Saz et al., 2022). *B. henselae* is a bacterium associated with Cat Scratch Disease (CSD), which is transmitted to humans through bites or scratches from an infected cat (Chomel et al., 2004): its seroprevalence in Spanish cats ranges from 24 to 71% (Solano-Gallego et al., 2006; Ayllón et al., 2012; Gracia et al., 2015; Ravicini et al., 2016). The transmission of *R. tiphy* goes through two cycles: the classic or murine

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https://doi.org/10.1016/j.vprsr.2024.101052
Received 18 January 2024; Received in revised form 20 May 2024; Accepted 31 May 2024
Available online 1 June 2024
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cycle between rats and fleas, and the extramurine cycle involving dogs, cats, opossums, raccoons, and their fleas (Azad, 1990). Very few seroprevalence studies exist on *R. tiphy* in cats, which has been found to range from 0 to 15% (Nogueras et al., 2013; Gracia et al., 2015). *R. felis* has been associated with several arthropods, primarily cat fleas, which serve as vectors for its transmission. The ecology and epidemiology of *R. felis* are not yet fully understood and require further investigation. Whether dogs and cats are competent hosts for this pathogen has not been determined. They do play an important role as the primary hosts for fleas, facilitating horizontal transmission among them (Angelakis et al., 2016). In Spain, Gracia et al. (2015) observed a seroprevalence of 16% of *R. felis* in domestic cats. Finally, although the canine species is the main reservoir for L. *infantum*, the same pathogen can parasitize other animal species, including felines. Seroprevalence of L. *infantum* in cats varies widely: from 2 to 70% (Martín-Sánchez et al., 2007; Alcover et al., 2021; Villanueva-Saz et al., 2022). It has been suggested that cats act as an urban reservoir for the disease, perpetuating the transmission of L. *infantum* and serving as primary and/or secondary hosts in transmission to canine species and humans in endemic areas (Solano-Gallego et al., 2009).

In summary, domestic and stray cats serve as reservoirs for various zoonotic pathogens. Their close interaction with pet owners, as well as their indirect contact with humans in urban environments, tend to increase the risk of transmission, which points out the need for achieving comprehensive knowledge and management of zoonotic diseases. Moreover, if we acknowledge the impact of feline health on animal welfare, we can better address a series of holistic aspects involved in public health and epidemiology.

In Spain, information regarding the seroprevalence of the above-mentioned pathogens in cats is scarce, and particularly scarce for stray cats. In the city of Zaragoza, the population of stray cats is abundant, thus representing an added risk of transmission to humans and domestic cats. Our study aimed to investigate the occurrence of antibodies to *B. henselae, T. gondii, R. typhi, R. felis*, and L. *infantum* in stray cats in Zaragoza, Spain. We also evaluated the influence of sex on these parasites' proportional occurrence in individual hosts. All of this should allow us to assess the potential risk to the human population.

2. Material and methods

2.1. CES project (TNR: trap-neuter-return)

The CES program of the City of Zaragoza singles out colonies (groups) of stray cats that live together in a specific area of a city: they are captured, sterilized, and returned to their territory. The program aims to control the proliferation of urban cats by sterilizing the majority of individuals in a colony with the aim of reducing their number to maintain the balance of the feline population in the area. The percentage of sterilization in the population of a colony is subsequently monitored to assess the colony's status and health. Such colonies can feature three types of cats: stray cats, abandoned or lost cats, and wild or feral cats.

Their presence in certain territories can cause substantial problems: not only rampant population increases, but also a series of sensory (auditory, visual, and olfactory) disturbances. Most of the problems cited below can be ascribed to two fundamental causes: 1) the hyperprolificacy of feline females and 2) continual human support, turning stray cats into subsidized inhabitants of a territory (Herrera-Coronado, 2023).

2.2. Animals and samples

We collected blood samples from a total of 88 stray cats (52 females and 36 males) that had been temporarily captured by the CES project administered by Zaragoza City Council. The animals were brought to University of Zaragoza Veterinary Hospital between 1st September 2019 and 30 November 2020 for surgical castration. A 2 mL blood sample was taken during pre-anesthesia by puncturing the jugular or cranial cephalic vein and transferred into an anticoagulant-free tube. To obtain the serum required for serological diagnosis, the blood samples were centrifuged at 1200 xg for 10 min. Serum samples were stored in 1.5 mL collection tubes with numbers assigned consecutively from 1 to 88, corresponding to the individual animals. Samples were kept frozen at −20 °C until analyses.

