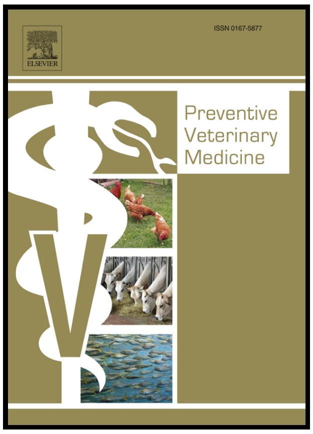
Journal Pre-proof



Seroprevalence of *Leptospira* antibodies in dogs and cats from Santa Fe, a city in East-Central Argentina endemic for leptospirosis

Tamara Ricardo, Ludmila R. Bazán Domínguez, Lucila Beltramini, Yanina Prieto, Anahí Montiel, Leticia Margenet, M. Fernanda Schmeling, Yosena T. Chiani, Marcelo L. Signorini, M. Andrea Previtali

PII: S0167-5877(24)00125-9

DOI: https://doi.org/10.1016/j.prevetmed.2024.106239

Reference: PREVET106239

To appear in: *Preventive Veterinary Medicine*

Received date: 10 April 2024
Revised date: 24 May 2024
Accepted date: 28 May 2024

Please cite this article as: Tamara Ricardo, Ludmila R. Bazán Domínguez, Lucila Beltramini, Yanina Prieto, Anahí Montiel, Leticia Margenet, M. Fernanda Schmeling, Yosena T. Chiani, Marcelo L. Signorini and M. Andrea Previtali, Seroprevalence of *Leptospira* antibodies in dogs and cats from Santa Fe, a city in East-Central Argentina endemic for leptospirosis, *Preventive Veterinary Medicine,* (2024) doi:https://doi.org/10.1016/j.prevetmed.2024.106239

Seroprevalence of *Leptospira* antibodies in dogs and cats from Santa Fe, a city in East-Central Argentina endemic for leptospirosis

Tamara Ricardo [a,b,1,*], Ludmila R. Bazán Domínguez [b], Lucila Beltramini [c], Yanina Prieto [c,d], Anahí Montiel [c], Leticia Margenet [e], M. Fernanda Schmeling [e], Yosena T. Chiani [e], Marcelo L. Signorini [f], M. Andrea Previtali [a,b,*]

[a] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Santa Fe, Argentina.

[b] Dpto. de Ciencias Naturales, Facultad de Humanidades y Ciencias (FHUC), Universidad Nacional del Litoral (UNL), Santa Fe, Argentina.

[c] Instituto Municipal de Salud Animal (IMuSA), Gobierno de la Ciudad de Santa Fe, Santa Fe, Argentina.

[d] Centro de Investigación sobre Endemias Nacionales (CIEN), Facultad de Bioquímica y Ciencias Biológicas (FBCB), Universidad Nacional del Litoral (UNL), Santa Fe, Argentina.

[e] Instituto Nacional de Enfermedades Respiratorias (INER) "Dr. E. Coni", ANLIS "Dr. C.G. Malbrán", Santa Fe, Argentina.

[f] Instituto de Investigación de la Cadena Láctea (INTA - CONICET), Rafaela, Santa Fe, Argentina.

* Corresponding authors:

andrea.previtali@gmail.com (M.A. Previtali), tamararicardo83@gmail.com (T. Ricardo).

[1] Current address: Instituto Nacional de Epidemiología (INE) "Dr. J.E. Jara", ANLIS "Dr. C.G. Malbrán", Mar del Plata, Buenos Aires, Argentina.

Abstract

This study examines household pets as potential epidemiological links between environments contaminated with pathogenic leptospires and humans in Santa Fe, Argentina. The aims of our study were: (a) to characterize the habits and exposure to environmental sources of leptospirosis in the population of dogs and cats attending to municipal spay and neutering campaigns in Santa Fe, Argentina, (b) to assess the seroprevalence of anti-*Leptospir*a antibodies in asymptomatic dogs and cats, (c) to evaluate factors that could increase seropositivity, and (d) to identify spatial clusters of seropositive dogs and cats in the capital city of Santa Fe.

From May to November 2022, a cross-sectional serosurvey was conducted during municipal spaying/neutering campaigns. Eligible household dogs and cats were over 6 months old, apparently healthy, and not vaccinated against leptospirosis in the past 6 months. We used microagglutination test to assess anti-*Leptospira* antibodies using a panel of 10 reference strains. We used generalized linear mixed effects models (GLMM) to examine individual and census tract-level risk factors for seropositivity, and local Moran's I statistic for spatial clusters.

Results showed higher leptospiral antibody prevalence in dogs (18.2%) than cats (3.6%) (p = 0.002). Dogs with street access had higher seropositivity probability (OR: 3.8, 95% CI: 1.2; 11.9), and areas with chronic poverty showed an elevated risk of presenting seropositive animals (RR: 4.0, 95% CI: 1.1; 14.4). Spatial analysis didn't reveal significant seropositivity clusters among census tracts.

These findings shed light on widespread *Leptospira* seropositivity in pets in this endemic region. Understanding seroprevalence and risk factors can guide public and veterinary health strategies, emphasizing increased leptospirosis vaccination for dogs in vulnerable areas and promoting responsible pet care.

Keywords: Epidemiology; Household pets; Leptospirosis; Risk factors; Santa Fe, Argentina

Declarations of interest: none

Introduction

Domestic dogs (*Canis lupus familiaris*), along with rats (*Rattus* spp.), cattle, and pigs, are considered the main reservoirs of pathogenic *Leptospira* species (Ellis, 2015; Sykes et al., 2023). In recent years, a meta-analysis reported a prevalence of pathogenic leptospires in the urine of cats (*Felis silvestris catus*) of 4% (Ricardo et al., 2023), which suggests that domestic cats also contribute to the dissemination of *Leptospira* spp. in the environment (Ricardo et al., 2023; Sykes et al., 2023).

The geographic distribution and environmental persistence of pathogenic leptospires are influenced by environmental and climatic features, characteristics of the animal reservoirs, and socioeconomic conditions of human populations (Costa et al., 2015; Ellis, 2015; Mwachui et al., 2015). The province of Santa Fe, located in northeastern Argentina, belongs to the Paraná flooded savanna ecoregion and has a humid subtropical climate, providing a favorable environment for the endemicity of leptospirosis (Costa et al., 2015). This is evidenced by the relatively high annual incidence of human cases during the rainy season and the occurrence of epidemics following floods (López et al., 2019; Vanasco et al., 2000).

In 2015, a publication estimated that 8% of the dog and cat population in Argentina, totaling 6,819,906 dogs and 2,407,026 cats, reside in the province of Santa Fe (Trabattoni and Ducommun, 2018). Many of these animals did not receive basic veterinary care, as indicated by the low rates of sterilization and mandatory annual rabies vaccination reported in the available literature (Azócar-Aedo et al., 2014). This lack of awareness of responsible pet care, coupled with some behavioral habits of dogs and cats, such as scavenging and hunting, makes them an important epidemiological link for the transmission of leptospires to humans (Ellis, 2015; Hartmann et al., 2013; Mwachui et al., 2015). However, only three studies assessed the prevalence of leptospiral antibodies in domestic dogs and cats from the province of Santa Fe (Seghesso Zabala et al., 2013; Yaafar et al., 2019; Francois et al., 2020). None of these studies were conducted in the city of Santa Fe. The first study, a cross-sectional study involving 156 dogs, reported a seroprevalence of 14.1% assessed using a microagglutination test (MAT) with a cut-off titer of 1:100 and a panel of six serovars (Seghesso Zabala et al., 2013). The other two studies focused on cats. Yaafar et al., 2019 is a case report of two cats with suspected clinical leptospirosis. Francois et al. (2020) conducted a cross-sectional study involving 160 asymptomatic cats and 160 with kidney disease and found seroprevalences of 3.8% and 31.3%, respectively, using a MAT with a cut-off titer of 1:50 and a panel of 11 serovars. The scarcity of studies and the inconsistencies found among them creates an information gap that constrains the abilities of government officials to characterize and comprehend the problem, identify the most vulnerable areas, and develop public policies to try to solve it. The aims of our study were: (a) to characterize the habits and exposure to environmental sources of leptospirosis in the population of dogs and cats attending to municipal spay and neutering campaigns in Santa Fe, Argentina, (b) to assess the seroprevalence of anti-*Leptospira* antibodies in asymptomatic dogs and cats, (c) to evaluate factors that could increase seropositivity, and (d) to identify spatial clusters of seropositive dogs and cats in the capital city of Santa Fe.

