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Environmental *Toxocara* spp. presence in crowded squares and public parks from San Juan Province, Argentina: A call for a "One Health" approach

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Introduction: Canine soil-transmitted helminth (cSTH) parasites need specific environmental conditions to complete their life cycle. *Toxocara canis* and *T. cati* are the most important zoonotic cSTH, since they are the causal agents of human toxocariasis. Canine STHs are dispersed in feces from infected domestic and wildlife canines. In this study, the presence of STH in canine feces was evaluated in 34 crowded public parks and squares from San Juan Province (Argentina).

Methods: Fecal samples were collected during different seasons in 2021–2022 and analyzed by standard coprological methods, including Sheather and Willis flotation and Telemann sedimentation. InfoStat 2020, OpenEpi V. 3.01 and R and RStudio® were used for statistical analysis and QGIS 3.16.10 for mapping.

Results: From a total of 1,121 samples collected, 100 (8.9%) were positive for at least one intestinal parasite (IP) and three cSTH species were detected: *Toxocara* spp., *Toxascaris leonina* and *Trichuris vulpis*. The most prevalent cSTH species was *T. vulpis* (64/1121; 0.057%), while the least prevalent was *Toxocara* spp. (19/1121; 0.017%). The detection of *Toxocara* spp. eggs was significantly different depending on the season. The geo-spatial variation of each cSTH per season is described.

Discussion: This is the first study in San Juan Province to identify environmental contamination of cSTHs in public areas. The specific localization of areas with the presence of cSTH eggs could provide information to guide strategies to reduce the cSTH infection burden in dogs and promote serological screening of the human population for *Toxocara* spp. Given the zoonotic nature of *Toxocara* spp. We hope this information will help to reinforce activities of control programs, focusing on the "One Health" approach.

KEYWORDS

Toxocara canis, soil transmitted helminths, One Health, spatial epidemiology, San Juan, Argentina

1. Introduction

The One Health approach recognizes that the health of humans, domestic and wild animals, and the wider environment (including ecosystems) are closely linked and interdependent; the term aims to sustainably balance and optimize the overall health of our planet and its inhabitants (1). The approach mobilizes multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems, while addressing the collective need for clean water, energy and air, safe and nutritious food, taking action on climate change, and contributing to sustainable development (2). The One Health approach supports global health security by collaboration and communication at the human-animal-environment interface to address shared health threats such as zoonotic diseases, and others. The zoonotic parasitic diseases transmitted by dogs' feces are considered under this approach, since it interferes with animal and human health, and its propagation generally occurs in the environment (3). In addition, the concept of one health contemplates the consequences produced by climate change (1), a determining factor in the transmission of canine soil-transmitted helminths (cSTH).

The high number of free-roaming dogs found in urban areas can serve as a source of pathogens which may be dangerous to humans; dogs can act as definitive hosts for a high number of parasites (3), some of which are considered zoonotic because they can cause disease in humans. *Toxocara canis*, *Toxocara cati*, and *Ancylostoma caninum* are, respectively, the primary species of zoonotic cSTHs. Other species of non-zoonotic cSTHs could also be present, e.g., *Toxascaris leonina*, and *T. vulpis* (4, 5), although they are not dangerous for humans, they do have an effect on animal health. For this reason, epidemiological studies aid in determining the parasitological status of the population, parasite burden and potential risk areas (6).

Toxocariasis is a parasitic disease transmitted usually from dogs and/or cats that are infected with *T. canis* and *T. cati* (3), to humans. Hosts include cats, dogs, foxes, coyotes, and wolves. These hosts harbor the nematodes in their guts, shedding the eggs in their feces. The embryonated eggs remain infectious for years outside the host. In the wild, carnivorous animals such as cats and dogs consume infected meat or simply soil containing the eggs, and the parasite persists in their gut. Additionally, transplacental transmission has been documented in dogs and cats (7, 8).