We calculated the minimum sample size using WinEpi v2.0 (http://www.winepi.net/winepi2) (Vallejo et al., 2013), applying the estimate proportion (random sampling and perfect diagnostic) option with an accepted error (or precision) of 10% and a confidence level of 95%. We obtained the estimated proportion for each pathogen from several previous studies performed in Spain. For *T. gondii*, the estimated proportion was 8.4%, obtained from a preliminary study conducted by members of our own research group in Zaragoza (Planas, 2019). For *B. henselae*, it was 30% (Ravicini et al., 2016), whereas for *R. felis* and *R. tiphy*, it was 26 and 0%, respectively (Gracia et al., 2015). Finally, the estimated population of *L. infantum* was 2.2% (Alcover et al., 2021). The corresponding minimum sample size we calculated was of 51, 88, 85, 30 and 32 individuals, respectively.

All practices involving animals were approved by the Ethics Committee for Animal Experiments from the University of Zaragoza (Project license PI62/18, date of approval: 5 February 2019).

2.3. Detection of antibodies

Serum samples were thawed to room temperature and assayed by indirect immunofluorescence assay (IFA) for all IgG antibodies to the above-mentioned pathogens.

T. gondii IgG antibodies were detected via the "Mega-FLUO®TOXOPLASMA g." in vitro assay (Diagnostik Megacor, Horbranz-Austria) with a 1:50 threshold. Following the kit's instructions, titers <50 were considered negative and titers ≥ 50 were considered positive. *B. henselae* IgG antibodies were detected with the "Mega-FLUO®*BARTONELLA henselae*" in vitro assay (Diagnostik Megacor, Horbranz-Austria) with a 1:64 threshold. Titers <64 were considered negative and titers ≥ 64 were considered positive. *R. felis* and *R. typhi* IgG antibodies were detected by using the "Flea-borne typhus IgG MIF kit" (Fuller Laboratories, California USA), featuring slides coated with cells infected with *R. felis* and *R. typhi* in the same well with a 1:16 threshold. Titers <16 were considered negative, while titers ≥ 16 were considered positive for both assays. Finally, *L. infantum* antibodies were detected with the "LEISHMANIA infantum Fuller kit" (Fuller Laboratories, California USA) with a 1:40 threshold. Titers <40 were considered negative, while titers ≥ 40 were considered positive.

In all pathogens under study, we used the positive and negative control provided by the respective kits for each slide.

2.4. Statistical analyses

All results, including identification number, sex, and antibody evaluation results, were collected in an Excel file. Prevalence was expressed as a percentage with a 95% confidence interval, using the free software "VassarStats: Website for Statistical Computation" (http://vassarstats.net/).

To determine eventual relationships between pathogens seropositivity and sex (male/female), we performed the categorical data test (positive/negative). The difference's significance was assessed using chi-square. Odds ratios in binary logistic regression were then calculated with the SPSS v24 (IBM Corporation, Armonk, NY, USA). A value of $p \leq 0.05$ was considered significant.

3. Results

3.1. Cat population characteristics

We tested a total of 88 cats (52 females and 36 males), all >1-year-old and of mixed breed. All were classified as apparently healthy. A general physical examination revealed one female with pyometra, one with mastitis, and one male with a testicular tumor. Seven females were pregnant.

3.2. Serological results

A total of 56/88 (63.6%) cats were seropositive for at least one pathogen, of which 26/56 (46.4%) presented co-infection. On the other hand, 32/88 (36.3%) were negative for all pathogens. The prevalence of *T. gondii* IgG antibodies in serum was 31.8% (95%CI = 22.5–42.7), 36.3% (26.5–47.3) IgG antibodies to *B. henselae,* 9% (4.2–17.6) for *R. tiphy*, 14.7% (8.4–24.3) for *R. felis*, and 10.2% (5–18.9) for L. *infantum*. Serology prevalence and antibody titer results are shown in Table 1.

3.3. Serological results associated with sex

Males were proportionally more seropositive than females for *T. gondii* and L. *infantum*. Regarding *B. henselae, R. tiphy*, and *R. felis*, females were more seropositive.