Materials and methods

Study design and setting

The city of Santa Fe, the capital of the province of Santa Fe, is the seventh most populated city in Argentina, with approximately 570,732 inhabitants in 2022 (Instituto Nacional de Estadísticas y Censos [INDEC], 2022). Santa Fe is geographically surrounded by rivers on the eastern, western, and southern sides and has a humid subtropical climate (Servicio Meteorológico Nacional, 2024). The city is divided into 399 census tracts, 29 census fractions, and eight administrative districts (Fig. 1). Socioeconomic conditions vary widely among the city districts, with a higher concentration of urban slums and informal settlements observed in the riverside areas (Fig. 1).

Between May and November 2022, we conducted a cross-sectional serological survey of anti-*Leptospira* antibodies in asymptomatic dogs and cats in Santa Fe city, Argentina. The sampling was carried out in two spaying/neutering units of the Municipal Institute of Animal Health (IMuSA) located in the West (IMuSA I) and North (IMuSA II) districts, as well as in the neighborhoods reached by the mobile sterilization units (Fig. 1). Each IMuSA unit receives between 10 and 20 dogs and cats per day for spaying/neutering, and about the same number of animals for anti-rabies vaccination, deworming, or post-sterilization veterinary control and most of these animals come from adjacent neighborhoods. The mobile units are generally placed in informal settlements or low-resource neighborhoods at the request of neighborhood referents (Fig. 1).

Eligibility criteria

We included dogs and cats aged six months and older that were scheduled for spaying/neutering, as well as dogs that came to IMuSA for rabies vaccination or post-sterilization veterinary controls. To be included, animals had to be household dogs and cats, apparently healthy based on the caretaker's statement provided prior to the intervention. We excluded from the study feral, stray, and shelter animals, those of less than six months of age, dogs vaccinated against leptospirosis in the previous six months, exhibiting aggressive behavior, as well as pregnant or lactating females. Our study did not include animals with signs of febrile illness, undergoing antibiotic treatment, or with comorbidities, as they did not qualify for spaying or neutering. To reduce the risk of pseudoreplication and improve the validity of results, we only sampled one dog or cat per household.

Sample size calculation

For the calculation of sample size, we estimated the number of dogs and cats in the city by multiplying the number of inhabitants by a coefficient of 0.17 for dogs and 0.06 for cats as suggested by Trabattoni and Ducommun (2018). These coefficients were estimated by dividing the total estimate of existing dogs in Argentina in 2014 by the number of inhabitants reported in the 2010 National Population Census (National Institute of Statistics and Census, INDEC) (Trabattoni and Ducommun, 2018). We assumed an expected seroprevalence of 14.1% for dogs (Seghesso Zabala et al., 2013), and 3.8% for cats (Francois et al., 2020). Considering a precision of 7.5% and a potential data loss of 15%, the desired sample size was 95 dogs and 95 cats. The number of sampled cats was below the estimated sample size due to the high frequencies of stray/feral cats and multiple cats from the same household taken for sterilization, as well as the refusal of some caretakers.

Data collection tool

The caretaker of the animal was requested to respond an interviewer-administered questionnaire to collect information on the following variables: animal species (dog, cat), sex (male, female), history of pregnancies (yes, no), history of abortions (yes, no), age in years, breed (defined breed, mixed breed), history of vaccination (yes, no, don't know), internal or external deworming (yes, no), type of immunization received (rabies, canine hexavalent, others), time since last vaccination (less than six months, between 6 and 12 months, more than 12 months), role/s of the animal in the household (companion, guarding, hunting/rodent control), type of food provided (commercial pet food formula, raw meat, homemade food, leftovers), street access (yes, no), unsupervised street access (yes, no), exposure to garbage dumps (yes, no), exposure to environmental water/mud (yes, no), hunting behavior (yes, no), contact with dogs (yes, no), cats (yes, no) and other animals (yes, no), origin of the contacted animals (own, neighbors, stray), number of dogs/cats in the household, febrile illness in the past year (yes, no), rodent sightings at the household (yes, no), frequency of rodent sightings (always, occasionally, never), where the animal is held (inside only, inside and outside, outside only), presence of yard in the house (yes, no), and frequency of cleaning the pet bed/kennel/house (daily, every other day, weekly, occasionally, never). The animals were palpated by members of the research team to assess body condition score (BCS) in a 1-5 scale, where a BCS of 1 indicates that the animal is very thin/emaciated, while a BCS of 5 indicates obesity (Burkholder, 2000). The postal address of the animal's caretaker was recorded, geo-referenced, and assigned to a census tract, census fraction, and administrative district for further analysis. The data collection instrument can be accessed in File S1.

Sample collection

Blood samples were obtained via venipuncture of either the antebrachial or jugular vein using a 21-gauge needle. The volume of blood extracted, which ranged from approximately 1.5 to 3 ml, was determined based on the species, age, and size of the animal. Samples were stored in standard blood tubes with separator gel. Blood extraction was performed while the animal was anesthetized for surgery, except for dogs under veterinary control or receiving anti-rabies vaccination, who were manually restrained by their caretakers or by veterinary assistants.

Following collection, blood samples were placed in cooling containers to maintain their integrity during transportation to the Biosafety Laboratory of the Department of Natural Sciences of the National University of El Litoral. Samples were then centrifuged for 10 minutes at 6000 rpm to separate the serum, which was stored at -20°C until further processing.

Laboratory analysis

Serological tests were conducted at the National Institute of Respiratory Diseases “Emilio Coni” (INER-ANLIS CG Malbrán). The microagglutination test (MAT) was employed to assess the presence of anti-*Leptospira* antibodies against ten of the most frequently detected serogroups in dogs and cats worldwide (Ricardo et al., 2024) (Table1). Samples exhibiting a 50% agglutination at a titer of ≥1:100 were considered positive. Seropositive samples were subsequently titrated using quantitative MAT, and the serogroup with the highest titer was designated as the presumably infecting serogroup. Samples reacting with the same titer to two or more serogroups were considered coagglutinations. The results of the blood tests, along with a brief explanation of the interpretation of the results and an informative flier on responsible pet care were sent to the caretakers via WhatsApp® messenger (File S2).

Data processing

Data were entered and geo-referenced using EpiInfo™ version 7.2.5 (CDC, 2021) and processed in R software, version 4.3.1 (R Core Team, 2023). In cases where we were unable to geolocate a sample or there was no postal address available, we used the location of the IMuSA mobile unit to georeference using Google Maps®. We took this approach considering that a significant portion of the neighborhoods visited by the mobile units are small settlements lacking named or numbered streets, with the mobile units strategically placed in neighborhood associations or health centers. If the provided address or location was outside the city limits of Santa Fe, the sample was discarded.

The life stages of the sampled animals were categorized as follows: junior (0.5-2 years for dogs, 0.5-3 years for cats), adult (2-7 years for dogs, 3-10 years for cats), and senior (>7 years for dogs and >10 years for cats) (Harvey, 2021; Vogt et al., 2010). Body condition score was categorized into three groups: very thin/thin, normal, and overweight/obese. The role of the animal in the household was categorized as companion only, companion and guarding/hunting, or guarding/hunting only. The type of feeding was categorized as pet food only, pet food and homemade food/leftovers, or homemade food/leftovers only. The variables number of dogs in the household and the number of cats in the household were updated by adding the sampled animal to the count. We created the variable 'rabies vaccine at sterilization' (yes, no) based on the comments from the vaccination section, and the variable 'number of dogs and cats in the household' (1-2 dogs/cats, 2-3 dogs/cats, 3-6 dogs/cats, 6+ dogs/cats) based on the quartiles of the sum of the number of dogs and the number of cats.