In general, individuals infected with these species are asymptomatic, but some develop clinical syndromes which include visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocariasis (NT) and covert/common toxocariasis (CT) and can associate with allergic, neurological and/or visual disorders, or cognitive and intellectual deficits in children (9). Recent epidemiological research has estimated that ~1.4 billion people worldwide (10), particularly in subtropical and tropical regions, are infected with, or exposed to *Toxocara* species, indicating that human toxocariasis is a neglected tropical disease (NTD). Diagnosis in humans is based on clinical, epidemiological, and serological data. Indirect IgG ELISA is a widely used serological method for toxocariasis and western blots can be used to confirm positive ELISA findings to reduce false-positive results (11).

Embryonated *Toxocara* spp. eggs in the environment are considered as the most important source of human toxocariasis. These eggs, however, are also a source of infection for definitive and

paratenic hosts (12). To become infective, *Toxocara* spp. eggs need specific conditions of temperature and soil (13, 14), which are present in public squares and parks from different tropical and subtropical countries (15). In this study, canine fecal samples from different parks and squares from San Juan Province, Argentina, were analyzed. The samples were collected during the four seasons (autumn, winter, spring, and summer) in each selected area, with the aim to estimate the association between seasons, weather, presence of cSTH eggs and zoonotic risk.

2. Methodology

2.1. Study area

This study was carried out in the urban area of San Juan Capital (−31.54, −68.52), in the homonymous province; the main squares and parks were included. This area encompasses a surface of 239.12 km². It has about 450,000 inhabitants with a population density of approximately 1880 inhabitants/km². The elevation of the city is ~650 m and it is located in a valley at the eastern border of the Andes Mountain range. According to the Köppen-Geiger climate classification (16, 17), San Juan has an arid climate (BWh/BWk), with low rainfall (<20 mm on any given month), significant diurnal and annual temperature variation (ranging from an average of 32°C in January to 8°C in July), while the average annual temperature is 18°C. The most populated areas were selected for sampling.

2.2. Sample collection and coprological analysis

During 2021 and 2022, fresh canine fecal samples from each square and park were collected during each season: autumn (Epidemiological Week – EW – 21 of 2021/May), winter (EW 32/2021/August), spring (EW 45/2021/November) and summer (EW 8/2022/February). The entire samples were collected in pre-labeled plastic bags and subsequently inactivated at −20°C for 2 weeks. Each sample was homogenized, and 10 grams were used and processed using three different concentration methods; two different flotation techniques, Sheather method (saturated sugar solution, 1.25 specific gravity) and Willis method (saturated NaCl solution, 1.20 specific gravity) as well as a sedimentation technique (Telemann method) (18). The techniques chosen for this study are standard concentration techniques that increase the chances of detecting intestinal parasitic structures, including helminth parasites such as *Toxocara* spp. Each sample was microscopically examined at 100× and 400× magnifications. The identification of *Toxocara* spp. eggs was performed using morphological reference (19). Samples were classified as positive if the presence of eggs was confirmed (20).

2.3. Statistical analysis

This is a descriptive, cross-sectional, and observational study. The aim was exploratory and descriptive, focused on finding possible associations between the presence of cSTH, specifically *Toxocara* spp. and location and characteristics of the squares and the seasons. The

association was examined through χ^2 tests, using InfoStat® V.19 software (21). The parasitic prevalence was calculated and their association with season, department and square/park was analyzed. The Risk Ratio (RR) and Odds Ratio (OR), with 95% Confidence Interval (CI), of statistically significant associations were obtained using OpenEpi V. 3.01 (22).

To explore the distribution characteristics of the most prevalent parasites found, a calculation between observed and expected cases was performed assuming a uniform distribution of positive cases, using R and RStudio®.

