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Evaluation of pathogens with zoonotic potential in dogs and cats of the Metropolitan District of Quito

Tesis en torno a una hipótesis o problema de investigación y su contrastación

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Abstract

Reports of zoonotic pathogens associated with gastrointestinal disorders transmitted by dogs and cats in Ecuador are scarce. In this study, we investigated the prevalence of *Campylobacter* spp., *Giardia lamblia*, and *Cryptosporidium parvum* in 349 animals (237 dogs and 112 felines). Fecal samples were collected from the animals during spay and neuter campaigns carried out in marginal neighborhoods of the Metropolitan District of Quito. Owners were asked to answer a short survey to gather demographic, health, and lifestyle information about the animals. For the identification of *Campylobacter* spp., fecal samples were analyzed using the loop-mediated isothermal amplification technique (LAMP), which is an isothermal reaction that makes use of 4 to 6 primers specially designed for the recognition of 8 different regions of the target DNA, combined with multiplex PCR for the identification of different species. For the identification of *G. lamblia* and *C. parvum*, an immunochromatographic test was used, which identifies *C. parvum* oocysts and *G. lamblia* antigens. Binomial regressions were used to assess the relationship between the presence of pathogens and a set of demographic, health, and lifestyle variables. The prevalence of *Campylobacter* was 30.6%. among the *Campylobacter* species, *C. jejuni* had the highest prevalence in dogs (13.5%) and cats (8.03%), followed by *C. coli* (5.9% dogs and 5.3%, cats), *C. upsaliensis* (2.1% dogs and 3.5% cats), and *C. lari* (1.2% dogs). In the binomial regression model age, cohabitation with dogs and cats, and consumption of commercial food had a significant effect on the prevalence of *Campylobacter* spp. Meanwhile, the prevalence of *G. lamblia* was low (8.6%). The binomial regression model showed that consumption of homemade food had a significant effect on the prevalence of this parasite. *C. parvum* was not detected in any sample. Overall, the results evidence that dogs and cats can be sources of transmission of pathogens to

humans and other animals, pointing to the importance of changing lifestyle variables to reduce the prevalence of *Campylobacter* and *Giardia* pathogens.

Keywords: Dogs, Cats, *Campylobacter* spp., *Giardia lamblia*, *Cryptosporidium parvum*, fecal samples, risk factors, Quito

Resumen

Los reportes de patógenos zoonóticos asociados con trastornos gastrointestinales transmitidos por perros y gatos en Ecuador son escasos. En este estudio investigamos la prevalencia de *Campylobacter* spp., *Cryptosporidium parvum* y *Giardia lamblia* en 349 animales (237 perros y 112 gatos). Se recolectaron muestras de heces de los animales durante campañas de esterilización llevadas a cabo en el Distrito Metropolitano de Quito. Se pidió a los propietarios que respondieran a una breve encuesta para recabar información demográfica, sanitaria y sobre el estilo de vida de los animales. Para la identificación de *Campylobacter* spp., las muestras fecales se analizaron mediante la técnica de amplificación isotérmica mediada por bucle (LAMP), que hace uso de 4 a 6 cebadores especialmente diseñados para el reconocimiento de 8 regiones diferentes del ADN diana, combinada con PCR multiplex para la identificación de diferentes especies. Para la identificación de *G. lamblia* y *C. parvum* se utilizó una prueba inmunocromatográfica que identifica los antígenos de ooquistes de *C. parvum* y de *G. lamblia*. Se emplearon regresiones binomiales para evaluar la relación entre la presencia de patógenos y un conjunto de variables demográficas, de salud y de estilo de vida. La prevalencia de *Campylobacter* fue del 30.6%. Entre las especies de *Campylobacter, C. jejuni* tuvo la mayor prevalencia en perros (13.5%) y gatos (8.03%), seguida de *C. coli* (5,9% perros y 5,3% gatos), *C. upsaliensis* (2,1% perros y 3,5% gatos) y *C. lari* (1,2% perros). En el modelo de regresión binomial, la edad, la convivencia con perros y gatos, y el consumo de una dieta comercial (pellets) tuvieron un efecto significativo en la prevalencia de *Campylobacter* spp. Respecto a *Giardia lamblia*, que tuvo una prevalencia baja (8.6%), la alimentación casera se relacionó significativa y positivamente con la presencia de este parásito. No se detectó *C. parvum* en las muestras. En general, los resultados evidencian que los perros y gatos pueden ser fuentes de transmisión de patógenos a humanos y otros animales.

Nuestros resultados también destacan la necesidad de comprender mejor cómo los patrones de estilo de vida podrían cambiar para reducir la prevalencia de los patógenos *Campylobacter* y *Giardia*.

Palabras-clave: Perros, Gatos, *Campylobacter* spp., *Giardia lamblia*, *Cryptosporidium parvum*, muestras fecales, Quito

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Introduction

The close contact that humans have with companion animals can lead to the transmission of bacteria and parasites that could cause gastrointestinal disorders, most of which are self-limiting but sometimes could affect children, the elderly, and immuno-compromised individuals (LaLonde-Paul et al., 2019; Leahy et al., 2017). Health problems related to these pathogens can also be considered occupational diseases for professionals working with companion animals, especially veterinarians (Bouzid et al., 2015; Torkan et al., 2018).

Campylobacter species stand out among other zoonotic intestinal pathogens because of their worldwide distribution. Some species of this genus represent the main causes of most prevalent gastrointestinal disorders, especially in developing countries, like Ecuador (Vinueza-Burgos et al., 2017). Nonetheless, most *Campylobacter* species constitute the commensal microbiota in the intestinal mucosa of mammals and birds (Facciolà et al., 2017). Humans can become infected with pathogenic species through the consumption of food contaminated with feces from infected animals or by direct contact with domestic animals, such as dogs and cats (Bojanić et al., 2017). This poses a particular risk to people with weakened immune systems, as they may experience prolonged diarrhea if infected (Heyworth, 2014). One of the effects of *Campylobacter* infections is the Guillain-Barré syndrome, an autoimmune reaction in the peripheral nervous system that destroys axons' myelin in peripheral nerves, causing a deficiency in nerve signals transmission to the muscles (Nguyen & Taylor, 2022). This neuropathy is progressive and could lead to a respiratory condition that can escalate or even cause death (Scallan Walter et al., 2020).

Other zoonotic intestinal pathogens are protozoa, such as *Giardia* and *Cryptosporidium,* which can cause acute gastrointestinal disorders in humans and animals (Navone et al., 2017). Since 2006, the World Health Organization (WHO) included parasitic diseases caused by *Giardia and Cryptosporidium* as neglected diseases due to the negative impact they have on the populations of developing countries (Bartelt & Platts-Mills, 2016).

The genus *Giardia* belongs to the order *Diplomonadida*. This flagellate protozoan is widely distributed worldwide, and could infect several species of animals, including birds, reptiles, and mammals, leading to intestinal diseases Transmission occurs through the consumption of water and food contaminated with *Giardia* cysts shed in animal feces, with the notable characteristic that the infective dose is low, corresponding to 10 cysts (Dunn & Juergens, 2022; Rumsey & Waseem, 2022). Recently, contact with pets, such as dogs and cats, has also been associated as a risk factor in the transmission of this pathogen to humans (Merigueti et al., 2022; Stull et al., 2015).

Clinical signs of *Giardia*sis include abdominal pain, flatulence, and diarrhea, which may become chronic, along with severe dehydration and long-term complications, especially in children, where malnutrition and reduced IQ may occur (Dupont, 2009; Fekete et al., 2021; Halliez & Buret, 2013; Lemos et al., 2021; OMS, 2022). However, the disease can also occur asymptomatically, contributing significantly to its spread, with asymptomatic infections estimated to range from 5% to 76% (Waldram et al., 2017).

Giardia species are generally considered species-specific; however, certain species, such as *Giardia lamblia,* have eight genotypes with zoonotic potential, capable of affecting humans, dogs, cats, cattle, rodents, and wildlife (Piekara-Stępińska et al., 2021). Due to the close contact between dogs, cats, and humans, these animals are increasingly involved in the transmission of this zoonotic pathogen. Genotypes A and B are considered zoonotic, but there are also dog-specific

assemblages, C and D; assemblage F is found in cats (Barbosa et al., 2023; Cai et al., 2021). The detection of A and B assemblages in fecal samples from dogs and cats that have a home life and low contact with other animals may be attributed to coprophagy and contact with humans (Barbosa et al., 2023). Type A assemblages have been found in both humans and dogs, indicating that pets may become significant sources of infection for this protozoan, posing a public health risk (Godínez-Galaz et al., 2019).

Infections with *G. lamblia* in companion animals have been documented in various countries worldwide, in animals kept in shelters, stray animals, and working animals, exhibiting high prevalence rates. This may be attributed to poor hygienic conditions in their environment, the age of the animals, or a weak immune system (Sun et al., 2023). Age has been identified as a crucial factor, with prevalence being higher in young animals and decreasing with increasing age. However, the zoonotic potential increases with the age of the animal, and the risk is higher as animals may or may not show clinical signs of the disease, depending on their immune status (Sun et al., 2023). Environmental contamination in recreational parks, combined with a high animal population density, predisposes to infection (Godínez-Galaz et al., 2019). It has been reported that infections with a mixture of different genotypes may occur in both animals and humans, suggesting possible transmission of *G. lamblia* species between humans and pets (Agresti et al., 2022).

Likewise, *Cryptosporidium* is a genus of enteric protozoans with a worldwide distribution. They are the main agent of diarrhea in children and a major cause of mortality in the world, with an estimated rate of 10-15% in developing countries (Janssen & Snowden, 2022). Transmission typically occurs through the consumption of contaminated water or fecal particles in food. The infective dose is low, with 10-25 cysts being sufficient to cause infection (Alseady et al., 2023; Ayana, 2023).

Outbreaks caused by *Cryptosporidium* spp. in the population through consumption of contaminated water and contact with infected animals have been reported in different countries (Ahmed & Karanis, 2020). It has been reported that 20 species of *Cryptosporidium* can affect humans, but the majority of infections is caused by human-specific *C. hominis*, which has anthropogenic transmission occurring in day care centers, schools, and nursing homes (McKerr et al., 2022; Ryan et al., 2014)). On the other hand, *C. parvum*, which possesses a zoonotic character, has also been reported to cause outbreaks (Murnik et al., 2022). In addition, *C. canis* and *C. felis* have been found in both immunocompromised and immunocompetent patients worldwide (Lucio-Forster et al., 2010; The ANOFEL Cryptosporidium National Network, 2010). *C. canis* is common in patients that have dogs; for example, Xiao et al., (2007), detected this species in infants in Peru who lived with a dog infected with *C. canis*, exhibiting gastrointestinal signs. Both, *C. canis* and *C. felis* are mostly reported in developing countries, whereas in European countries, the prevalence is usually higher for *C. parvum* and *C. hominis* (Chalmers et al., 2009; Xiao, 2010).