We obtained spatial layers of socio-economic indicators based on the results of the 2010 National Census from the Plataforma Abierta de Datos Espaciales de la Argentina [POBLACIONES] (https://poblaciones.org/). The following indicators, expressed as the number of households per census tract, were considered relevant for the study: at least one indicator of unsatisfied basic needs (UBN), the incidence of chronic poverty, overcrowding, poor housing, absence of sewage, pit or cesspool drainage without a chamber, absence of piped-water, presence of paved roads, and regular garbage collection. Raw indicators were transformed into percentages by dividing by the number of households in the census tract. The incidence of chronic poverty at the household level was estimated by dividing the number of households with indicators of chronic poverty by the overall number of households at the census tract (POBLACIONES, 2022). We categorized the incidence of chronic poverty at the sampled census tracts into four groups: Very low (<1%), Low (1-5%), Moderate (5-10%), and High (>10%).

Data analysis

Data were cleaned and analyzed in R software, version 4.3.1 (R Core Team, 2023). Statistical significance in all analyses was set at $p \leq 0.05$. The frequencies of categorical variables between dogs and cats were compared using the package *gtsummary* (Sjoberg et al., 2021).

We assessed potential risk factors for seropositivity to pathogenic *Leptospira* antibodies by fitting mixed-effects logistic regression models (GLMM). Due to the small number of seropositive cats, the models were only fit for dogs with complete epidemiological data. To address sample interdependence, we tested as possible random intercepts the administrative district, the neighborhood of residence, and the nested effect of the administrative district and neighborhood. The three models containing only the random intercepts were compared based on their Akaike's Information Criteria (AIC) and Bayesian Information Criteria (BIC) using the *performance* package (Lüdecke et al., 2024). We fitted a series of univariate GLMMs using the most parsimonious random intercept and one of the following explanatory variables: sex, age in years, age category, role/s of the animal in the household, type of food provided, street access, unsupervised street access, exposure to garbage dumps, exposure to environmental water/mud, hunting behavior, rodent hunting, sylvan animal hunting, contact with dogs, contact with cats, contact with other animals, contact with stray dogs/cats, contact with neighbor dogs/cats, contact with own dogs/cats, categorized number of owned dogs/cats, rodent sightings at the household, frequency of rodent sightings, where the animal is held, presence of yard in the house, and frequency of cleaning the pet bed/kennel/house. The variables with a p-value ≤ 0.10 in univariate analyses were retained as candidate explanatory variables in a multivariate model. A manual step-backward procedure was then implemented to select explanatory variables based on the

Akaike's Information Criteria (AIC). Inferences were drawn from the final model and interpreted in terms of odds ratios (OR) and its 95% confidence interval (95% CI).

We fitted a series of univariate GLMMs with a Poisson distribution to evaluate factors that can increase the risk of seropositivity at the census tract level, using the number of seropositive dogs and cats per census tract as the response variable. In these models, the incidence of chronic poverty and the percentage of households with one of the selected socio-economic indicators at the census tract level were used as candidate explanatory variables. Indicators with a p-value ≤ 0.10 in univariate analyses were assessed for correlation using Pearson's correlation coefficient, and those with high correlation (r >0.5) were not included in the same multivariate model. We compared the performances of the fitted models and drew inferences from the best-fit model in terms of risk ratios (RR) and its 95% CI.

We assessed the presence of spatial autocorrelation among the census tracts in which samples were collected using Moran's I statistic. A value of 0 indicates no spatial autocorrelation, values between 0 and -1 indicate spatial dispersion and values between 0 and 1 indicate spatial autocorrelation (Moraga, 2023). We used the local Moran's I (I_i) as a Local Indicator of Spatial Association (LISA) to assess the presence of spatial clusters. Higher values of I_i indicate that one area is part of a cluster of a similar number of observations, and lower values indicate that the area is different from its neighbors (Moraga, 2023). The results of the analysis were presented as choropleth maps. An interactive version of the generated maps can be accessed from: https://issengard83.github.io/R_data_IMUSA/

Ethics statement

The blood extraction procedures were carried out by veterinarians from the research team or the IMuSA units. Before the collection of blood samples, the caretaker of the animal was requested to read and sign an informed consent form (File S3). In cases where the caretaker was a minor or illiterate, a representative adult was asked to sign the consent on their behalf. Data confidentiality and anonymity were ensured. The work protocol had been approved by the Ethics and Safety Committee for Research at the College of Biochemistry and Biological Sciences, National University of the Litoral (Acta FBCB 03/17).

Results

Characteristics of the sampled animals

We collected a total of 209 samples, of which 15 were discarded due to the following reasons: dogs that had received the hexavalent vaccine less than six months before the sampling date (n = 3), caretakers leaving the sterilization unit without completing the questionnaire (n = 3), and residences located outside the city limits (n = 9). The remaining 194 samples comprised 110 dogs (56.4%) and 84 cats (43.6%) from 108 census tracts across the eight administrative districts. The district "La Costa" had the highest number of samples (30.4%), followed by the districts Southwest (13.9%) and Northwest (12.9%, Fig. 1).

Among the sampled animals, 59.1% of dogs and 45.1% of cats were females, with no significant differences in sex distribution ($p = 0.055$). Female dogs had a significantly higher proportion of pregnancies compared to cats (49.2% and 28.9%, respectively; $p = 0.044$). However, there were no significant differences in the frequencies of abortions or stillbirths between both species (12.3% and 5.3%, respectively; $p = 0.3$).

The sampled cats had a median age of 0.8 years (IQR: 0.60, 1.00 years), with 98.8% categorized as juniors and 1.2% as adults. Sampled dogs had a median age of 2.5 years (IQR: 1.20, 4.75 years), with 49.1% classified as juniors, 40.9% as young adults, and 10% as seniors. The majority of sampled animals were of mixed breeds (96.4% of cats and 62.4% of dogs) and had a normal body condition score (63.9% and 60.2%, respectively). Statistically significant differences were observed between dogs and cats regarding age and breed ($p < 0.001$).

Most caretakers mentioned that their dog or cat served solely as a companion animal (80.9% and 96.4%, respectively) and were fed a combination of commercial pet food and homemade food or leftovers (79.1% and 67.9%, respectively). Of the remaining animals, 10.9% of dogs and 27.4% of cats were solely fed commercial pet food, while 10% of dogs and 4.8% of cats were solely fed homemade foods or leftovers; none were exclusively fed raw meat. Approximately 42% of cat caretakers and 33.6% of dog caretakers reported not knowing or remembering whether their pet was ever vaccinated or which vaccines they had received. However, dogs had a significantly higher frequency of receiving at least one instance of vaccination (65.5%) compared to cats (50%, $p = 0.009$). Additionally, significant differences were observed between dogs and cats regarding their roles in the household and the type of food they received ($p < 0.05$).

Prevalence of *Leptospira* antibodies and potential risk factors

Out of the 194 animals tested, 23 were found to be seropositive for pathogenic *Leptospira* species. The seroprevalence in dogs (18.2%, 95% CI: 12.1-26.4%) was significantly higher than in cats (3.6%, 95% CI: 1.2-10.0%, $p = 0.002$). Fourteen out of the 20 seropositive dogs (70%, 95% CI: 48.1-85.5%) and all three seropositive cats (100%) reacted to *L. interrogans* serogroup Autumnalis. Two dog samples presented coagglutinations: one was seropositive for *L. interrogans* serogroup Canicola and *L. borgpetersenii* serogroup Ballum, while the other was seropositive for *L. interrogans* serogroups Canicola and Pyrogenes as well as for *L. borgpetersenii* serogroup Ballum. *L. interrogans* serogroups Icterohaemorrhagiae, Pomona and Pyrogenes, as well as *L. borgpetersenii* serogroup Tarassovi were the only reactive serogroups in four canine samples. The highest titer recorded was 1:200 for *L. interrogans* serogroup Autumnalis (in two samples); *L. borgpetersenii* serogroup Ballum, and *L. interrogans* serogroup Icterohaemorrhagiae (in one sample each), respectively. Notably, none of the sampled animals tested seropositive the other tested serogroups. All three seropositive cats had MAT titers of 1:100.