Considering the weight of the sex factor, we observed that infection with *T. gondii* was higher in males; this association was statistically significant ($p = 0.034$). Infection with *B. henselae* was higher in females; this association was likewise statistically significant ($p = 0.002$). No significant association was detected between sex and infection for *R. tiphy* ($p = 0.337$), *R. felis* ($p = 0.421$), or *L. infantum* ($p = 0.820$) (Table 2).

To study the sex factor further in-depth, we only included *T. gondii* and *B. henselae* in the subsequent logistic regression step. Based on the Odds ratio, the occurrence of anti-*T. gondii* antibodies was 2.6 times higher ($p = 0.037$) in male than in female cats. The occurrence of anti-*B. henselae* antibodies was 4.16 times higher (p = 0.002) in female than in male cats. To summarize, males were more likely to be infected with *T. gondii*, and females were more likely to be infected with *B. henselae*.

Table 2
Number, percentage and statistical association of seropositive cats according to sex.

Pathogen	Sex	No. (%) seropositive by sex	p
T. gondii	Male	16/36 (44.4%)	0.034
	Female	12/52 (23%)	
B. henselae	Male	12/36 (33.3%)	0.002
	Female	20/52 (38.4%)	
R. tiphy	Male	2/36 (5.5%)	0.337
	Female	6/52 (11.5%)	
R. felis	Male	4/36 (11.1%)	0.421
	Female	9/52 (17.3%)	
L. infantum	Male	5/36 (13.8%)	0.820
	Female	4/52 (7.6%)	

4. Discussion

This study addressed the importance of certain zoonotic pathogens in cats. The seroprevalence rates we obtained were 36.3% for *B. henselae,* 31.8% for *T. gondii*, 14.7% for *R. felis*, 9% for *R. typhi*, and 10.2% for L. *infantum*. The number of animals we sampled exceeded the required number; our results can thus be regarded as representative of the population.

Compared to domestic cats, stray cats are generally exposed to a higher risk of contracting pathogens and are more likely to be pathogen carriers. This is due to their feeding and lifestyle habits, such as drinking water from stagnant areas, hunting small wild animals that may carry diseases, and exposure to disease-carrying arthropod vectors (Liberg, 2000).

B. henselae is distributed worldwide in cats, but with varying antibody and infection prevalence rates depending on geographical location, the assays used, and animal status (stray or household pet). The bacterium is transmitted among felines through the bite of the flea *Ctenocephalides felis*, to which stray cats are more highly predisposed (Razgūnaitė et al., 2021). Other studies on the subject of *B. henselae* cat infection in Spain have found that a cat's status and/or living environment (stray or domestic) does not exert an influence on the seroprevalence of *B. henselae*. In a study conducted by members of our research group on 86 domestic cats across Spain, the assayed prevalence was 50% (Gracia et al., 2015), which is considerably higher than the prevalence measured in the present study (36.3%). A study in Catalonia and Majorca found a prevalence of 71% of *B. henselae* in a population of 168 domestic cats (Solano-Gallego et al., 2006). However, other studies conducted across various Spanish territories have reported lower prevalence rates, as in Madrid, where the prevalence assayed in a population of 680 domestic and stray cats was 24.7% (Ayllón et al., 2012), or of 29.6% in 116 shelter cats in Catalonia (Ravicini et al., 2016). These differences in prevalence are likely due to the cats' exposure to fleas. The overall prevalence of *B. henselae* in the Spanish cat population is expected to decrease over time, as cats covered by current population control programs are internally and externally dewormed to prevent the transmission of diseases by flea vectors.

In the 88 cats we studied, we observed a seroprevalence of 31.8% for *T. gondii*. Similar results were obtained by Miró et al. (2004) in a study conducted across Spain, where the assayed seroprevalence in a sample of 317 stray cats was 36.9%. Higher rates were found elsewhere, with seroprevalences of nearly 50% in 131 and 53 stray cats analyzed in Barcelona and Andalusia, respectively (Gauss et al., 2003; Millán et al., 2009a). A considerably higher seroprevalence rate was observed in Majorca, reaching 84.7% in a population of 59 free-roaming cats (Millán et al., 2009b). However, in a study conducted on 114 stray cats in Zaragoza, a prevalence of only 12.2% was obtained (Villanueva-Saz et al., 2022). In this case, the difference vs. our study may be due to the IFA technique cutoff point, which was lower in our case. Regarding the seroprevalence of *T. gondii* in domestic cats, Miró et al. (2004) observed a prevalence of 25.5% in a total of 220 cats analyzed across Spain, whereas Gauss et al. (2003) found a prevalence of 34.8% in 89 cats analyzed in Barcelona. Both studies obtained statistically significant differences between stray and domestic cats. Furthermore, the

Table 1
Total seroprevalence and antibody titer results for all pathogens under study.