In Ecuador, the prevalence of these zoonotic intestinal pathogens has been studied in some rural areas. To facilitate the comparison among studies, in the following review we present the sample sizes of each study since they varied widely. Atherton et al. (2013) identified the presence of *Giardia lamblia* by ELISA and PCR in 26% of the fecal samples from people of a rural community in the province of Esmeraldas (n=592). However, this study did not include samples from animals. Meanwhile, Gingrich et al. (2010) analyzed 97 dog fecal samples in three areas of the Galapagos Islands and reported the presence of *Giardia* spp. (5.2%), *Cryptosporidium* spp. (1%), *Toxocara canis* (16.5%), *Isospora canis* (4.1%), *Ancylostoma caninum* (57.7%), and *Sarcocystis canis* (4.1%), raising concern about the risk of zoonotic transmission to the inhabitants of the archipelago. Vasco et al. (2016) evaluated the presence of zoonotic enteropathogens in stool

samples from 64 asymptomatic children and 203 domestic animals of 62 households in Yaruquí, a semirural community in the Quito district, between June and August 2014. The authors detected *Campylobacter jejuni* (30.7%), *C. coli* (11.6%), *Giardia lamblia* (13.1%), and *Cryptosporidium parvum* (1.1%) in the feces of children (47% females and 53% males). In the feces of dogs (n=40), *C. jejuni* was found in 25% (10/40), *C. coli* in 2.5% (1/40), and *G. lamblia* in 12.5% (5/40). In cats (n=6), *C. jejuni* was detected in 33.3% (2/6) and *C. coli* in 16.7% (1/6). No positive samples were obtained for protozoa such as *G. lamblia* and *C. parvum*. In a study in the area of Loja, in southern Ecuador, Toledo et al. (2018) identified different *Campylobacter* species in 250 fecal samples of dogs, cows, pigs, and chickens . They reported a prevalence of *C. jejuni* in 78.6% of the samples, followed by *C. coli* (21.4%), where 10 isolates were resistant to multiple antibiotics. Nonetheless, this study did not include cats, which also represent a risk of contagion as noted by Sandberg et al. (2002). *Giardia* and other parasites were also reported in different species of domestic animals (cattle, sheep, horses, donkeys, lamas, pigs, rabbits, chickens, guinea pigs, and dogs), in the rural community of San Andrés in the province of Chimborazo by González-Ramirez et al. (2021). However, once again, domestic cats were not included in their sample. It is important to mention that most of these studies did not assess the risk factors for pathogens' prevalence in dogs or cats, which can significantly affect pathogens' prevalence in the human population. Understanding such risk factors forcompanion animals could help to define effective strategies to control zoonoses.

In a large urban area like Quito, with 2,679.722 inhabitants (INEC, 2023), the risk of zoonotic transmission is expected to be high, especially in marginalized and low-income neighborhoods, where financial constraints hinder access to veterinary care for animal owners. Companion animals in these areas are often allowed to roam freely, scavenging for food, and hunting native wildlife. Although previous studies elsewhere have found that these behaviors could

increase parasite transmission within and between species (Curi et al., 2017; Karama et al., 2019), the risk factors, routes of transmission, and epidemiology have not yet been determined in Quito.

In this context, the present study aims to report the prevalence of these pathogens in dogs and cats from marginal, low-income neighborhoods in Quito, and to assess environmental, health, and lifestyle risk factors associated with this prevalence.

Methodology

Sample collection

The sample size was calculated based on an infinite population because current information on the number of dogs and cats in the Metropolitan District of Quito is scarce (Cárdenas et al. 2021). The estimated sample size needed to produce a 95% confidence interval with a 5% sampling error was 384. However, due to limited accessibility to neutering campaigns, 349 animals (238 dogs and 113 felines) were sampled over 2 years (2021-2023).

Samples were collected from dogs and cats that attended free spay and neuter campaigns in 12 neighborhoods in eleven parishes of the Metropolitan District of Quito (see Figure 1). The owners of the selected animals belonged to Quintiles 1 and 2, representing the vulnerable population segment, which corresponds to the 20% of the population with the lowest income. Owners were informed about the research objectives and protocols and asked to authorize the sample collection and answer a short survey about the conditions in which the animals live, their state of health, and their feeding habits (see Annex 1). Owners were provided with an explanation of how to fill in the surveys, particularly regarding the type of food and water that the animals

consume. It was emphasized that they should select the option that best represented the animals' most frequent consumption.

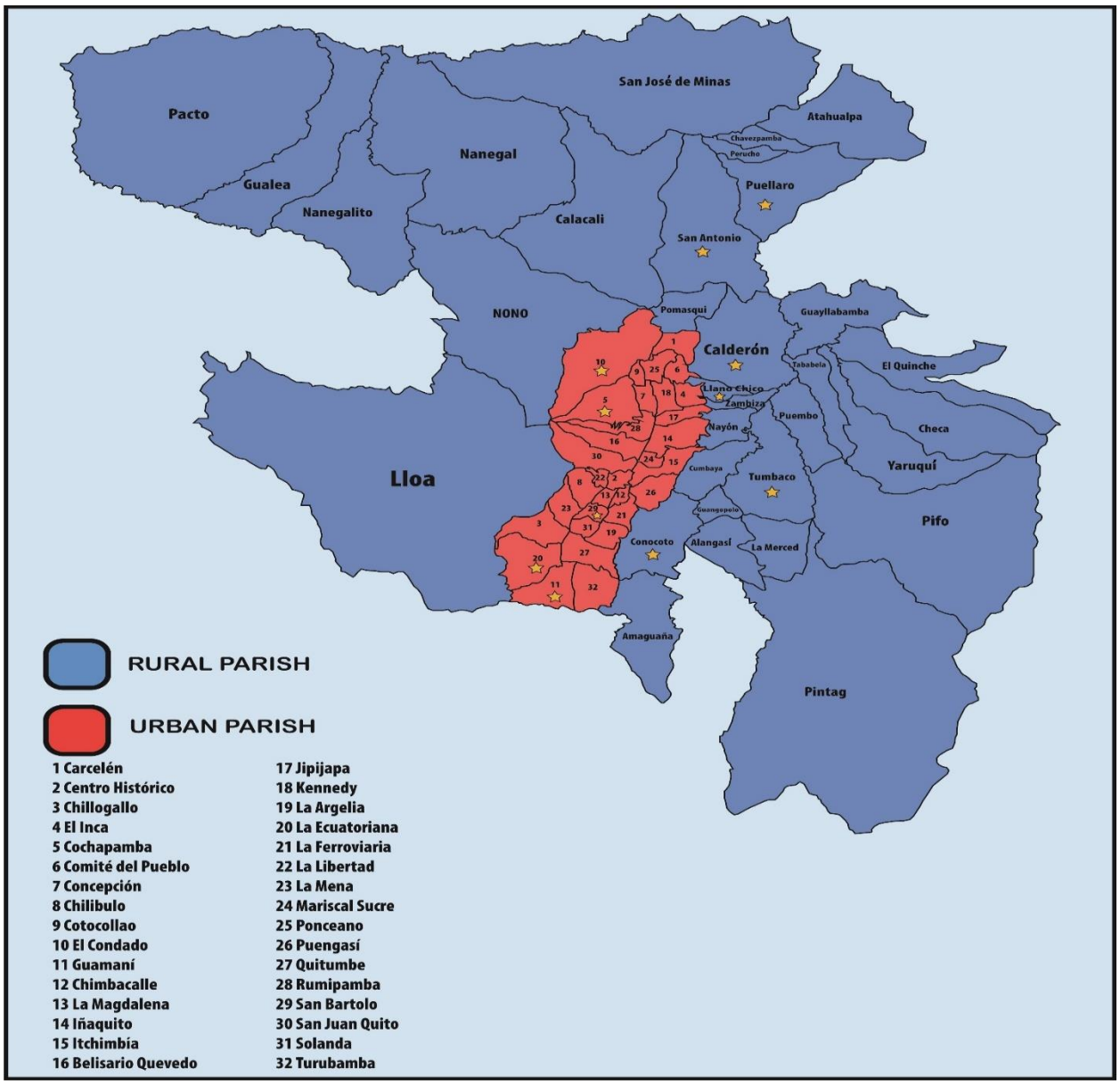


Figure 1. Map of the Metropolitan District of Quito, illustrating urban parishes (in red) and rural parishes (in blue). The stars show the sites where the samples were taken. Adapted from the official property map of the D.M.Q, (https://geoquito.quito.gob.ec/portal/apps/webappviewer/index.html?id=b7480a6986264efebce9135c30ffe58e). For the specific location coordinates of the sample sites, see Supplementary Table S1

Fecal samples were collected by direct swabbing when the animals were in anesthetic recovery. Subsequently, samples were placed in cryovial tubes, kept under refrigeration, and then transported to the Institute of Microbiology at USFQ (IM-USFQ), where they were stored at -20°C. This research was approved by the USFQ Committee of Ethics in the Use of Animals in Research and Teaching (Approval 2017-011; see Annex 2).

Campylobacter species detection

For the detection of *Campylobacter* spp., a pre-enrichment phase was first performed using a culture medium supplied by the manufacturer 3M Molecular Detection Assay 2 - *Campylobacter* MDA2CAM96 (Food Safety, 2021), which selectively enhanced the growth of *Campylobacter* species. Once the culture broth was distributed in falcon tubes, the stool samples were swabbed and homogenized in each of the tubes and then incubated at 42°C for 24 hours under aerobic conditions (Ha et al., 2021). After the incubation time, *Campylobacter* isolates were detected following the manufacturer's protocol (Food Safety, 2021; Ha et al., 2021). Further molecular analyses were carried out to validate the preliminary results.

For molecular identification of *Campylobacter* species in positive samples, the multiplex PCR protocol established by Klena et al., (2004) was followed, with a slight modification in the annealing time from 30 to 35s (Tables 1 and 2). The primers were previously developed by Klena et al. (2004) and then recommended by EUCAST guidelines.

Table 1. Primers set used for species detection of *Campylobacter* and their related information.