The low number of seropositive cats precluded us from fitting statistical models at the individual level to the available data, therefore, individual-level analyses were only conducted for 106 dogs with complete epidemiological data. The most parsimonious random intercept was the one including only the neighborhood of residence and it was used in the fit of all univariate and multivariate models. At this level, univariate binomial GLMMs revealed a significant association between seroprevalence in dogs and street access (p = 0.013), or unsupervised street access (p = 0.038, Table 2). Associations with contact with garbage dumps (p = 0.072) and with always seeing rodents in the household (p = 0.067) were not statistically significant but we considered that they were relevant enough to be included in the multivariate GLMM (Table 2). We fitted two multivariate binomial GLMMs containing as explanatory variables: sex, age group, contact with garbage dumps, frequency of rodent sight and either street access or unsupervised street access. The most parsimonious model was the one including street access, and manual *step-backwards* variable selection was performed from this model. The final model retained only street access as an explanatory variable. Based on the results from this model, dogs with street access were 4.85 times more likely to be infected than dogs without such access (95% CI: 1.39, 16.9). The model residuals met the assumptions of normality and homogeneity of variances. This model accounted for 22.0% of the observed variability (conditional R^2), with 14.9% being explained by the fixed effect term (marginal R^2).

At the census tract level, univariate Poisson GLMMs showed significant associations between the number of seropositive dogs and cats and higher levels of incidence of chronic poverty (*P* = 0.032), the % of households with at least one indicator of NBI (*P* = 0.008), and the % of households with poor housing conditions (*P* = 0.044). All socio-economic indicators presented high correlation coefficients, except for households with poor housing conditions (%) and households with regular garbage collection (%) (*r* = -0.453). Based on these results, we compared the performance of the four univariate models with a multivariate model including the additive effect of poor housing and regular garbage collection. Of these, the best-fit model was the one including the incidence of chronic poverty as an explanatory variable. In this model, animals living in census tracts with high/very high incidence of chronic poverty had 4 times (95% CI: 1.13, 14.4) more risk of being infected with pathogenic *Leptospira* species than those from census tracts with lower incidences. The residuals met the assumptions of normality and homogeneity of variances and there was no evidence of overdispersion or zero-inflation of the data. This model accounted for 23.4% of the observed variability (conditional R^2), with 13.7% being explained by the fixed effect term (marginal R^2).

Spatial analysis

Seropositive samples came from 19 census tracts within six of the eight administrative districts of the city, with no positive animals detected in the districts North and Center. The maps show that most of the seropositive animals are from areas of the city characterized by unfavorable socio-economic indicators and deficient access to basic sanitary services (Figs. 2 and 3).

The results of the global Moran's test indicate that there is no significant evidence of spatial autocorrelation in the number of seropositive animals per census tract (I = -0.025, P = 0.598). The results of the LISA revealed that the majority of the census tracts displayed non-significant spatial autocorrelation (Fig. 4). However, one small census tract exhibited negative spatial autocorrelation (Fig. 4). Furthermore, the results identified a spatial cluster of low values surrounded by areas with higher values (Fig. 4).

Discussion

The climatic and geographic characteristics of the city of Santa Fe, combined with high socioeconomic inequity, inadequate infrastructure and deficient access to basic sanitary services observed in the northern-western part of the city, as well as in neighborhoods and settlements near the riverbanks, create a favorable environment for the transmission of leptospirosis (Cristaldi et al., 2022; Reis et al., 2008; Ricardo et al., 2018). In fact, a recent study that considered socioeconomic and environmental determinants of leptospirosis based on expert knowledge showed that the suitability for human leptospirosis in the city of Santa Fe increases from downtown areas towards peri-urban and suburban areas (Cristaldi et al., 2022). However, only a few studies have addressed in Santa Fe the epidemiology of human (Cudós et al., 2014; Ricardo et al., 2018; Vanasco et al., 2008, 2000) and animal leptospirosis (Francois et al., 2020; Seghesso Zabala et al., 2013; Vanasco et al., 2003; Yaafar et al., 2019) and most of them were published more than ten years ago. In this context, our study brings new insights to the epidemiology of animal leptospirosis in an endemic area and can contribute to filling some knowledge gaps.

In our study, we observed a seroprevalence of leptospiral antibodies in asymptomatic dogs and cats of 18.2% and 3.6%, respectively. The seroprevalence found in dogs is consistent with a previous report from this area (Seghesso Zabala et al., 2013), as well as with estimators of seroprevalence at a global scale (18.5%; 95%CI: 15.8, 21.5%) and for Latin America and the Caribbean region (21%; 95% IC: 17, 25%) reported in a recent meta-analysis published by our research group (Ricardo et al., 2020). The seroprevalence found in cats was similar to the overall seroprevalence and to the seroprevalence of *L. interrogans* serogroup Autumnalis reported for asymptomatic cats from Rosario, a city that also belongs to the state of Santa Fe, Argentina by Francois et al. (Francois et al., 2020). However, it was lower than the seroprevalence found for asymptomatic cats from Latin America and the Caribbean (6.6 %; 95% CI: 4.2, 10.2 %) and for cats at a global scale (10.9 %, 95% IC: 7.8-15.1 %) reported in another meta-analysis from our research team (Ricardo et al., 2023). It should be noted that the cut-off titer of 1:100 used in our study is higher than the cut-off titer used in some of the studies reporting a higher prevalence of leptospiral antibodies in domiciled cats (Dickeson and Love, 1993; Francois et al., 2020; Holzapfel et al., 2021; Mylonakis et al., 2005; Shophet, 1979; Sprißler et al., 2019). Therefore, the interpretation of this parameter has to be taken with caution due to this caveat. Agreeing on the standard MAT cut-off titer and carrying out more serological studies of asymptomatic cats will help solve this drawback.

Most of the seropositive dogs and cats from our study were reactive to *L. interrogans* serogroup Autumnalis. This serogroup is thought to be maintained by rats (*Rattus* spp.), wild rodents, and opossums and there is also evidence of chronic infections in dogs (Boey et al., 2019; Grimm et al., 2020; Koizumi et al., 2020). Serogroup Autumnalis was previously detected in asymptomatic dogs and cats from Argentina (Francois et al., 2020), Chile (Azócar-Aedo et al., 2014), Colombia (Salazar et al., 2019) and Brazil (Azevedo et al., 2011; Miotto et al., 2018). There are reports of cross-reactions or coagglutination between *L. interrogans* serogroups Autumnalis, Pomona, Canicola, and Icterohaemorrhagiae (André-

Fontaine and Triger, 2018). However, none of the seropositive animals in our study exhibited coagglutinations between Autumnalis and any of the other tested serogroups, and all of them had not been vaccinated against leptospirosis in the previous six months. Additionally, of the 17 animals seropositive to *L. interrogans* serogroup Autumnalis, 64.7% had unsupervised street access, 35.3% had exposure to rodents in the household, 29.4% hunted rodents and/or wild mammals, and 23.5% had contact with livestock or poultry. Taking this into account, and considering that this serogroup is not included in any of the available dog vaccines, we believe that efforts should be directed toward promoting responsible pet care to prevent zoonotic spillover from wild small mammals to companion animals.

Most of the sampled dogs resided in households with yards (82.6%) and had either an outdoors-only (29.3%) or mixed indoors/outdoors (45.9%) lifestyle. The results of the logistic regression model indicated that dogs with street access are nearly four times more likely to be exposed to pathogenic leptospires than dogs confined indoors, regardless of whether their street access is supervised. These findings are in concordance with the results of our meta-analysis on canine leptospirosis, which identified the street as a risk factor for seropositivity (Ricardo et al., 2020). They are also consistent with recent epidemiological studies conducted elsewhere that found associations between outdoor exposure and the prevalence of *Leptospira* spp antibodies in dogs (Bernardino et al., 2021). However, it is important to note that this model explained only a limited amount of the observed variability, and it is probable that the variable "street access" may be masking other exposure factors not covered in our survey instrument. Additionally, responses to some questions regarding environmental exposure and contact with potential maintenance hosts may be influenced by recall bias or social acceptance bias.

At the spatial level, we did not find evidence of the existence of spatial clusters of seropositivity, but the number of seropositive animals was significantly associated with the incidence of chronic poverty. This may be explained by considering that all seropositive animals resided in urban or periurban slums and that the deficiencies in basic public services were similar among sampled census tracts. However, when considering more complex indicators, such as the NBI and the incidence of chronic poverty, there might appear small differences in the socioeconomic conditions of residents that impact the exposure of their domestic animals to environmental sources of infection (Reis et al., 2008). It should be noted that we had a small number of samples from neighborhoods with better socioeconomic indicators because most of the people who accessed municipal spaying/neutering campaigns live in impoverished neighborhoods or the outskirts of the city. Considering this, future research should be aimed at sampling dogs and cats from both public and private veterinary clinics to better capture the spatial and demographic gradient of exposure to pathogenic leptospires in house pets.