	T. gondii		*B. henselae*		*R. tiphy*		*R. felis*		*L. infantum*	
	Ab titers	No.	Ab titers	No.	Ab titers	No.	Ab titers	No.	Ab titers	No.
	<50	60	<64	56	<16	68	<64	64	<40	79
	50	18	64	21	16	12	16	11	40	5
	100	4	128	8	32	5	32	9	80	2
	200	3	256	2	≥64	3	≥64	4	160	0
	≥400	3	≥562	1					≥320	2
Seroprevalence (95%CI)	31.8% (22.5–42.7)		36.3% (26.5–47.3)		9% (4.2–17.6)		14.7% (8.4–24.3)		10.2% (5–18.9)	

preliminary study performed by members of our own research group in Zaragoza observed no positive cases in 35 domestic cats, but a seroprevalence of 8.4% in 24 stray cats (Planas, 2019).

Regarding *R. typhi* and *R. felis*, studies worldwide have found higher degrees of seroprevalence in stray cats than in domestic ones. For example, studies conducted in California and Wisconsin by Case et al. (2006), as well as in Florida by Luria et al. (2004), observed this trend. In a study on *R. tiphy* in southern Spain, no difference was observed between stray and domestic in a sample of 221 cats, with an overall prevalence of 15.8% (Nogueras et al., 2013). The prevalence found in our study in Zaragoza was somewhat lower: 9% in a sample of 88 cats. It is worth noting that a study conducted in 2015 by Gracia et al. (2015) did not detect any trace of *R. typhi* among 86 domestic cats analyzed across Spain. This discrepancy among studies might be due to the different origins of the cats involved (veterinary clinics vs. shelters and the street), or to the chosen sampling area. To our knowledge, ours is the first study to have detected *R. typhi* in naturally infected cats in Zaragoza. However, regarding *R. felis*, we detected antibodies in 14.7% of the 88 cats, a value similar to a previous report from Spain with a prevalence of 16.3% in domestic cats (Gracia et al., 2015). It is important to bear in mind that *R. typhi* and *R. felis* exhibit a significant serological cross-reaction; it is sometimes challenging to determine which pathogen is responsible for seroreactivity unless the agent has been identified with a finer measurement technique such as PCR (Ebani et al., 2021). Rickettsial DNA has been detected in the blood of dogs and cats, but researchers have not determined whether the infection is long-lasting and stable enough to make those animals competent hosts. Dogs and cats do play an important role as the primary hosts of fleas, facilitating horizontal transmission. In general, in vertebrates, infection can occur when an infected flea excretes feces that contaminate wounds and abrasions (Angelakis et al., 2016). This is why studies on seroprevalence in cats have often observed the presence of rickettsial DNA in fleas (Nogueras et al., 2011; Nogueras et al., 2013; Gracia et al., 2015). We could not perform that step in our study, as we only had access to sera.

The prevalence of feline leishmaniosis tends to be lower than that of canine leishmaniosis in the same geographic area; however, in endemic zones, it is increasing. In Spain, seropositivity values for L. *infantum* vary widely: from 2.8 to 70.6%. Two studies conducted on 179 and 144 stray cats revealed L. *infantum* seropositivity rates of 2.8% and 12.2%, respectively (Alcover et al., 2021; Villanueva-Saz et al., 2022). Similar results were obtained in our current study, showing a prevalence of 10.2%. Higher values were observed in a study conducted in southern Spain: in a sample of 183 domestic cats, 70.6% of the feline population was, or could be, infected (Martín-Sánchez et al., 2007). This significant difference may again be due to the origin of the animal subjects (domestic) and due to population control programs where cats are externally parasitized, thereby reducing their exposure to disease-transmitting vector bites.