Gen target	Sequence (5' -3')	Strain	PCR product
IpxA	**(F 5'-3')** AGA CAA ATA AGA GAG AAT CAG **(R 3'-5')** CTG ATT CTC TCT TAT TTG TCT	*Campylobacter coli*	391 pb
IpxA	**(F 5'-3')** ACA ACT TGG TGA CGA TGT TGT A **(R 3'-5')** TGT TGA ACC ACT GCT ACA ACA T	*Campylobacter jejuni*	331 pb
IpxA	**(F 5'-3')** TRC CAA ATG TTA AAA TAG GCC A **(R 3'-5')** AYG GTT TAC AAT TTT ATC CGG T	*Campylobacter lari*	233 pb
IpxA	**(F 5'-3')** AAG TCG TAT ATT TTC YTA CGC TTG TGT G **(R 3'-5')** TTC AGC ATA TAA AAG RAT GCG AAC ACA C	*Campylobacter upsaliensis*	206 pb

Adapted from: Klena et al., 2004.

Table 2. PCR settings for *Campylobacter* species detection.

Temperature	Stage	Time
95°C	Initial denaturation	2:00
95°C	Denaturation	0:30
50°C	Annealing	0:35
72°C	Elongation	0:45
72°C	Final elongation	5:00

Adapted from: Klena et al., 2004.

Detection of *Giardia lamblia* and *Cryptosporidium* spp.

For the detection of *G. lamblia*, *Cryptosporidium parvum*, and *C. hominis*, we used the commercial kit *Giardia*-Crypto STRIP®. Briefly, this immunochromatographic test detects antigens of *G. lamblia* and *Cryptosporidium* oocysts in stool samples that are not concentrated. The sensitivity and specificity values for *G. lamblia* are 89.2% and 99.3%, respectively, and for *C. parvum* are 86.7% and 100%, respectively. This kit allows a trustful detection when compared to the direct microscopy technique which is known to show sensitivity and specificity values of 73% and 99.3%, respectively (Bitilinyu-Bangoh et al., 2019; Goudal et al., 2019). *G. lamblia*-

positive samples were analysed for genotyping in collaboration with the University of Reims in France.

Statistical Analysis

The data from the molecular tests, as well as the information gathered in the surveys, were recorded in a Microsoft Excel© spreadsheet for sorting and subsequent analysis. Statistical analyses were performed using the free software R version 4.3.2 (R Core Team, 2023). To evaluate the association between the set of demographic, health, and lifestyle variables and the prevalence of the pathogens, binomial regressions were conducted using the backward stepwise regression approach with the base package "pacman" (https://cran.r-project.org/web/packages/pacman/) (Rinker, 2018).

This model utilizes the natural logarithm (ln), which calculates the exponent to which the base must be raised to obtain (x). In logistic regression, it is employed with a link function to establish a relationship between the predictor variables and the response variables (Schober & Vetter, 2021). The alpha-to-remove significance level was 0.05.

Definition of the initial model

The initial model encompassed all variables considered for the analysis. The presence of *Campylobacter* spp., *Giardia lamblia*, and *Cryptosporidium parvum* served as the response variables. Age, sex, species, recent (less than 1 week) deworming, recent antibiotic treatment, presence of vomit or diarrhea, feeding on commercial food or homemade food, drinking potable or untreated water, cohabitation with other dogs or cats, cohabitation with other animals, and allowance to roam freely, served as candidate predictor variables, or potential risk factors.

Results

A total of 237 dogs and 112 cats that attended spay/neuter campaigns in marginal neighborhoods of the Quito district were sampled. These animals ranged in age from 2 to 108 months (median 12 months). The sex ratio was biased towards females in dogs (1:4) and cats (1:3). In the surveys, owners reported that 5.48% of canines and 3.57% of felines experienced recent episodes of diarrhea, 22.36% of canines and 5.35% of felines underwent recent deworming, 2.10% of canines and 0.89% of felines received recent antibiotic treatment, and 22.36% of canines and 17.85% of felines were vaccinated.

Most sampled dogs (81.85%) and cats (74%) lived with other dogs or cats, while 33.75% of canines and 37.5% of felines cohabited with other domestic animal species (cattle, pigs, and poultry). Pellets (commercial food) were the most consumed food (73.41% of canines and 65.17% of felines), while 26.58% of canines and 34.82% of felines consumed homemade diets. Regarding water consumption, 91.56% of canines and 97.32% of felines consumed potable water, while 8.43% of canines and 2.67% of felines consumed untreated water. Additionally, 27.84% of canines and 2.67% of felines consumed/hunted other animals, and 38.81% of canines and 47.32% of felines were allowed to roam freely (see Table 3).

Table 3. Demographic, health, and lifestyle characterization of the sample of dogs and cats.

DIARRHEA EPISODES	DOGS	SEX		CATS	SEX	
		MALE	FEMALE		MALE	FEMALE
YES	**13**	1	12	**4**	2	2
NO	**224**	46	178	**108**	36	72
DEWORMING						
YES	**53**	12	41	**17**	6	11
NO	**184**	35	149	**95**	32	63
ANTIBIOTIC TREATMENT						
YES	**5**	1	4	**1**	0	1
NO	**232**	46	186	**111**	38	73
COHABITATION WITH DOGS AND CATS						
YES	**194**	35	159	**83**	26	57
NO	**43**	12	31	**29**	12	17
COHABITATION WITH OTHER ANIMALS						
YES	**80**	17	63	**42**	15	27
NO	**157**	30	127	**70**	23	47
COMMERCIAL DIET						
YES	**174**	31	143	**73**	21	52
NO	**63**	16	47	**39**	17	22
HOMEMADE DIET						
YES	**63**	16	47	**39**	17	22
NO	**174**	31	143	**73**	21	52
VACCINATE						
YES	**53**	11	42	**20**	5	15
NO	**184**	36	148	**92**	33	59
POTABLE WATER						
YES	**217**	44	173	**109**	36	73
NO	**20**	3	17	**3**	2	1
UNTREATED WATER						
YES	**20**	3	17	**3**	2	1
NO	**177**	37	140	**109**	36	73
CONSUMPTION/HUNTING OF OTHER ANIMAL						
YES	**66**	10	56	**49**	17	32
NO	**171**	37	134	**63**	21	42
MOBILITY						
FREE- ROAMING	**92**	22	70	**53**	21	32
SUPERVISED	**145**	25	120	**59**	17	42

Campylobacter prevalence was found in 31.65% (95% CI: 25.7% - 37.5%) of dog samples (n= 237) and 28.75% (20.2% - 36.9%) of cat samples (n= 112). Among the *Campylobacter* species, *C. jejuni* had the highest prevalence in dogs (13.5%) and cats (8.03%), followed by *C. coli, C. upsaliensis,* and *C. lari*. A low percentage (2.8%) of the samples was found to be co-infected with *C. jejuni, C. coli,* and *C. lari.* Species-level identification was not possible in 11.39% of dog samples and 13.3% of cat samples (see Table 4).

The prevalence of *G. lamblia* was 8.44% in dogs and 8.9% in cats. The results provided showed that the assemblages belonged to groups C and D. No positive results were obtained for *C. parvum* in both species.

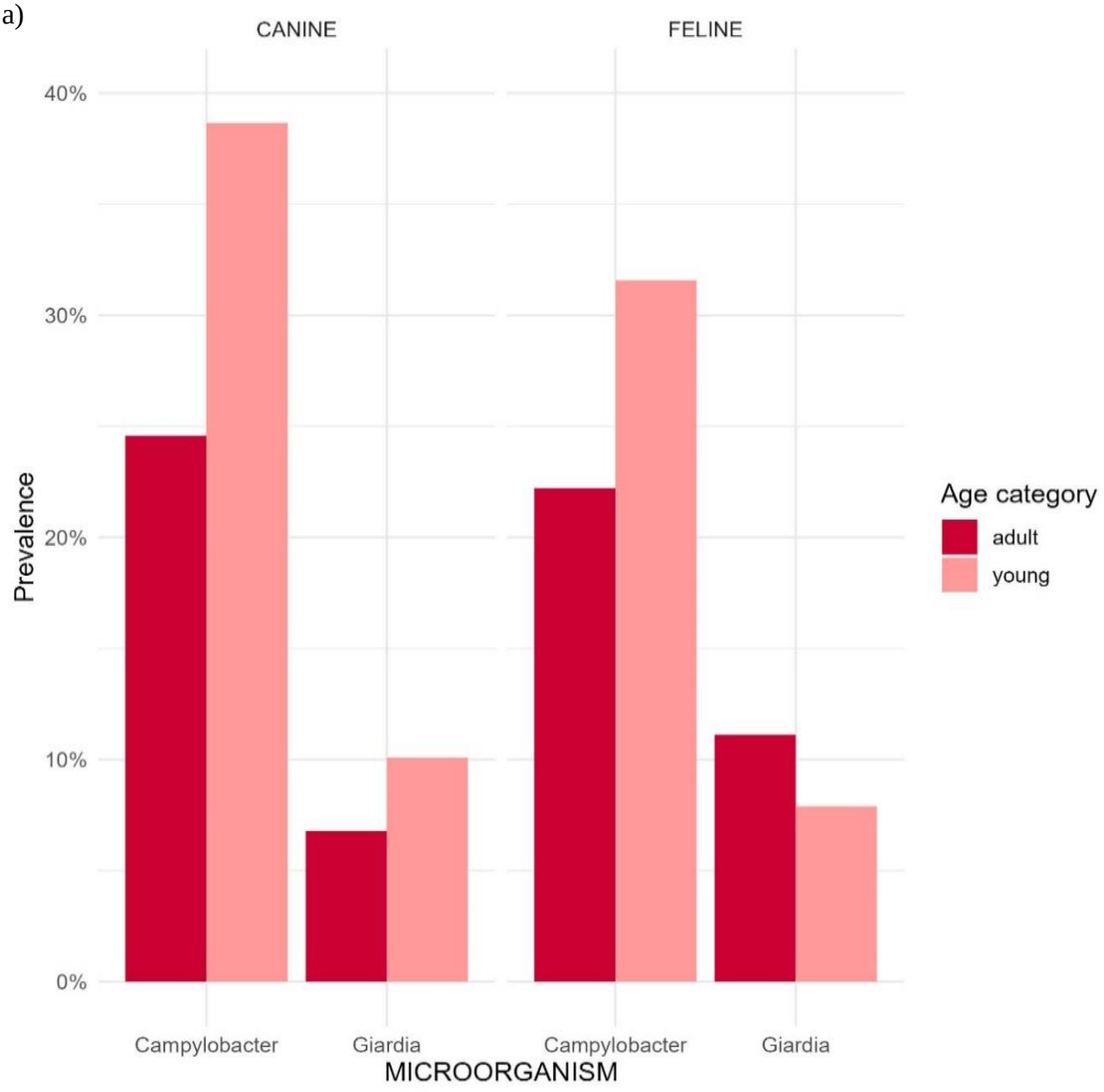
Table 4. Prevalence (in percentage) of *Campylobacter* species, *Giardia lamblia*, and Cryptosporidium *parvum* in juvenile and adult dogs and cats.