This study has some limitations worth noting. Firstly, the sample exhibits bias towards young adult animals, female dogs, and slum settlements. In some neighborhoods, participation in spaying/neutering campaigns was very low, despite the absence of nearby private veterinary clinics. Another limitation of the study was the omission of questions regarding household conditions in the survey instrument. This was done to reduce the time required to respond, but hindered the collection of relevant spatial-level data. Finally, although we used ten of the most frequently detected serogroups in dogs and cats based on the available literature (Ricardo et al., 2024), it is acknowledged that a larger antigen panel, including other serogroups and local isolates, would likely enhance the results of the serological analysis.

Despite the aforementioned limitations, we consider that our results make a significant contribution to the understanding of the epidemiology of animal leptospirosis in an area with a high incidence of annual human cases (López et al., 2019). Few studies in Argentina have explored the relationship between the seroprevalence of leptospirosis in domestic canines and felines and extensively detailed socioeconomic indicators to identify risk factors. Georeferencing the samples, also enhances precision and facilitates informed decision-making in environmental management. We consider that our findings may serve as a foundation for future epidemiological studies in this area, ideally employing a One Health approach to address the issue of urban leptospirosis. Moreover, we believe that our conclusions can be extrapolated to areas with similar environmental and socioeconomic conditions, either within Argentina or in other South American countries. Finally, we consider that our study can be valuable for both veterinary and public health practitioners, as well as decision-makers, to identify areas and animals with higher vulnerability to pathogenic leptospires. This information can aid in reinforcing prevention strategies to reduce exposure in dogs and cats and mitigate the potential transmission to humans.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Chat GPT-3.5 in order to check the grammar and spelling of some parts of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

References

André-Fontaine, G., Triger, L., 2018. MAT cross-reactions or vaccine cross-protection: retrospective study of 863 leptospirosis canine cases. Heliyon 4, e00869.

https://doi.org/10.1016/j.heliyon.2018.e00869

Azevedo, S.S. de, Fernandes, A.R.F., Queiroga, I.M.B.N., Alves, C.J., Morais, Z.M. de, Santos, C. de S.A.B., Vasconcellos, S.A., 2011. Ocorrência e fatores de risco associados à leptospirose em cães atendidos em hospital veterinário no semiárido paraibano. Braz. J. Vet. Res. Anim. Sci. 48, 161–166. https://doi.org/10.11606/S1413-95962011000200009

Azócar-Aedo, L., Monti, G., Jara, R., 2014. *Leptospira* spp. in Domestic Cats from Different Environments: Prevalence of Antibodies and Risk Factors Associated with the Seropositivity. Anim. Open Access J. MDPI 4, 612–626. https://doi.org/10.3390/ani4040612

Azócar-Aedo, L., Smits, H.L., Monti, G., 2014. Leptospirosis in dogs and cats: Epidemiology, clinical disease, zoonotic implications and prevention. Arch. Med. Vet. 46, 337–348. https://doi.org/10.4067/S0301-732X2014000300002

Bernardino, M.G.S., Costa, D.F., Nogueira, D.B., Silva, M.L.C.R., Silva, E.G., Carreiro, A.N., Alves, C.J., Azevedo, S.S., 2021. Cross-sectional survey for canine leptospirosis in an Atlantic Rainforest area of the semiarid of Paraíba state, Northeastern Brazil. Pesqui. Veterinária Bras. 41, e06640. https://doi.org/10.1590/1678-5150-PVB-6640

Boey, K., Shiokawa, K., Rajeev, S., 2019. *Leptospira* infection in rats: A literature review of global prevalence and distribution. PLoS Negl. Trop. Dis. 13, e0007499. https://doi.org/10.1371/journal.pntd.0007499

Burkholder, W.J., 2000. Use of body condition scores in clinical assessment of the provision of optimal nutrition. J. Am. Vet. Med. Assoc. 217, 650–654. https://doi.org/10.2460/javma.2000.217.650

Costa, F., Hagan, J.E.J.E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M.S.M.S., Stein, C., Abela-Ridder, B., Ko, A.I.A.I., 2015. Global Morbidity and Mortality of Leptospirosis: A Systematic Review. PLoS Negl. Trop. Dis. 9, e0003898. https://doi.org/10.1371/journal.pntd.0003898

Cristaldi, M.A., Catry, T., Pottier, A., Herbreteau, V., Roux, E., Jacob, P., Previtali, M.A., 2022. Determining the spatial distribution of environmental and socio-economic suitability for human leptospirosis in the face of limited epidemiological data. Infect. Dis. Poverty 11, 86. https://doi.org/10.1186/s40249-022-01010-x

Cudós, M.C., Landolt, N., Jacob, P., Schmeling, M.F., Chiani, Y., Brazza, S., Celora, A.G., Anchart, E., Uboldi, M.A., Vanasco, N.B., 2014. Vigilancia Intensificada De Leptospirosis En Santa Fe Y Entre Ríos (2012-2013). Rev Argent Salud Pública 5, 24–30.

Dickeson, D., Love, D.N., 1993. A serological survey of dogs, cats and horses in south-eastern Australia for leptospiral antibodies. Aust. Vet. J. 70, 389–390. https://doi.org/10.1111/j.1751-0813.1993.tb00823.x

Ellis, W.A., 2015. Animal Leptospirosis, in: Adler, B. (Ed.), Current Topics in Microbiology and Immunology. Springer Verlag, pp. 99–137. https://doi.org/10.1007/978-3-662-45059-8_6

Francois, S., Poli, G., Yaafar, N., Prado, N., Adrien-Rüeger, M., Gorordo, M., 2020. Estudio serológico de la infección por *Leptospira* spp. en gatos (*Felis silvestris catus*) en el sur de la provincia de Santa Fe, Argentina. Clin Infecto Vet 6, 2–9.

Grimm, K., Rivera, N.A., Fredebaugh-Siller, S., Weng, H.-Y., Warner, R.E., Maddox, C.W., Mateus-Pinilla, N.E., 2020. Evidence of *Leptospira* serovars in wildlife and leptospiral DNA in water sources in a natural area in east-central Illinois, USA. J. Wildl. Dis. 56, 316–327.

Hartmann, K., Egberink, H., Pennisi, M.G., Lloret, A., Addie, D., Belák, S., Boucraut-Baralon, C., Frymus, T., Gruffydd-Jones, T., Hosie, M.J., Lutz, H., Marsilio, F., Möstl, K., Radford, A.D., Thiry, E., Truyen, U., Horzinek, M.C., 2013. *Leptospira* Species Infection in Cats: ABCD guidelines on prevention and management. J. Feline Med. Surg. 15, 576–581. https://doi.org/10.1177/1098612X13489217

Harvey, N.D., 2021. How Old Is My Dog? Identification of Rational Age Groupings in Pet Dogs Based Upon Normative Age-Linked Processes. Front. Vet. Sci. 8, 643085. https://doi.org/10.3389/fvets.2021.643085

Holzapfel, M., Taraveau, F., Djelouadji, Z., 2021. Serological and molecular detection of pathogenic *Leptospira* in domestic and stray cats on Reunion Island, French Indies. Epidemiol. Infect. 149, e229. https://doi.org/10.1017/S095026882100176X

Koizumi, N., Izumiya, H., Ohnishi, M., 2020. Genetic relatedness of *Leptospira interrogans* serogroup Autumnalis isolated from humans, dogs, and mice in Japan. BMC Res. Notes 13, 369. https://doi.org/10.1186/s13104-020-05211-1

López, M.S., Müller, G.V., Lovino, M.A., Gómez, A.A., Sione, W.F., Aragonés Pomares, L., 2019. Spatio-temporal analysis of leptospirosis incidence and its relationship with hydroclimatic indicators in northeastern Argentina. Sci. Total Environ. 694, 133651. https://doi.org/10.1016/j.scitotenv.2019.133651

Lüdecke, D., Makowski, D., Ben-Shachar, M.S., Patil, I., Waggoner, P., Wiernik, B.M., Thériault, R., Arel-Bundock, V., Jullum, M., gjo11, Bacher, E., 2024. performance: Assessment of Regression Models Performance.