2.4. Spatial analysis

Given the low number of *Toxocara* spp. positive cases found during the study, a correlation analysis was performed using only *Toxocara* spp. positive parks ($N=12$). The correlation between its presence and a composite remote sensed index, which can be identified as a proxy for tree shadow, was analyzed. This new index, specifically created for this study, was named the Tree Magnitude Index (TMI) and it is calculated through the multiplication of the Topographic Index Position (TPI) obtained from a Digital Surface Model (DSM) and the Normalized Difference Vegetation Index (NDVI) obtained from satellite imagery. The TMI was treated both as a response variable and as an explanatory one, using the difference between the observed and expected value of positive cases, assuming a homogeneous distribution. High TPI values suggest surface objects that stand out from their surroundings; high NDVI values suggest vigorous vegetation. As a result, when these two factors are multiplied, high values of TMI would indicate high-rise vegetation, such as trees, whereas low values would indicate low and flat lands with little to no vegetation. TMI might therefore be thought of as a tree magnitude index and as a proxy for tree shadows. Only positive NDVI values were taken into consideration to prevent positive outcomes brought on by both negative indices.

The DSM used had a 5m resolution, generated from a photogrammetric aerial survey by the Argentinian IGN (23). The bandwidth for TPI was 100m. NDVI was retrieved from Google Earth Engine (24), and it was computed using Sentinel 2 Level-2A imagery (10m spatial resolution); the values of the image represent the analysis time-span average. The values of TPI (resampled at 10m) and NDVI

were extracted for each pixel, then the product of the two terms was calculated, and finally, the average value of the multiplication of these two indices was computed for each square and park.

2.5. Weather data analysis

Weather data from the nearest weather station, San Juan Airport (12km east of the city center) (25), was retrieved to gauge weather conditions during the 4 weeks of analysis. Seven variables were retrieved: mean temperature (daily average temperature), Diurnal Temperature Variation (DTV), accumulated precipitation, air humidity, cloud cover, solar energy, and wind speed. For every variable, a value for each of the 4 weeks of the analysis was quantified. Values were the weekly average of the rolling mean of the previous 21 days, except for precipitation data, which was the weekly mean accumulation of the previous 21 days. This range was selected due to previous reports stating there were no significant differences detected in the viability of eggs until day 21 (20); maximum infectivity of larvae eggs has been reported up to day 30 of incubation (26, 27).

3. Results

3.1. Sample collection and coprological analysis

After less than a year of sampling, 1,121 samples were collected, 271 samples in Autumn, 280 samples in Winter, 342 samples in Spring and 228 samples in the Summer. In 8.9% (100/1121) of them, at least one type of cSTH (*Toxocara* spp., *T. vulpis* and *T. leonina*) was found. *Toxocara* spp. eggs were detected in 0.017% (19/1121) of the samples (Table 1). With respect to the other cSTHs found, the most prevalent species was *Trichuris vulpis* (0.0571%; 64/1121), followed by *Toxascaris leonina* (0.0259%; 29/1121). Twelve samples showed almost one type of co-infection (Table 1). Figure 1 shows the overall number of positive samples of the three STH found per sampling location and the general study area that was included in this study. During the study, 67.6% (23/34) of the squares and parks sampled showed environmental contamination with at least one type of cSTH. The presence of *Toxocara* spp. was detected in 12 of the analyzed squares and parks (35.3%).

TABLE 1 Description of the canine fecal samples collected, prevalence of canine soil-transmitted helminths (cSTH) found in total and per season in San Juan City, San Juan, Argentina (2021–2022).

	Autumn (N=271)	Winter (N=280)	Spring (N=342)	Summer (N=228)	Total (N=1,121)	χ^2 ; d.f.=3	p
Parasite presence (%)	28 (10.33%)*	32 (11.43%)**	25 (7.31%)	15 (6.58%)	100 (8.9%)	5.46	0.1410
<i>Trichuris vulpis</i> presence (%)	13 (4.80%)	24 (8.57%)	16 (4.68%)	11 (4.82%)	64 (5.7%)	5.69	0.1279
<i>Toxascaris leonina</i> presence (%)	11 (4.06%)	9 (3.21%)	6 (1.75%)	3 (1.32%)	29 (2.6%)	5.17	0.1597
<i>Toxocara</i> spp. presence (%)	12 (4.43%)	3 (1.07%)	3 (0.88%)	1 (0.44%)	19 (1.7%)	16.34	<0.001

*There were eight samples which presented co-infection: three between *T. vulpis* and *T. leonina* and 2 between *T. vulpis* and *Toxocara* spp.