	Total DOGS	**DOGS ≤ 1 year**	**DOGS ≥ 1 year**	**Total CATS**	**CATS ≤ 1 year**	**CATS ≥ 1 year**
PATÓGENS	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**
C. jejuni	13.50	18.48	8.47	8.03	7.89	8.33
C. coli	5.90	5.88	5.93	5.35	7.89	0
C. upsaliensis	2.10	2.52	1.69	3.57	3.94	2.77
C. lari	1.26	5.52	0	0	0	0
Other *Campylobacter* spp.	11.39	13.44	9.32	13.39	13.15	13.88
Giardia lamblia	8.43	10.08	6.77	8.92	7.89	11.11
Cryptosporidium parvum	0	0	0	0	0	0
	(n=237)	**(n=119)**	**(n=118)**	**(n=112)**	**(n=76)**	**(n=36)**

In the backward stepwise binomial regression to assess the risk factors of *Campylobacter* prevalence, the variables included in the final model were age, coexistence with other dogs or cats,

and type of food. Young animals of both species were 2.06 times more likely to be infected than adults, while animals living with other dogs or cats were 2.58 times more likely to be infected than animals living alone. Quantitatively, the odds ratio calculated for 'homemade food' stands at 0.53 (< 1 connotes a reduced likelihood), indicating a decrease in the odds of *Campylobacter* prevalence in comparison to animals fed with pellets (Table 5, Figure 2).

a)



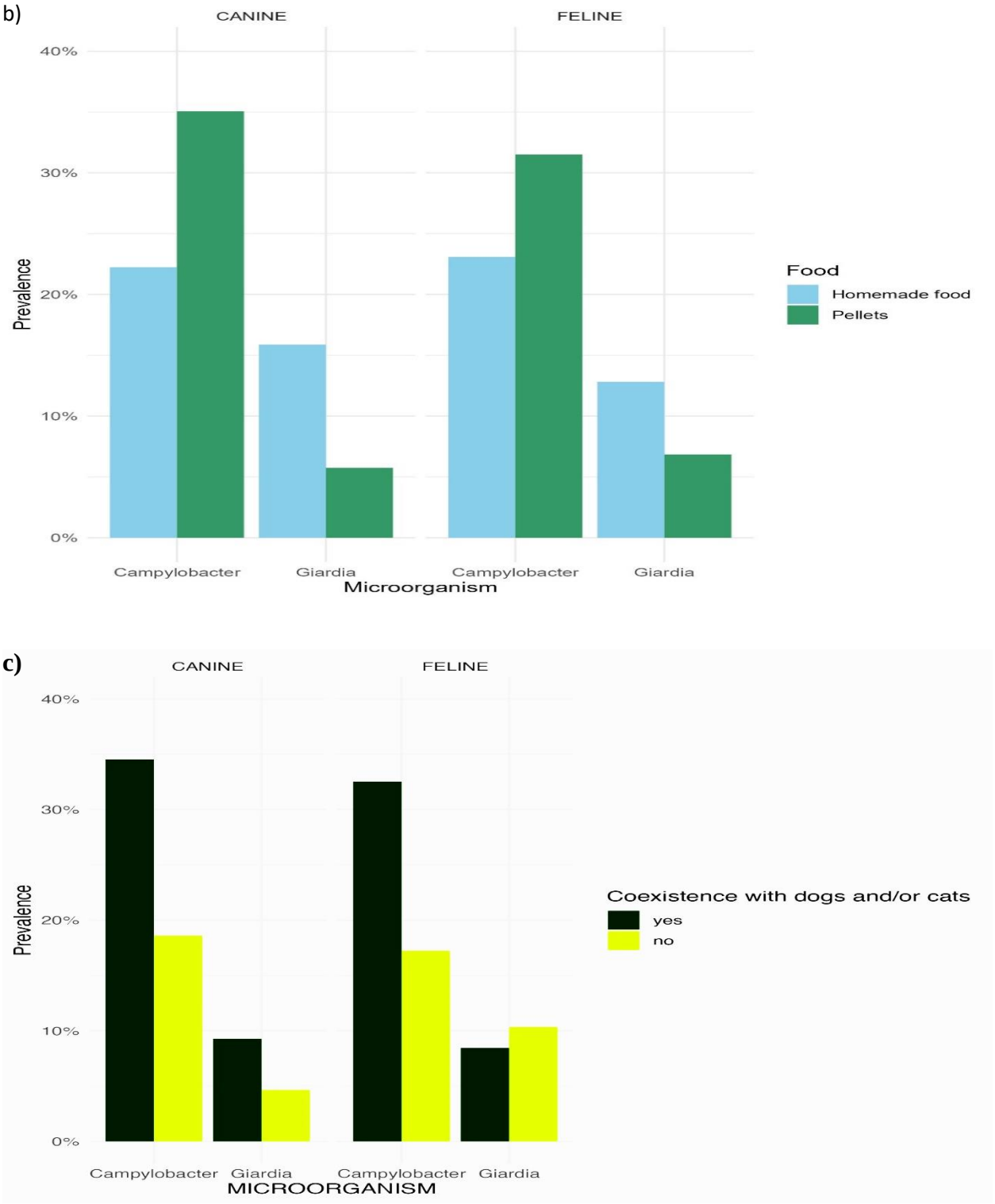


Figure 2. Variation in the prevalence of the pathogens detected in the present study as a function of: (a) age of the animals (<12 months young; >12 months adults), (b) type of diet (homemade or pelleted), and (c) cohabitation with other dogs or cats.

Table 5. Odds ratios of the final model with the predictor variables of *Campylobacter* prevalence.

PREDICTOR	Odds ratio (95% CI)	p-value
Coexistence with dogs and/or cats		
Absence	-	
Presence	2.58 (1.37-5.19)	0.005
Homemade food		
Absence	-	
Presence	0.53 (0.30-0.90)	0.021
Age category		
Adult	-	
Geriatric	1.18 (0.35-3.46)	0.8
Young	2.06 (1.25-3.44)	0.005

Regarding the prevalence of *Giardia lamblia*, the only variable included in the final model was the type of food. Animals consuming homemade food were 2.67 times more likely to contract *G. lamblia* compared to those fed commercial food (Table 6).

Table 6. The odds ratio of the final model with the predictor variable of *Giardia lamblia* prevalence.

PREDICTOR	Odds ratio (95% CI)	p-value
Homemade food		
Absence	-	
Presence	2.67 (1.24-5.72)	0.011

Discussion

The results of this study highlight the importance of demographic and lifestyle factors on the prevalence of intestinal pathogens in dogs and cats of marginal areas in the Quito district. In our model, age (OR 2.06), cohabitation with other pets (OR 2.58), and commercial diet were identified as significant risk factors for the prevalence of *Campylobacter* spp. in dogs and cats. Additionally, a homemade diet was found to be a significant risk factor (OR 2.67) for the prevalence of *Giardia lamblia*. These findings underscore the importance of considering risk factors in the prevention of intestinal disorders in companion animals, given their close relationship with humans.

The findings revealed that 31.6% of dogs and 28.7% of cats were carriers of *Campylobacter* species, while the prevalence of *Giardia lamblia* was 8.4 and 8.9% in dogs and cats, respectively. The heightened prevalence of *Campylobacter* in young animals could be related to their not-fully developed immune systems, coupled with risk factors such as the consumption of contaminated water and food, poor nutrition, and inadequate veterinary care as has been found in previous studies (Acke, 2018; Murnik et al., 2023; Veyna-Salazar et al., 2023). We also found that animals that ate pellets were more likely to be infected with *Campylobacter*. This result suggests that pellets may not be the only food of these animals or that the food was stored and delivered in poor hygienic conditions. Additionally, cohabitation with dogs and cats may increase the risk of contagion due to direct and continuous contact among animals. The influence of these lifestyle variables was also pointed out by Karama et al. (2019), who observed that dogs of varying ages, engaging in behaviors such as semi-wild living with contact with other animals, and consumption of contaminated food and water, exhibited a higher likelihood of infection.

The prevalence of *Campylobacter* spp. in our sample of dogs (31.6%) and cats (28.7%) is consistent with the prevalences reported in previous studies (Ahmed et al., 2018; Bojanić et al., 2017; Ju et al., 2023; Leahy et al., 2017; Thépault et al., 2020; Torkan et al., 2018). To mention a few, Thépault et al. (2020) analyzed 304 fecal samples from dogs and cats from different locations such as veterinary clinics, kennels, and shelters in Côtes d'Armor, France, over one year (2014-2015), obtaining a prevalence of 38% in dogs and 10% in cats. Ju et al. (2023) analyzed 325 fecal samples from dogs, cats, and domestic foxes in the Shenzhen province, China, determining a prevalence of 35% in dogs, and 30.1% in cats. Since prevalence may vary depending on demographic and environmental conditions, reports of lower or higher *Campylobacter* prevalences are expected. Leahy et al. (2017) found a prevalence of 75% in dogs living in shelters around Texas, revealing that 70% of *Campylobacter*-positive animals had no gastrointestinal disorders. This result aligns with the findings of the present study since none of the variables providing information on the health status of the animals, including the presence of episodes of diarrhea or vomiting, was identified as a predictor in our models. Animals did not manifest diarrheal syndrome; nevertheless, they tested positive for *Campylobacter* spp.

Campylobacter can colonize the mucosa of the lower gastrointestinal tract, facilitated by an ample supply of nutrients and the presence of microbiota that promote its growth and expression (Hofreuter, 2014). Its interaction with different hosts varies, exhibiting a malleable nature across species. In certain hosts, such as birds and mammals, it establishes a commensal relationship, classifying them as reservoirs, while in humans, it leads to gastrointestinal disorders (Cribb et al., 2022; Olvera-Ramírez et al., 2023). Despite high colonization rates, animals often do not display clinical signs. This phenomenon is largely attributed to the microbiota, which influences resistance to colonization by preventing pathogen attachment and inhibiting virulence expression. However,

the precise mechanisms underlying this relationship remain unclear (Fu et al., 2021). Another explanation for asymptomatic colonization in animals may be the absence of receptors on intestinal cells, confining *Campylobacter* to the intestinal lumen (Burnham & Hendrixson, 2018). The discrepancy in clinical presentation may also stem from the development of a tolerant immune response in these species, effectively regulating the inflammatory response and minimizing its impact. Additionally, *Campylobacter*'s extensive sequence variability contributes to strain-dependent pathogenicity and specific colonization capabilities. Asymptomatic carriers are often infected by non-toxigenic strains (Al-Banna et al., 2018; Kreling et al., 2020).