Miotto, B.A., Guilloux, A.G.A., Tozzi, B.F., Moreno, L.Z., da Hora, A.S., Dias, R.A., Heinemann, M.B., Moreno, A.M., Filho, A.F. de S., Lilenbaum, W., Hagiwara, M.K., 2018. Prospective study of canine leptospirosis in shelter and stray dog populations:

Identification of chronic carriers and different *Leptospira* species infecting dogs. PloS One 13, e0200384. https://doi.org/10.1371/journal.pone.0200384

Moraga, P., 2023. Spatial Statistics for Data Science: Theory and Practice with R. Chapman & Hall/CRC.

Mwachui, M.A., Crump, L., Hartskeerl, R., Zinsstag, J., Hattendorf, J., 2015. Environmental and Behavioural Determinants of Leptospirosis Transmission: A Systematic Review. PLoS Negl. Trop. Dis. 9, e0003843. https://doi.org/10.1371/journal.pntd.0003843

Mylonakis, M.E., Bourtzi-Hatzopoulou, E., Koutinas, A.F., Petridou, E., Saridomichelakis, M.N., Leontides, L., Siochu, A., 2005. Leptospiral seroepidemiology in a feline hospital population in Greece. Vet. Rec. 156, 615–616. https://doi.org/10.1136/vr.156.19.615

Reis, R.B., Ribeiro, G.S., Felzemburgh, R.D.M., Santana, F.S., Mohr, S., Melendez, A.X.T.O., Queiroz, A., Santos, A.C., Ravines, R.R., Tassinari, W.S., Carvalho, M.S., Reis, M.G., Ko, A.I., 2008. Impact of Environment and Social Gradient on *Leptospira* Infection in Urban Slums. PLoS Negl. Trop. Dis. 2, e228. https://doi.org/10.1371/journal.pntd.0000228

Ricardo, T., Azócar-Aedo, L.I., Previtali, M.A., Monti, G., 2024. Seroprevalence of pathogenic *Leptospira* serogroups in asymptomatic domestic dogs and cats: systematic review and meta-analysis. Frontiers in Veterinary Science 11.

Ricardo, T., Azócar-Aedo, L., Signorini, M., Previtali, M., 2023. Leptospiral infection in domestic cats: Systematic review with meta-analysis. Prev. Vet. Med. 212, 105851. https://doi.org/10.1016/j.prevetmed.2023.105851

Ricardo, T., Bergero, L.C., Bulgarella, E.P., Previtali, M.A., 2018. Knowledge, attitudes and practices (KAP) regarding leptospirosis among residents of riverside settlements of Santa Fe, Argentina. PLoS Negl. Trop. Dis. 12, e0006470.

https://doi.org/10.1371/journal.pntd.0006470

Ricardo, T., Previtali, M.A.A., Signorini, M., 2020. Meta-analysis of risk factors for canine leptospirosis. Prev. Vet. Med. 181, 105037. https://doi.org/10.1016/j.prevetmed.2020.105037

Salazar, L.L.C., Diez, L.C.L., Nodarse, R.S., Gonzalez, W.S., Henao, E., Angel, M.O., 2019. Prevalencia de presentación de algunos agentes zoonóticos transmitidos por caninos y felinos en Medellín, Colombia. Rev. MVZ Córdoba 24, 7119–7126. https://doi.org/10.21897/rmvz.1524

Seghesso Zabala, A., Anthony Omezzolli, L.M., Poli Lovagnini, G., Francois Barbagelata, S., 2013. Seropositividad a *Leptospira interrogans* en perros de la ciudad de Rosario, Argentina. Rev. Cubana Med. Trop. 65, 185–190.

Servicio Meteorológico Nacional, 2024. Atlas Climático [WWW Document]. URL https://www.smn.gob.ar/clima/atlasclimatico (accessed 2.23.24).

Shophet, R., 1979. A serological survey of leptospirosis in cats. N. Z. Vet. J. 27, 236, 245–246. https://doi.org/10.1080/00480169.1979.34662

Sjoberg, D.D., Whiting, K., Curry, M., Lavery, J.A., Larmarange, J., 2021. Reproducible Summary Tables with the *gtsummary* Package. R J. 13, 570–580. https://doi.org/10.32614/RJ-2021-053

Sprißler, F., Jongwattanapisan, P., Luengyosluechakul, S., Pusoonthornthum, R., Prapasarakul, N., Kurilung, A., Goris, M., Ahmed, A., Reese, S., Bergmann, M., Dorsch, R., Klaasen, H.L.B.M., Hartmann, K., 2019. *Leptospira* infection and shedding in cats in Thailand. Transbound. Emerg. Dis. 66, 948–956. https://doi.org/10.1111/tbed.13110

Sykes, J.E., Francey, T., Schuller, S., Stoddard, R.A., Cowgill, L.D., Moore, G.E., 2023. 2023 Updated ACVIM consensus statement on leptospirosis in dogs. J. Vet. Intern. Med.

jvim.16903. https://doi.org/10.1111/jvim.16903

Trabattoni, E, Ducommun, M, 2018. Conocimiento de la población canina y felina como punto de partida de los programas de vacunación antirrábica, Vet Comunicaciones.

Vanasco, N.B., Schmeling, M.F., Lottersberger, J., Costa, F., Ko, A.I., Tarabla, H.D., 2008. Clinical characteristics and risk factors of human leptospirosis in Argentina (1999-2005). Acta Trop. 107, 255–258. https://doi.org/10.1016/j.actatropica.2008.06.007

Vanasco, N.B., Sequeira, G., Dalla Fontana, M.L., Fusco, S., Sequeira, M.D., Enría, D., 2000. [Report on a leptospirosis outbreak in the city of Santa Fe, Argentina, March-April 1998]. Rev. Panam. Salud Publica Pan Am. J. Public Health 7, 35–40. https://doi.org/10.1590/s1020-49892000000100006

Vanasco, N.B., Sequeira, M.D., Sequeira, G., Tarabla, H.D., 2003. Associations between leptospiral infection and seropositivity in rodents and environmental characteristics in Argentina. Prev. Vet. Med. 60, 227–235. https://doi.org/10.1016/s0167-5877(03)00144-2

Vogt, A.H., Rodan, I., Brown, M., Brown, S., Buffington, C.A.T., Forman, M.J.L., Neilson, J., Sparkes, A., 2010. AAFP-AAHA: Feline Life Stage Guidelines. J. Feline Med. Surg. 12, 43–54. https://doi.org/10.1016/j.jfms.2009.12.006

Yaafar, N., Prado, M.A., Favot, N., Poli, G.L., Sarradell, J.E., Anthony, L.M., Francois, S.E., 2019. Possible clinical leptospirosis in two cats (*Felis silvestris catus*) from the south of the Santa Fe province.

Acknowledgements

The authors would like to thank María Pilar Ponse MV, Luciano Busaniche MV, Patricio Ramos MV, and all the other veterinarians and veterinary assistants from IMuSA, as well as the veterinary students that collaborated with the sampling. We also would like to thank all the caretakers of dogs and cats involved in the fieldwork for their invaluable help in sampling the animals included in this study.

Funding

This research was funded by the National Agency for Scientific and Technological Promotion (ANPCYT), ANPCyT awarded to M. Andrea Previtali (PICT 2017-4280), and by the National Council of Scientific and Technical Research (CONICET). The funders had no role in study design, data collection, andanalysis, decision to publish, or preparation of the manuscript.

Conflicts of interest

None of the authors have a conflict of interest to disclose.