After thorough comparison of our study with others conducted in our country, it appears that there is no clear difference between stray and domestic cats. This may be because in large cities such as Zaragoza, an increasing number of projects not only foresee the sterilization of captured animals but also feed stray animals with commercial feeds, ensure hygienic-sanitary care of registered colonies, conduct periodic checks on sanitary conditions, and organize appropriate distribution of animals to avoid overcrowding and unhygienic conditions. This underscores the imperative to persist in providing vigilant care for stray cats with the purpose of mitigating the dissemination of pathogens within feline populations and, consequently, averting the potential transmission of harmful diseases to humans.

Although several studies found no significant differences between males and females in terms of seroprevalence of *T. gondii* (Lopes et al., 2008; Coelho et al., 2011), other studies suggested that there is a higher risk in females (Sroka et al., 2018; Besné-Mérida et al., 2008; Troncoso et al., 2015). In our study, the occurrence of anti-*T. gondii* antibodies was 2.6 times higher ($p = 0.037$) in male than in female cats. This may be attributed to the fact that male cats typically cover a broader exploration territory and are more inclined to roam, thus increasing their likelihood of visiting contaminated areas. This heightened activity may elevate their exposure to the parasite or its oocyst in the environment. In this context, it is worth noting that cats are natural hunters. Furthermore, it has been demonstrated that neutered and fed cats do not reduce their predation frequency, as predation is a natural component of their behavior that cannot be controlled by merely providing them with food (Cecchetti et al., 2021).

Regarding *B. henselae*, we detected a higher proportion of seropositive females compared to males, similar to results obtained by Zaror et al. (2002). In our study, the occurrence of *B. henselae* was 4.16 times higher ($p = 0.002$) in female than in male cats. *B. henselae* is primarily transmitted by fleas, and females may have more fleas due to their behavior while caring for their offspring. They tend to create nests that can serve as perfect habitats for the development of a large number of fleas, thus increasing female cats' probability of contracting flea-borne diseases. This differs from the results obtained by Bergmans et al. (1996), who, in the Netherlands, reported a slightly higher seropositivity percentage in males (55%) than females (47%). That study is apparently the only one to report such a tendency. We found a higher prevalence of rickettsioses in females (although not to a statistically significant degree); this, as well, could be explained by females' nesting behavior as described above.

This study has certain limitations that can be taken into account for future research. It would have been interesting to compare the seroprevalence of these diseases in stray cats with those obtained from domestic cats with and without outdoor access. Alternative diagnostic methods would have been helpful in distinguishing between infection and disease, as well as in assessing whether exposure to the pathogen is indicative of past or present (active) infection.

5. Conclusions

This research substantiates the presence of antibodies against *T. gondii, B. henselae, R. typhi, R. felis*, and L. *infantum* in stray cats in Zaragoza, Spain. We suggest that male cats are more predisposed to contracting *T. gondii*, while females are more prone to acquiring *B. henselae*. Such predispositions may be attributed to their distinct behavioral patterns. As the high prevalence of these pathogens continues to emerge in studies such as ours, we suggest that it would be advisable to maintain and even increase general zoonotic control measures, for the sake of feline welfare and in the interest of the broader public health landscape.

Funding

Funding for this research was provided by the research groups “Reproducción Asistida y Sanidad Animal (Ref: A17_17R)” and “Grupo de referencia enfermedades priónicas, vectoriales y zoonosis emergentes (Ref: A05_17R)”.

Ethical statement

The care and use of animals were performed according to the Spanish Policy for Animal Protection RD 53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes. All practices involving animals were approved by the Ethic Committee for Animal Experiments from the University of Zaragoza (Project license PI62/18, date of approval: 5th February 2019).

CRediT authorship contribution statement

Sandra Planas: Methodology, Investigation. **Jon Langa:**

Methodology, Investigation. **Alicia Laborda:** Supervision, Resources, Methodology. **Juan Antonio Castillo:** Supervision, Resources, Project administration. **María Jesús Gracia:** Writing – review & editing, Supervision, Resources, Project administration, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank their colleagues at the University of Zaragoza Veterinary Hospital for collaborating on this study. We would also like to thank and commend the City Council of Zaragoza for its initiative to control cat colonies.

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