Campylobacter species identified as more prevalent in earlier studies elsewhere were *C. upsaliensis* and *C. helveticu*s, frequently isolated from dogs and cats, particularly from young animals (Gras et al., 2013; Torkan et al., 2018). However, in the current study, *C. upsaliensis* had a relatively low prevalence of only 2.6%, whereas, *C. jejuni* emerged as the species with the highest prevalence (11.7%). This finding is consistent with the findings of Yildiz et al. (2023) in their study in Turkey, with 126 fecal samples from dogs and cats. Thépault et al. (2020) also identified *C. jejuni* as the most prevalent species in Britain, France. Several authors report that 60% of animals under three months of age are carriers of *Campylobacter* spp., with the prevalence increasing to 100% at one year of age (Acke et al., 2006; Carbonero et al., 2012). However, as the age of the animals increases, the prevalence starts to decrease. Hald et al. (2004), for example, determined that *C. upsaliensis* has a higher prevalence in animals aged around 13 to 15 months, and for *C. jejuni*, between 3 and 12 months. Moreover, Giacomelli et al. (2015) identified different *Campylobacter* species in dogs and cats from various localities in Veneto, northern Italy. *C. jejuni* (55.2% in dogs and 53.3% in cats, n= 29 dogs and 15 cats) and *C. upsaliensis* (27.6% in dogs and 40% in cats) were the most prevalent species. In a study conducted in Quito, Ecuador, in 2017,

samples from canines (n=271) were analyzed. It was determined that the most frequent species was *C. jejuni* (20%), followed by *C. coli* (5%) (Andrade & Mena, 2017). The difference in the prevalence of these two species compared to our study could be attributed to the geographical location and lifestyle of the companion animals.

Mixed colonization of *Campylobacter*, particularly *C. jejuni* and *C. coli*, has been documented in various studies. For instance, Santaniello et al. (2021), reported findings from a study conducted in Italy, where animals used for therapy were found to be colonized by both *C. jejuni* and *C. coli*, which is consistent with our results. Furthermore, other species have been observed colonizing dogs. Subejano & Penuliar. (2018) conducted a study in the Philippines, revealing that 15.8% of sampled animals harbored both *C. upsaliensis* and *C. jejuni* simultaneously. These findings underscore the potential for companion animals, such as dogs and cats, to carry diverse *Campylobacter* species. Finally, the present study found that the prevalence of *C. lari* and *C. coli* was low, being consistent with numerous other studies reporting these species as infrequently isolated (Giacomelli et al., 2015; Murawska et al., 2022; Thépault et al., 2020).

Other pathogens, such as *Giardia* spp., have also been reported to be more prevalent in young animals, especially those whose owners have limited financial resources, as these animals may face challenges in accessing preventive and curative veterinary care (Epe et al., 2010; Mohamed et al., 2013). Previous studies have indicated that this protozoan is predominantly detected in dogs and cats compared to other parasitic species (Yun et al., 2023). Various factors have been identified to influence its prevalence, including age, the presence of animals exhibiting diarrheal syndrome, and the origin of the animals, whether they are domestic, homeless, or sheltered, due to their continuous contact with other animals (Barbosa et al., 2023). In contrast, in our research, age did not emerge as a significant predictor of the prevalence of *G. lamblia*, possibly

due to the relatively low prevalence of this pathogen in our sample (10.08% and 7.89% in canines and felines, respectively). Diet was the only significant risk factor, with animals feeding with homemade food showing a higher probability of being infected. Consumption of homemade diets with inadequate cooking or those consisting of raw food is a crucial factor that has recently gained attention (Hellgren et al., 2019). It has been observed that these diets are often nutritionally deficient and may increase the likelihood of pets contracting pathogens, some of which may be zoonotic (Davies et al., 2019), as may be the case with *G. lamblia* in our study. Interestingly, the assemblages of our samples belonged to groups C and D that are predominantly found in dogs (Barbosa et al., 2023; Cai et al., 2021). Increasing the sample size in future studies could help to elucidate these findings.

The prevalence of *G. lamblia* in the present study was somewhat lower than those reported by previous studies. For example, Lara-Reyes et al. (2021), reported a prevalence of 13.4% in dogs with owners in the city of Toluca, Mexico, while Ponce-Macotela et al. (2005) sampling stray dogs in Mexico City, Mexico, reported a prevalence of 46.5%. Veyna-Salazar et al. (2023) observed a higher prevalence of *G. lamblia* in young cats (less than one-year-old) compared to adult animals in the city of Queretaro, Mexico. Notably, the prevalence was greater in owned cats (28%) than in unowned cats (21%), aligning with the findings of Iturbe Cossío et al. (2021), who determined that the presence of *G. lamblia* and *C. parvum* in Mexico city was not only associated with the age of the animals but also with the suboptimal husbandry practices of their owners. Palmer et al. (2008) reported that the prevalence of *G. lamblia* was higher in dogs (16.1%) and cats (11.4%) in Australia compared to other parasitic species.

In Ecuador, Vasco et al. (2016) identified parasitic forms of *Giardia lamblia* and *Cryptosporidium parvum* in fecal samples from dogs and cats in a rural area, probably related to

the practice of using cattle feces as soil fertilizer. A study conducted by González-Ramírez et al., (2021) revealed a prevalence rate of 4.8% for *Giardia* spp. and 6% for *Cryptosporidium* spp., among other parasites, in dogs residing in the rural area of San Andres, Chimborazo, Ecuador. In the present study, no positive results for *Cryptosporidium* spp. were obtained. This lack of detection may be attributed to the sensitivity limitations of the immunochromatography test employed. Since the test served as a screening tool, it is possible that the samples contained levels of oocysts below the test's recognition threshold or that different *Cryptosporidium* species, such as *C. canis* and *C. felis*, were not detected by the test (Manouana et al., 2020). In addition, most of the samples came from urban areas, which presents another plausible explanation for the absence of this pathogen in the samples. *Cryptosporidium* spp. is predominantly associated with rural regions, mainly due to the presence of cattle and pigs. These animals, especially young ones, may excrete parasitic forms in their feces, leading to contamination of soil and water and possible transmission to dogs and cats (Robertson et al., 2014).

Overall, the results indicate that dogs and cats are potential sources of intestinal zoonotic pathogens, particularly *Campylobacter* spp., when their owners do not practice responsible ownership. This lack of responsibility can result in the contamination of public spaces, such as recreational parks where both people and animals coexist. In the city of Quito, the number of urban parks is increasing (Puente Amán et al., 2022). These green spaces promote leisure and recreation and have become areas where animals can walk with their owners (Ortega-Paredes et al., 2019). However, these places can become focal points of infection, as pointed out by A. Wang et al. (2012) who found that animals frequenting public parks had a higher probability of being infested with *Giardia* spp. and *Cryptosporidium* spp. Given the risk of transmission of pathogens from pets to humans, especially among individuals with compromised immune systems, it is crucial to apply

hygienic practices in the preparation of pet food and management of pet excreta. In addition, maintaining a clean environment for pets and ensuring they are supervised to prevent free roaming and access to garbage can further reduce the risk of infection. Ultimately, it is imperative to educate pet owners about responsible pet ownership and "One Health" principles.

Conclusions

The analysis revealed that about one-third of the samples from dogs and cats of marginal neighborhoods in Quito tested positive for *Campylobacter* upon examination using LAMP and multiplex PCR. Factors such as age and cohabitation with other animals were identified as significant contributors to the likelihood of *Campylobacter* infection. *C. jejuni* and *C. coli* were the predominant species found in both canine and feline samples, followed by *C. upsaliensis*, while *C. lari* exhibited the lowest prevalence. Additionally, the immunochromatography test detected *G. lamblia* in 8% of the sampled animals, whereas none tested positive for *Cryptosporidium parvum*. Insights derived from the survey of pet owners shed light on demographic variables and lifestyle factors that influence animals' susceptibility to these microorganisms, illustrating their potential as carriers.

Overall, the findings indicated that young animals, especially cohabiting with other dogs or cats and eating food stored or prepared in poor hygienic conditions, faced a higher risk of infection. Further analysis of gastrointestinal microorganisms in these animals is crucial, given the limited information available on these species and their potential impact on human health upon close contact.

Due to the absence of reliable estimations of the size of the populations of dogs and cats in Quito, and the limitations we faced on accessibility to neutering campaigns, the pathogens' prevalences we are reporting may not reflect the situation of companion animals in marginal neighborhoods in the city.

Considering that a significant proportion of the positive samples for *Campylobacter* could not be identified, using alternative primers in future research is a must. Furthermore,

quantification, sequencing, and detection of antimicrobial resistance factors should be conducted in a larger sample, particularly in free-roaming animals or those in shelters, to facilitate a more comprehensive analysis.

Longitudinal studies could shed light on the dynamic interplay of microorganisms between animals and humans, offering deeper insights into potential risk factors, such as the hygiene practices of owners. Governmental institutions responsible for epidemiological surveillance could develop a guide outlining sanitary standards for pet owners, promoting good animal ownership practices to prevent the transmission of pathogens. Guidelines could be developed alongside awareness campaigns and training programs for animal guardians, in collaboration with health professionals from various fields, to raise awareness about the importance and risks that companion animals can pose to humans. Such educational strategies have the potential to improve coexistence with companion animals over the long term, enhance sanitary practices, and minimize the risks associated with close contact with them.