Figure captions

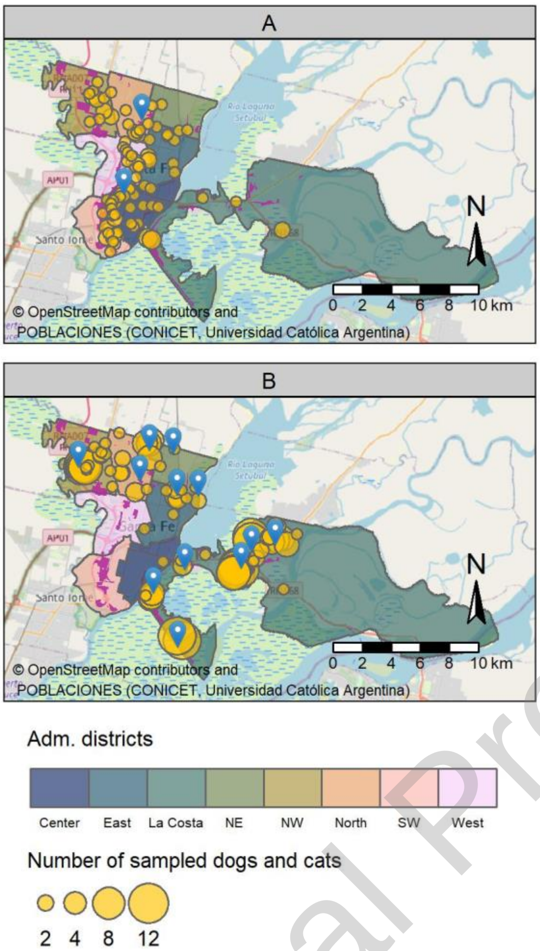


Figure 1. Map of the study area showing (A) the origin of animals sampled at the fixed veterinary care units or (B) the origin of animals sampled at the mobile sterilization units. The shading on the map indicates the location of each administrative district. Magenta polygons highlight the presence of urban slums and informal settlements. The diameter of the yellow bubbles represents the number of dogs and cats sampled per census tract. Municipal sterilization units are shown as blue pin icons.

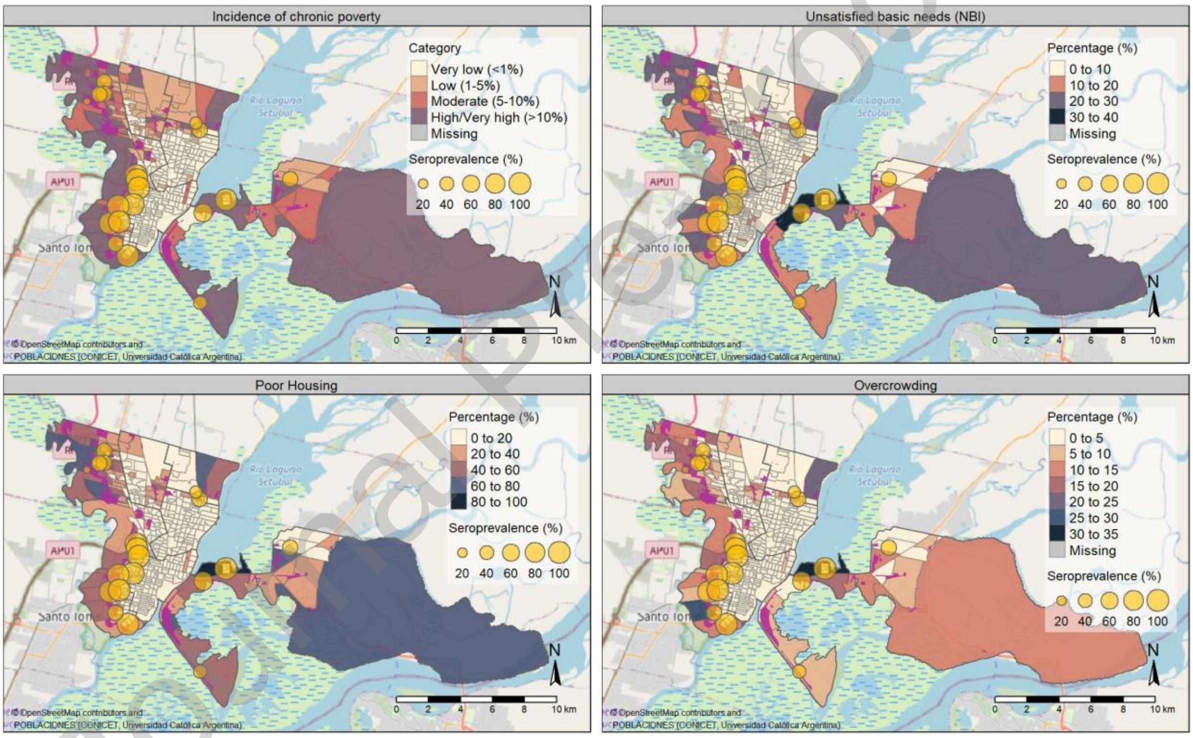


Figure 2. Choropleth maps of the distribution of seropositive samples according to socioeconomic indicators. Map shading indicates the incidence level of chronic poverty or the proportion (%) of households with the selected indicator at the census tract level. Magenta polygons indicate the placement of urban slums and informal settlements. The diameter of the yellow bubbles indicates the seroprevalence per census tract.

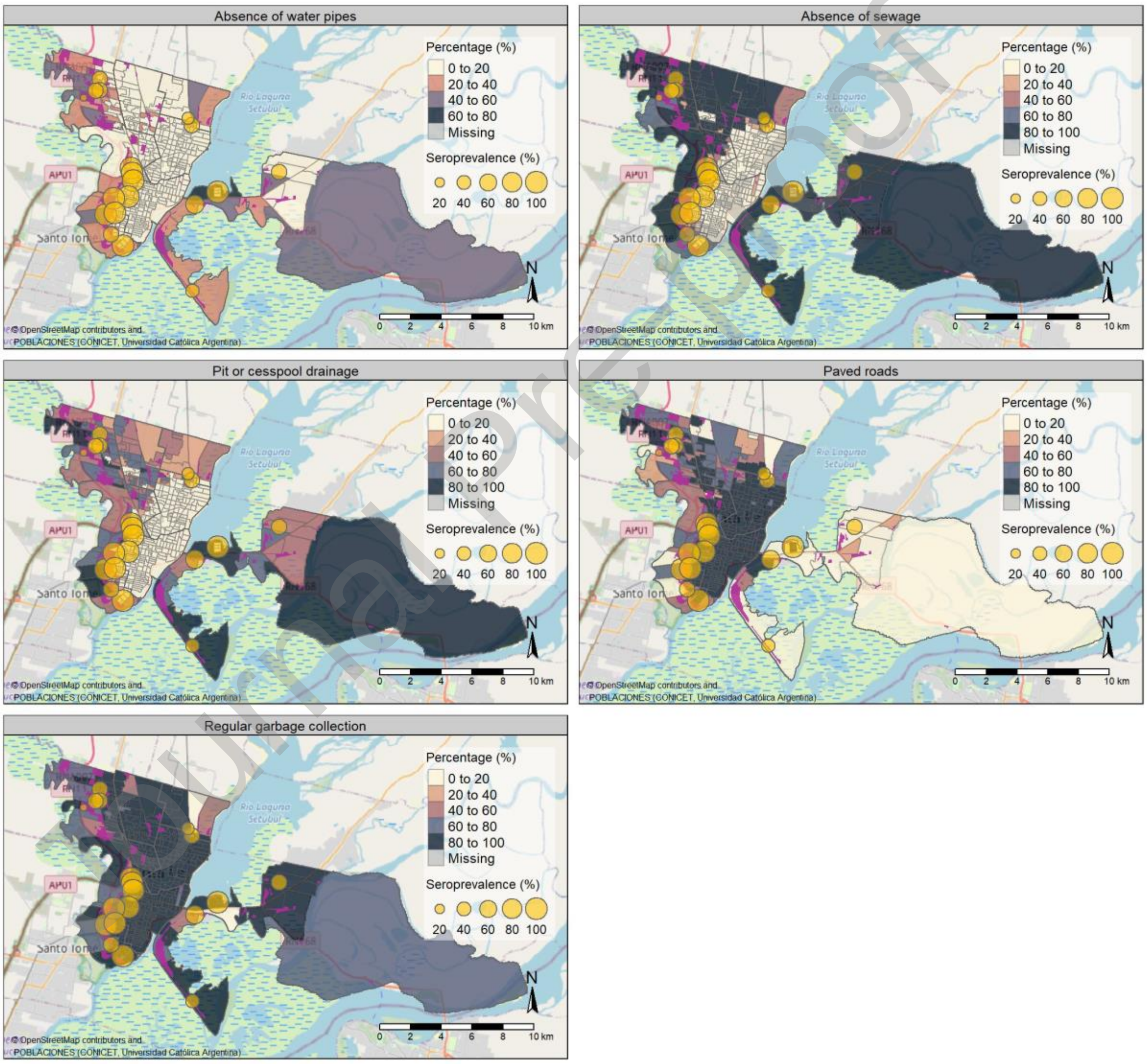


Figure 3. Choropleth maps of the distribution of seropositive samples according to access of basic sanitary services. Map shading indicates the incidence level of chronic poverty or the proportion (%) of households with the selected indicator at the census tract level. Magenta polygons indicate the placement of urban slums and informal settlements. The diameter of the yellow bubbles indicates the seroprevalence per census tract.