References

Acke, E. (2018). Campylobacteriosis in dogs and cats: a review. In *New Zealand Veterinary Journal* (Vol. 66, Issue 5, pp. 221–228). N Z Vet J. https://doi.org/10.1080/00480169.2018.1475268

Acke, E., Whyte, P., Jones, B. R., McGill, K., Collins, J. D., & Fanning, S. (2006). Prevalence of thermophilic Campylobacter species in cats and dogs in two animal shelters in Ireland. *The Veterinary Record*, *158*(2), 51–54. https://doi.org/10.1136/VR.158.2.51

Agresti, A., Berrilli, F., Maestrini, M., Procesi, I. G., Loretti, E., Vonci, N., & Perrucci, S. (2022). Prevalence, Risk Factors and Genotypes of Giardia duodenalis in Sheltered Dogs in Tuscany (Central Italy). *Pathogens*, *11*(1). https://doi.org/10.3390/PATHOGENS11010012

Ahmed, I., Verma, A. K., & Kumar, A. (2018). Prevalence, associated risk factors and antimicrobial susceptibility pattern of Campylobacter species among dogs attending veterinary practices at Veterinary University, Mathura, India. *Veterinary and Animal Science*, *6*, 6–11. https://doi.org/10.1016/j.vas.2018.07.001

Ahmed, S. A., & Karanis, P. (2020). Cryptosporidium and cryptosporidiosis: The perspective from the gulf countries. In *International Journal of Environmental Research and Public Health* (Vol. 17, Issue 18, pp. 1–34). Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/ijerph17186824

Al-Banna, N. A., Cyprian, F., & Albert, M. J. (2018). Cytokine responses in campylobacteriosis: Linking pathogenesis to immunity. In *Cytokine and Growth Factor Reviews* (Vol. 41, pp. 75–87). Pergamon. https://doi.org/10.1016/j.cytogfr.2018.03.005

Alseady, H. H., Al-Dabbagh, S. M. K., & Marhash, A. D. (2023). Prevalence and molecular characterization of Giardia intestinalis isolated from children and calves in Babylon province, Iraq. *Veterinary World*, *16*(9), 1781–1789. https://doi.org/10.14202/vetworld.2023.1781-1789

Andrade, S. D., & Mena, R. P. (2017). *Determinación de la prevalencia y la resistencia antimicrobiana de Campylobacter spp. en caninos de las parroquias urbanas de la ciudad de Quito* [Quito: UCE]. http://www.dspace.uce.edu.ec/handle/25000/10150

Atherton, R., Bhavnani, D., Calvopiña, M., Vicuña, Y., Cevallos, W., & Eisenberg, J. (2013). Molecular identification of giardia duodenalis in ecuador by polymerase chain reaction-estriction fragment length polymorphism. *Memorias Do Instituto Oswaldo Cruz*, *108*(4), 512–515. https://doi.org/10.1590/S0074-02762013000400019

Ayana, D. (2023). Giardiasis of domestic animals and its zoonotic significance: A review. *Ethiopian Veterinary Journal*, *27*(1), 1–30. https://doi.org/10.4314/evj.v27i1.1

Barbosa, A. D., Egan, S., Feng, Y., Xiao, L., & Ryan, U. (2023). Cryptosporidium and Giardia in cats and dogs: What is the real zoonotic risk? In *Current Research in Parasitology and Vector-Borne Diseases* (Vol. 4, p. 100158). Elsevier. https://doi.org/10.1016/j.crpvbd.2023.100158

Bartelt, L. A., & Platts-Mills, J. A. (2016). Giardia: a pathogen or commensal for children in high prevalence settings? *Current Opinion in Infectious Diseases*, *29*(5), 502. https://doi.org/10.1097/QCO.0000000000000293

Bitilinyu-Bangoh, J., Voskuijl, W., Thitiri, J., Menting, S., Verhaar, N., Mwalekwa, L., De Jong, D. B., Van Loenen, M., Mens, P. F., Berkley, J. A., Bandsma, R. H. J., & Schallig, H. D. F. H. (2019). Performance of three rapid diagnostic tests for the detection of Cryptosporidium

spp. and Giardia duodenalis in children with severe acute malnutrition and diarrhoea. *Infectious Diseases of Poverty*, *8*(1). https://doi.org/10.1186/S40249-019-0609-6

Bojanić, K., Midwinter, A. C., Marshall, J. C., Rogers, L. E., Biggs, P. J., & Acke, E. (2017). Isolation of Campylobacter spp. from Client-Owned Dogs and Cats, and Retail Raw Meat Pet Food in the Manawatu, New Zealand. *Zoonoses and Public Health*, *64*(6), 438–449. https://doi.org/10.1111/ZPH.12323

Bouzid, M., Halai, K., Jeffreys, D., & Hunter, P. R. (2015). The prevalence of Giardia infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. *Veterinary Parasitology*, *207*(3–4), 181–202. https://doi.org/10.1016/J.VETPAR.2014.12.011

Burnham, P. M., & Hendrixson, D. R. (2018). Campylobacter jejuni: collective components promoting a successful enteric lifestyle. In *Nature Reviews Microbiology* (Vol. 16, Issue 9, pp. 551–565). Nature Publishing Group. https://doi.org/10.1038/s41579-018-0037-9

Cai, W., Ryan, U., Xiao, L., & Feng, Y. (2021). Zoonotic giardiasis: an update. *Parasitology Research*, *120*(12), 4199–4218. https://doi.org/10.1007/S00436-021-07325-2

Carbonero, A., Torralbo, A., Borge, C., García-Bocanegra, I., Arenas, A., & Perea, A. (2012). Campylobacter spp., C. Jejuni and C. Upsaliensis infection-associated factors in healthy and ill dogs from clinics in Cordoba, Spain. Screening tests for antimicrobial susceptibility. *Comparative Immunology, Microbiology and Infectious Diseases*, *35*(6), 505–512. https://doi.org/10.1016/j.cimid.2012.05.002

Chalmers, R. M., Elwin, K., Thomas, A. L., Guy, E. C., & Mason, B. (2009). Long-term Cryptosporidium typing reveals the aetiology and species-specific epidemiology of human cryptosporidiosis in England and Wales, 2000 to 2003. *Euro Surveillance : Bulletin Européen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin*, *14*(2), 19086. https://doi.org/10.2807/ESE.14.02.19086-EN/CITE/REFWORKS

Cribb, D. M., Varrone, L., Wallace, R. L., McLure, A. T., Smith, J. J., Stafford, R. J., Bulach, D. M., Selvey, L. A., Firestone, S. M., French, N. P., Valcanis, M., Fearnley, E. J., Sloan-Gardner, T. S., Graham, T., Glass, K., & Kirk, M. D. (2022). Risk factors for campylobacteriosis in Australia: outcomes of a 2018–2019 case–control study. *BMC Infectious Diseases*, *22*(1), 1–11. https://doi.org/10.1186/S12879-022-07553-6/TABLES/3

Curi, N. H. A., Paschoal, A. M. O., Massara, R. L., Santos, H. A., Guimarães, M. P., Passamani, M., & Chiarello, G. (2017). Fatores de risco para infecções parasitárias gastrointestinais em cães do entorno de áreas protegidas da Mata atlântica: Implicações para a saúde humana e da vida selvagem. *Brazilian Journal of Biology*, *77*(2), 388–395. https://doi.org/10.1590/1519-6984.19515

Davies, R. H., Lawes, J. R., & Wales, A. D. (2019). Raw diets for dogs and cats: a review, with particular reference to microbiological hazards. *The Journal of Small Animal Practice*, *60*(6), 329. https://doi.org/10.1111/JSAP.13000

Dunn, N., & Juergens, A. L. (2022). Giardiasis. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK513239/

Epe, C., Rehkter, G., Schnieder, T., Lorentzen, L., & Kreienbrock, L. (2010). Giardia in symptomatic dogs and cats in Europe—Results of a European study. *Veterinary Parasitology*, *173*(1–2), 32–38. https://doi.org/10.1016/J.VETPAR.2010.06.015

Facciolà, A., Riso, R., Avventuroso, E., Visalli, G., Delia, S. A., & Laganà, P. (2017). Campylobacter: from microbiology to prevention. *Journal of Preventive Medicine and Hygiene*, *58*(2), E79. /pmc/articles/PMC5584092/

Food Safety, M. (2021). *3M ™ Molecular Detection Assay 2-Campylobacter and 3M ™ Campylobacter Enrichment Broth Primary detection method used by the USDA Food Safety and Inspection Service 1*.

Fu, Y., Alenezi, T., Almansour, A., Wang, H., Jia, Z., Sun, X., Fu, Y., Alenezi, T., Almansour, A., Wang, H., Jia, Z., & Sun, X. (2021). The Role of Immune Response and Microbiota on Campylobacteriosis. *Campylobacter*. https://doi.org/10.5772/INTECHOPEN.96755

Giacomelli, M., Follador, N., Coppola, L. M., Martini, M., & Piccirillo, A. (2015). Survey of Campylobacter spp. in owned and unowned dogs and cats in Northern Italy. *The Veterinary Journal*, *204*(3), 333–337. https://doi.org/10.1016/J.TVJL.2015.03.017

Gingrich, E. N., Scorza, A. V., Clifford, E. L., Olea-Popelka, F. J., & Lappin, M. R. (2010). Intestinal parasites of dogs on the Galapagos Islands. *Veterinary Parasitology*, *169*(3–4), 404–407. https://doi.org/10.1016/J.VETPAR.2010.01.018

Godínez-Galaz, E. M., Veyna-Salazar, N. P., Olvera-Ramírez, A. M., Milián-Suazo, F., Perea-Razo, C. A., Bernal-Reynaga, R., & Cantó-Alarcón, G. J. (2019). Prevalence and zoonotic potential of giardia intestinalis in dogs of the central region of Mexico. *Animals*, *9*(6), 325. https://doi.org/10.3390/ani9060325

González-Ramírez, L. C., Vázquez, C. J., Chimbaina, M. B., Djabayan-Djibeyan, P., Prato-Moreno, J. G., Trelis, M., & Fuentes, M. V. (2021). Ocurrence of enteroparasites with zoonotic potential in animals of the rural area of San Andres, Chimborazo, Ecuador. *Veterinary Parasitology: Regional Studies and Reports*, *26*, 100630. https://doi.org/10.1016/J.VPRSR.2021.100630

Goudal, A., Laude, A., Valot, S., Desoubeaux, G., Argy, N., Nourrisson, C., Pomares, C., Machouart, M., Le Govic, Y., Dalle, F., Botterel, F., Bourgeois, N., Cateau, E., Leterrier, M., Lavergne, R. A., Beser, J., Le Pape, P., & Morio, F. (2019). Rapid diagnostic tests relying on antigen detection from stool as an efficient point of care testing strategy for giardiasis and cryptosporidiosis? Evaluation of a new immunochromatographic duplex assay. *Diagnostic Microbiology and Infectious Disease*, *93*(1), 33–36. https://doi.org/10.1016/J.DIAGMICROBIO.2018.07.012

Gras, L. M., Smid, J. H., Wagenaar, J. A., Koene, M. G. J., Havelaar, A. H., Friesema, I. H. M., French, N. P., Flemming, C., Galson, J. D., Graziani, C., Busani, L., & Van Pelt, W. (2013). Increased risk for Campylobacter jejuni and C. coli infection of pet origin in dog owners and evidence for genetic association between strains causing infection in humans and their pets. *Epidemiology and Infection*, *141*(12), 2526. https://doi.org/10.1017/S0950268813000356

Ha, J., Seo, Y., Cho, E., Choi, S., Kim, H., & Yoon, Y. (2021). Improvement of the detection efficiency of 3M™ molecular detection system for Campylobacter in poultry using nitrogen-doped carbon nanodots. *Journal of Microbiological Methods*, *184*. https://doi.org/10.1016/J.MIMET.2021.106211

Hald, B., Pedersen, K., Wainø, M., Jørgensen, J. C., & Madsen, M. (2004). Longitudinal Study of the Excretion Patterns of Thermophilic Campylobacter spp. in Young Pet Dogs in Denmark. *Journal of Clinical Microbiology*, *42*(5), 2003–2012. https://doi.org/10.1128/JCM.42.5.2003-2012.2004

Hellgren, J., Hästö, L. S., Wikstrom, C., Fernström, L. L., & Hansson, I. (2019). Occurrence of Salmonella, Campylobacter, Clostridium and Enterobacteriaceae in raw meat-based diets for dogs. *Veterinary Record*, *184*(14), 442–442. https://doi.org/10.1136/vr.105199

Heyworth, M. F. (2014). Immunological aspects of Giardia infections. *Parasite*, *21*.

https://doi.org/10.1051/parasite/2014056
Hofreuter, D. (2014). Defining the metabolic requirements for the growth and colonization capacity of Campylobacter jejuni. *Frontiers in Cellular and Infection Microbiology*, *4*(SEP). https://doi.org/10.3389/FCIMB.2014.00137
INEC. (2023). *Instituto Nacional de Estadística y Censos*. https://www.ecuadorencifras.gob.ec/institucional/home/
Iturbe Cossío, T. L., Montes Luna, A. D., Ruiz Mejia, M., Flores Ortega, A., Heredia Cárdenas, R., & Romero Núñez, C. (2021). Risk factors associated with cat parasites in a feline medical center. *JFMS Open Reports*, *7*(2). https://doi.org/10.1177/20551169211033183
Janssen, B., & Snowden, J. (2022). *Cryptosporidiosis*. https://www.ncbi.nlm.nih.gov/books/NBK448085/
Ju, C., Ma, Y., Zhang, B., Zhou, G., Wang, H., Yu, M., He, J., Duan, Y., & Zhang, M. (2023). Prevalence, genomic characterization and antimicrobial resistance of Campylobacter spp. isolates in pets in Shenzhen, China. *Frontiers in Microbiology*, *14*, 1152719. https://doi.org/10.3389/FMICB.2023.1152719/BIBTEX
Karama, M., Cenci-Goga, B. T., Prosperi, A., Etter, E., El-Ashram, S., McCrindle, C., Ombui, J. N., & Kalake, A. (2019). Prevalence and risk factors associated with Campylobacter spp. occurrence in healthy dogs visiting four rural community veterinary clinics in South Africa. *The Onderstepoort Journal of Veterinary Research*, *86*(1). https://doi.org/10.4102/OJVR.V86I1.1673
Klena, J. D., Parker, C. T., Knibb, K., Claire Ibbitt, J., Devane, P. M. L., Horn, S. T., Miller, W. G., & Konkel, M. E. (2004). Differentiation of Campylobacter coli, Campylobacter jejuni, Campylobacter lari, and Campylobacter upsaliensis by a Multiplex PCR Developed from the Nucleotide Sequence of the Lipid A Gene lpxA. *Journal of Clinical Microbiology*, *42*(12), 5549. https://doi.org/10.1128/JCM.42.12.5549-5557.2004
Kreling, V., Falcone, F. H., Kehrenberg, C., & Hensel, A. (2020). Campylobacter sp.: Pathogenicity factors and prevention methods—new molecular targets for innovative antivirulence drugs? *Applied Microbiology and Biotechnology*, *104*(24), 10409. https://doi.org/10.1007/S00253-020-10974-5
LaLonde-Paul, D., Cummings, K. J., Rodriguez-Rivera, L. D., Wu, J., & Lawhon, S. D. (2019). Ciprofloxacin resistance among Campylobacter jejuni isolates obtained from shelter dogs in Texas. *Zoonoses and Public Health*, *66*(3), 337–342. https://doi.org/10.1111/ZPH.12544
Lara-Reyes, E., Quijano-Hernández, I. A., Rodríguez-Vivas, R. I., Ángel-Caraza, J. Del, & Martínez-Castañeda, J. S. (2021). Factors associated with endoparasites and ectoparasites in domiciled dogs in the metropolitan area of Toluca, México. *Biomedica*, *41*(4), 556–572. https://doi.org/10.7705/BIOMEDICA.6013
Leahy, A. M., Cummings, K. J., Rodriguez-Rivera, L. D., Hamer, S. A., & Lawhon, S. D. (2017). Faecal Campylobacter shedding among dogs in animal shelters across Texas. *Zoonoses and Public Health*, *64*(8), 623–627. https://doi.org/10.1111/ZPH.12356
Lucio-Forster, A., Griffiths, J. K., Cama, V. A., Xiao, L., & Bowman, D. D. (2010). Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. In *Trends in Parasitology* (Vol. 26, Issue 4, pp. 174–179). Trends Parasitol. https://doi.org/10.1016/j.pt.2010.01.004
Manouana, G. P., Lorenz, E., Ngwese, M. M., Moure, P. A. N., Ascofaré, O. M., Akenten, C. W., Amuasi, J., Rakotozandrindrainy, N., Rakotozandrindrainy, R., Mbwana, J., Lusingu, J., Byrne, N., Melhem, S., Zinsou, J. F., Adegbite, R. B., Hogan, B., Winter, D., May, J., Kremsner, P. G., … Adegnika, A. A. (2020). Performance of a rapid diagnostic test for the

detection of Cryptosporidium spp. in African children admitted to hospital with diarrhea. *PLoS Neglected Tropical Diseases*, *14*(7), 1–12. https://doi.org/10.1371/JOURNAL.PNTD.0008448

McKerr, C., Chalmers, R. M., Elwin, K., Ayres, H., Vivancos, R., O'Brien, S. J., & Christley, R. M. (2022). Cross-sectional household transmission study of Cryptosporidium shows that C. hominis infections are a key risk factor for spread. *BMC Infectious Diseases*, *22*(1). https://doi.org/10.1186/s12879-022-07086-y

Merigueti, Y. F. F. B., Giuffrida, R., Silva, R. C. da, Kmetiuk, L. B., Santos, A. P. Dos, Biondo, A. W., & Santarém, V. A. (2022). Dog and Cat Contact as Risk Factor for Human Toxocariasis: Systematic Review and Meta-Analysis. In *Frontiers in Public Health* (Vol. 10, p. 854468). Frontiers Media S.A. https://doi.org/10.3389/fpubh.2022.854468

Mohamed, A. S., Glickman, L. T., Camp, J. W., Lund, E., & Moore, G. E. (2013). Prevalence and risk factors for Giardia spp. infection in a large national sample of pet dogs visiting veterinary hospitals in the United States (2003-2009). *Veterinary Parasitology*, *195*(1–2), 35–41. https://doi.org/10.1016/j.vetpar.2012.12.049

Murawska, M., Sypecka, M., Bartosik, J., Kwiecień, E., Rzewuska, M., & Sałamaszyńska-Guz, A. (2022). Should We Consider Them as a Threat? Antimicrobial Resistance, Virulence Potential and Genetic Diversity of Campylobacter spp. Isolated from Varsovian Dogs. *Antibiotics*, *11*(7). https://doi.org/10.3390/ANTIBIOTICS11070964/S1

Murnik, L. C., Daugschies, A., & Delling, C. (2022). Cryptosporidium infection in young dogs from Germany. *Parasitology Research*, *121*(10), 2985. https://doi.org/10.1007/S00436-022-07632-2

Murnik, L. C., Daugschies, A., & Delling, C. (2023). Gastrointestinal parasites in young dogs and risk factors associated with infection. *Parasitology Research*, *122*(2), 585–596. https://doi.org/10.1007/s00436-022-07760-9

Navone, G. T., Zonta, M. L., Cociancic, P., Garraza, M., Gamboa, M. I., Giambelluca, L. A., Dahinten, S., & Oyhenart, E. E. (2017). Estudio transversal de las parasitosis intestinales en poblaciones infantiles de Argentina. *Revista Panamericana de Salud Pública*, *41*. https://doi.org/10.26633/RPSP.2017.24

Nguyen, T. P., & Taylor, R. S. (2022). Guillain Barre Syndrome. *StatPearls*. http://www.ncbi.nlm.nih.gov/pubmed/30335287

Olvera-Ramírez, A. M., McEwan, N. R., Stanley, K., Nava-Diaz, R., & Aguilar-Tipacamú, G. (2023). A Systematic Review on the Role of Wildlife as Carriers and Spreaders of Campylobacter spp. *Animals*, *13*(8). https://doi.org/10.3390/ANI13081334/S1

Ortega-Paredes, D., Haro, M., Leoro-Garzón, P., Barba, P., Loaiza, K., Mora, F., Fors, M., Vinueza-Burgos, C., & Fernández-Moreira, E. (2019). Multidrug-resistant Escherichia coli isolated from canine faeces in a public park in Quito, Ecuador. *Journal of Global Antimicrobial Resistance*, *18*, 263–268. https://doi.org/10.1016/j.jgar.2019.04.002

Palmer, C. S., Thompson, R. C. A., Traub, R. J., Rees, R., & Robertson, I. D. (2008). National study of the gastrointestinal parasites of dogs and cats in Australia. *Veterinary Parasitology*, *151*(2–4), 181–190. https://doi.org/10.1016/J.VETPAR.2007.10.015

Piekara-Stępińska, A., Piekarska, J., Gorczykowski, M., & Bania, J. (2021). Genotypes of Giardia duodenalis in Household Dogs and Cats from Poland. *Acta Parasitologica*, *66*(2), 428–435. https://doi.org/10.1007/s11686-020-00292-1

Ponce-Macotela, M., Peralta-Abarca, G. E., & Martínez-Gordillo, M. N. (2005). Giardia intestinalis and other zoonotic parasites: Prevalence in adult dogs from the southern part of

Mexico City. *Veterinary Parasitology*, *131*(1–2), 1–4. https://doi.org/10.1016/j.vetpar.2005.03.027

Puente Amán, J. S., Orbe Ornaza, D. F., Salazar Proaño, E. E., Betancourt Cubillo, E. P., & Sinde Gonzalez, I. (2022). *Distribución, accesibilidad y equidad territorial de las áreas verdes urbanas en el Distrito Metropolitano de Quito*. https://www.ciuq.ec/Distribucion accesibilidad y equidad territorial de las areas verdes urbanas en el Distrito Metropolitano de Quito.pdf

R Core Team. (2023). *A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.* https://www.r-project.org/

Rinker TW, K. D. (2018). *pacman: Package Management for R. version 0.5.0*. https://cran.r-project.org/web/packages/pacman/