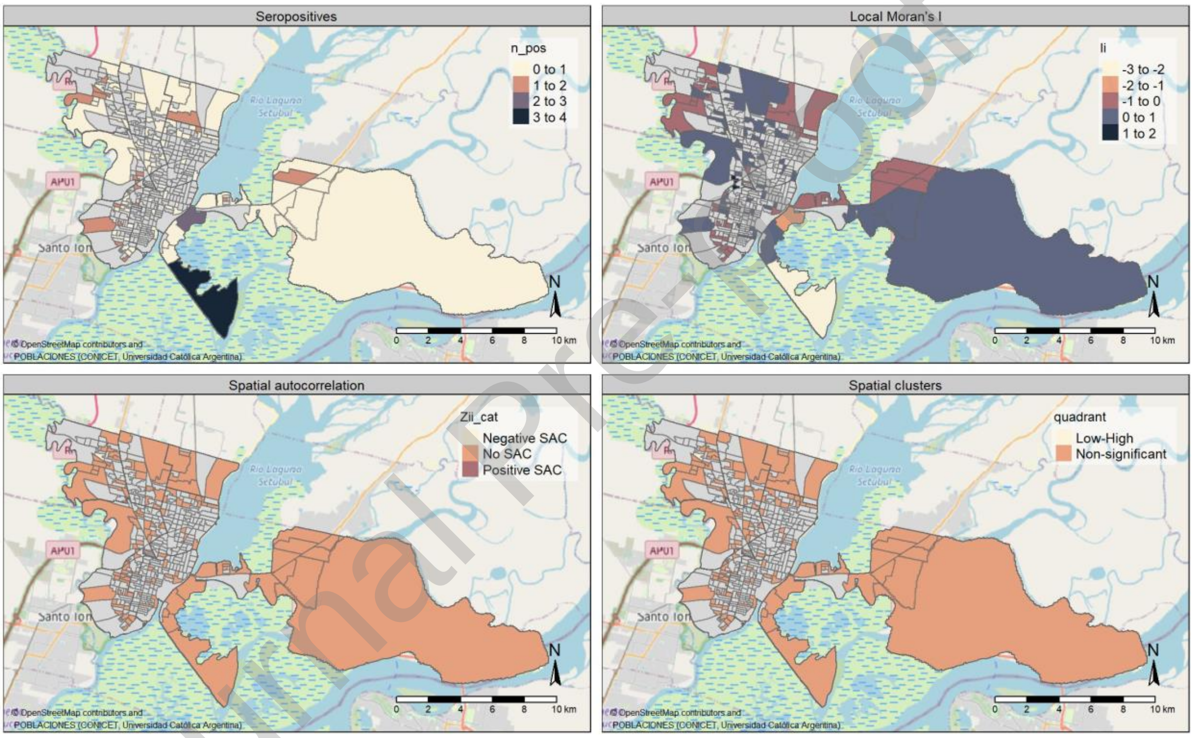


Figure 4. Results of the LISA analysis. (A) Seroprevalence per census tract; (B) raw I_i test values; (C) statistical significance of I_i test; (D) spatial clusters detected. The gray shading represents census tracts with no samples collected.

Supplementary information

File S1. Questionnaire implemented in the study [English] (PDF).

File S2. Informative flier given to the dogs or cats caregivers [Spanish] (PDF).

File S3. Informed consent form [Spanish] (PDF).

Table 1. Serovar panel used in the microagglutination test (MAT) for detecting *Leptospira* antibodies in asymptomatic dogs and cats, Santa Fe, Argentina (2022).

Species	Serogroup	Serovar	Reference strain
L. interrogans	Australis	Australis	Ballico
	Autumnalis	Autumnalis	Akiyami A
	Canicola	Canicola	Hond Utrecht IV
	Icterohaemorrhagiae	Copenhageni	M20
	Sejroe	Hardjo	Hardjoprajitno
	Pomona	Pomona	Pomona
	Pyrogenes	Pyrogenes	Salinem
L. borgpetersenii	Ballum	Castellonis	Castellón 3

	Tarassovi	Tarassovi	Perepeletsin
L. kirschneri	Grippotyphosa	Grippotyphosa	Moskva V

Table 2. Results of the univariate binomial GLMMs to assess potential predictors of seropositivity in domestic dogs using neighborhood of residence as a random intercept, Santa Fe, Argentina (2022).

Characteristic	**OR**	**95% CI**[1]	***p*-value**
Sex			
Female	—	—	
Male	1.08	0.38, 3.13	0.9
Age (years)	0.93	0.72, 1.20	0.6
Age category			
Junior	—	—	
Adult	0.68	0.23, 2.05	0.5
Senior	0.40	0.04, 4.00	0.4
BCS			
Very thin/thin	—	—	
Normal	0.93	0.24, 3.54	>0.9
Overweight/obese	1.56	0.31, 7.94	0.6
Role in the household			
Companion	—	—	
Companion and hunting/guarding	0.25	0.03, 2.11	0.2
Hunting/guarding	4.43	0.23, 86.0	0.3
Type of feeding			
Commercial pet food	—	—	
Pet food and homemade/leftovers	2.16	0.25, 18.9	0.5
Homemade/leftovers	6.65	0.56, 78.8	0.13

Characteristic	OR	95% CI[1]	p-value
Street access	4.85	1.39, 16.9	0.013**
Unsupervised street access	3.21	1.07, 9.63	0.038**
Hunting behavior	0.96	0.27, 3.45	>0.9
Hunting: rodents	1.19	0.22, 6.54	0.8
Hunting: wild animals	5.02	0.51, 49.7	0.2
Exposure to dumpyards	2.83	0.91, 8.81	0.072*
Exposure to environmental water/mud	0.78	0.26, 2.36	0.7
Contact with other dogs	0.70	0.06, 8.01	0.8
Contact with cats	0.88	0.31, 2.51	0.8
Contact with other animals	0.55	0.14, 2.16	0.4
Contact with own dogs/cats	0.97	0.18, 5.33	>0.9
Contact with neighbor dogs/cats	1.43	0.50, 4.06	0.5
Contact with stray dogs/cats	2.79	0.73, 10.6	0.13
Number of owned dogs/cats			
1-2 dogs/cats	—	—	
2-3 dogs/cats	1.28	0.25, 6.43	0.8
3-6 dogs/cats	1.33	0.38, 4.70	0.7
6+ dogs/cats	1.03	0.21, 4.95	>0.9
Rodent sightings	1.26	0.44, 3.60	0.7
Frequency of rodent sightings			
Never	—	—	

Characteristic	OR	95% CI[1]	p-value
Occasionally	0.85	0.25, 2.87	0.8
Always	4.53	0.90, 22.8	0.067*
Presence of a yard	1.90	0.37, 9.83	0.4
Type of housing			
Inside	—	—	
Inside and outside	0.80	0.24, 2.70	0.7
Outside	0.55	0.13, 2.32	0.4
Frequency of cleaning the kennel/bed			
Daily	—	—	
Every other day	0.67	0.12, 3.65	0.6
Weekly	2.65	0.69, 10.1	0.2
Occasionally	0.55	0.06, 4.94	0.6

[1]CI = Confidence Interval; ** $p \leq 0.05$; * $p \leq 0.10$

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- The higher *Leptospira* seroprevalence in household dogs with street access provides insight for the development of targeted public health interventions aimed at enhancing leptospirosis vaccination coverage in vulnerable areas.

- The study results provide essential data for formulating veterinary health guidelines. These guidelines can serve as a basis for promoting responsible pet ownership practices, ultimately reducing the incidence of infection with pathogenic *Leptospira* in pets and minimizing the potential for human-animal transmission.
- Understanding the spatial distribution of pets seropositive to *Leptospira* within a specific region informs future research directions and helps refine surveillance and monitoring strategies for zoonotic diseases, potentially preventing localized outbreaks.