Robertson, L. J., Björkman, C., Axén, C., & Fayer, R. (2014). Cryptosporidiosis in Farmed Animals. *Cryptosporidium: Parasite and Disease*, 149. https://doi.org/10.1007/978-3-7091-1562-6_4

Rumsey, P., & Waseem, M. (2022). Giardia Lamblia Enteritis. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK531495/

Ryan, U., Fayer, R., & Xiao, L. (2014). Cryptosporidium species in humans and animals: Current understanding and research needs. In *Parasitology* (Vol. 141, Issue 13, pp. 1667–1685). Parasitology. https://doi.org/10.1017/S0031182014001085

Sandberg, M., Bergsjo, B., Hofshagen, M., Skjerve, E., & Kruse, H. (2002). Risk factors for Campylobacter infection in Norwegian cats and dogs. *Preventive Veterinary Medicine*, *55*(4), 241–253. https://doi.org/10.1016/S0167-5877(02)00095-8

Santaniello, A., Varriale, L., Dipineto, L., Borrelli, L., Pace, A., Fioretti, A., & Menna, L. F. (2021). Presence of Campylobacterjejuni and C. coli in Dogs under Training for Animal-Assisted Therapies. *International Journal of Environmental Research and Public Health*, *18*(7), 3717. https://doi.org/10.3390/IJERPH18073717

Scallan Walter, E. J., Crim, S. M., Bruce, B. B., & Griffin, P. M. (2020). Incidence of Campylobacter-Associated Guillain-Barré Syndrome Estimated from Health Insurance Data. *Foodborne Pathogens and Disease*, *17*(1), 23–28. https://doi.org/10.1089/FPD.2019.2652

Schober, P., & Vetter, T. R. (2021). Logistic Regression in Medical Research. *Anesthesia and Analgesia*, *132*(2), 365. https://doi.org/10.1213/ANE.0000000000005247

Stull, J. W., Brophy, J., & Weese, J. S. (2015). Reducing the risk of pet-associated zoonotic infections. *CMAJ : Canadian Medical Association Journal = Journal de l'Association Medicale Canadienne*, *187*(10), 736–743. https://doi.org/10.1503/CMAJ.141020

Subejano, M. S. E. P., & Penuliar, G. M. (2018). Prevalence and risk factors for colonization of Campylobacter spp. In household dogs in Metro Manila, Philippines. *Tropical Biomedicine*, *35*(3), 744–754.

Sun, J., Qin, Z., Fu, Y., Qin, H., Sun, M., Dong, H., Chao, L., Zhang, L., & Li, J. (2023). Assessment of potential zoonotic transmission of Giardia duodenalis from dogs and cats. *One Health*, *17*, 100651. https://doi.org/10.1016/J.ONEHLT.2023.100651

The ANOFEL Cryptosporidium National Network, C. (2010). Laboratory-based surveillance for Cryptosporidium in France, 2006–2009. *Eurosurveillance*, *15*(33), 19642. https://doi.org/10.2807/ese.15.33.19642-en

Thépault, A., Rose, V., Queguiner, M., Chemaly, M., & Rivoal, K. (2020). Dogs and cats: Reservoirs for highly diverse Campylobacter jejuni and a potential source of human

exposure. *Animals*, *10*(5), 838. https://doi.org/10.3390/ani10050838

Toledo, Z., Simaluiza, R. J., & Fernández, H. (2018). Occurrence and antimicrobial resistance of Campylobacter jejuni and Campylobacter coli isolated from domestic animals from Southern Ecuador. *Undefined*, *48*(11), 11. https://doi.org/10.1590/0103-8478CR20180003

Torkan, S., Vazirian, B., Khamesipour, F., & Dida, G. O. (2018). Prevalence of thermotolerant Campylobacter species in dogs and cats in Iran. *Veterinary Medicine and Science*, *4*(4), 296–303. https://doi.org/10.1002/vms3.117

Vasco, K., Graham, J. P., & Trueba, G. (2016). Detection of zoonotic enteropathogens in children and domestic animals in a semirural community in ecuador. *Applied and Environmental Microbiology*, *82*(14), 4218–4224. https://doi.org/10.1128/AEM.00795-16

Veyna-Salazar, N. P., Cantó-Alarcón, G. J., Olvera-Ramírez, A. M., Ruiz-López, F. J., Bernal-Reynaga, R., Bárcenas-Reyes, I., & Durán-Aguilar, M. (2023). Occurrence of Giardia duodenalis in Cats from Queretaro and the Risk to Public Health. *Animals*, *13*(6), 1098. https://doi.org/10.3390/ANI13061098/S1

Vinueza-Burgos, C., Wautier, M., Martiny, D., Cisneros, M., Van Damme, I., & De Zutter, L. (2017). Prevalence, antimicrobial resistance and genetic diversity of Campylobacter coli and Campylobacter jejuni in Ecuadorian broilers at slaughter age. *Poultry Science*, *96*(7), 2366. https://doi.org/10.3382/PS/PEW487

Waldram, A., Vivancos, R., Hartley, C., & Lamden, K. (2017). Prevalence of Giardia infection in households of Giardia cases and risk factors for household transmission. *BMC Infectious Diseases*, *17*(1), 1–7. https://doi.org/10.1186/s12879-017-2586-3

Wang, A., Ruch-Gallie, R., Scorza, V., Lin, P., & Lappin, M. R. (2012). Prevalence of Giardia and Cryptosporidium species in dog park attending dogs compared to non-dog park attending dogs in one region of Colorado. *Veterinary Parasitology*, *184*(2–4), 335–340. https://doi.org/10.1016/J.VETPAR.2011.08.019

Xiao, L. (2010). Molecular epidemiology of cryptosporidiosis: an update. *Experimental Parasitology*, *124*(1), 80–89. https://doi.org/10.1016/J.EXPPARA.2009.03.018

Xiao, L., Cama, V. A., Cabrera, L., Ortega, Y., Pearson, J., & Gilman, R. H. (2007). Possible transmission of Cryptosporidium canis among children and a dog in a household. *Journal of Clinical Microbiology*, *45*(6), 2014–2016. https://doi.org/10.1128/JCM.00503-07

Yildiz, M., Sahin, O., & Adiguzel, M. C. (2023). Prevalence and antimicrobial resistance of Campylobacter species in shelter-housed healthy and diarrheic cats and dogs in Turkey. *Veterinary Medicine and Science*. https://doi.org/10.1002/vms3.1327

Yun, C. S., Moon, B. Y., Lee, K., Kang, S. M., Ku, B. K., & Hwang, M. H. (2023). The detection and phylogenetic characterization of Cryptosporidium, Cystoisospora, and Giardia duodenalis of cats in South Korea. *Frontiers in Cellular and Infection Microbiology*, *13*, 1296118. https://doi.org/10.3389/fcimb.2023.1296118

Annexes

Annex 1

OFICIO: 2017-011

Dra. Stella de la Torre USFQ
Dr. Antonio Machado USFQ,
Santiago Andrade USFQ
Dra. Sonia Zapata USFQ
Presente.-

Estimados investigadores:

Por medio de la presente, tengo a bien informarle que se ha procedido a la evaluación de su protocolo de toma de muestras titulado "***Factores ambientales relacionados con la prevalencia de parásitos con potencial zoonótico en perros y gatos del Distrito Metropolitano de Quito***", y después de evaluar el caso a partir de las aclaratorias solicitadas en relación con el cumplimiento de las Normas de Bienestar Animal y de las recomendaciones de Reemplazo, Reducción y Refinamiento en la investigación con animales, propuestas por Russell y Burch (1959), se ha decidido **APROBAR** el mencionado proyecto.

Así mismo, se le informa que **este aval cubre únicamente los aspectos relacionados con el respeto a los principios y normativas vigentes acerca del bienestar animal en la investigación y docencia, tal como se redactaron en el protocolo aprobado**. Si se incumple o cambia sin previo aviso cualquier aspecto de su diseño experimental de manera que afecte las Normas y Recomendaciones arriba mencionadas, el aval se considerará nulo. Otros cambios en el diseño metodológico no anulan este aval por no corresponder a las competencias de este comité.

Sin otro particular al cual hacer referencia, y deseándole todo el éxito posible, me despido,

Atentamente,

Francisco Cabrera
Presidente
Comité de Ética en el Uso de Animales en Investigación y Docencia de la USFQ

Annex 2

Fecha **Encuesta**

Edad del animal **Nombre del animal**

Especie **Sexo**

Por favor marque con una X en la casilla correspondiente

	SI	NO
1. Su animal ha tenido vómitos y/o diarreas en las dos últimas semanas		
2. En las dos últimas semanas su animal tuvo tratamiento con antibióticos		
3. Su animal fue desparasitado hace 1 mes más o menos		
4. Su animal convive con más perros/gatos		
5. Su animal convive con otros animales (aves, vacas o cerdos)		
6. Su animal consume alimento balanceado		
7. Su animal consume comida hecha en casa		
8. Su animal fue vacunado hace un mes más o menos		
9. Su animal tiene un recipiente para tomar agua y comida		
10. Su animal toma agua de otras fuentes (ríos, sequias o agua empozada)		
11. Su animal ha capturado alguna vez aves, ratones, otros animales o carroña		
12. Su animal sale solo, sin una persona que lo supervise, a la calle o al campo		

Supplementary material

Supplementary Table S1. Location of sampling points collected in the present study.

PARISH	NEIGHBOURHOOD	STREET	COORDINATES
PUELLARO	PUELLARO	24 DE MAYO	-0.065230, -78.402983
TUMBACO	TUMBACO	DE LAS AMAPOLAS	-0.182730, -78.388852
CONOCOTO	CONOCOTO	3 TRANSVERSAL	-0.310756, -78.465763
GUAMANI	GUAMANI	OE4A	-0.340372, -78.555810
COCHAPAMBA	ATUCUCHO	CESAR BAQUERO	-0.124756, -78.513623
CALDERON	ZABALA	ROSA MONTUFAR	-0.083486, -78.408157
EL CONDADO	RANCHO ALTO	ECOVIA -CALLE A	-0.104793, -78.525436
LLANO CHICO	LLANO CHICO	TOBIAS REINOSO	-0.129926, -78.447325
SAN BARTOLO	QUITO SUR	GASPAR CUJIAS	-0.259792, -78.532069
CALDERON	MONCAYO	OE23A	0.079201, -78.528603
SAN ANTONIO	ALTAR DEL PULU-LAHUA	DEL TURISMO	0.012747, -78.450349
LA ECUATORIANA	EL TRANSITO	ANDRES PEREZ	-0.301998, -78.571946