**There were four samples which presented co-infection: three between *Toxocara* spp. and *T. leonina*, 3 between *T. vulpis* and *T. leonina* and 1 between *T. vulpis* and *Toxocara* spp.

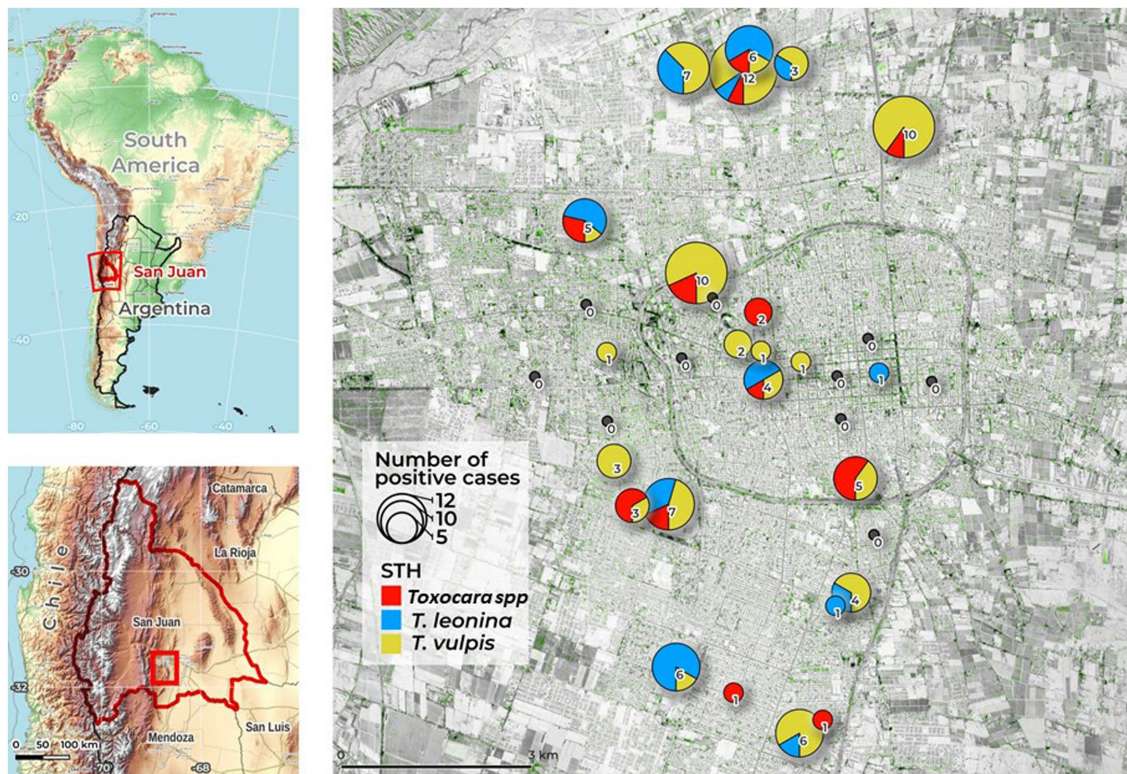


FIGURE 1 Study area of San Juan City (San Juan, Argentina) in the context of South America. Main Map: Cumulative number of canine soil-transmitted helminths (cSTH) found in each sampling location (2021–2022) by species. Map backgrounds: main map from ©2022 Google; inset map from ©OpenStreetMap, ©OpenTopoMap (CC-BY-SA). Map data: August 09, 2022.



FIGURE 2 Mean Tree Magnitude Index (TMI) for four of the 34 sampled squares from San Juan City (San Juan, Argentina).

3.2. Statistical and spatial analysis

The distribution of cSTHs per season was not uniform, being *T. vulpis* the most prevalent in the samples collected in the autumn, winter, and summer, with *T. leonina* being the most prevalent in the spring. The highest prevalence of *Toxocara* spp. was observed in autumn (4.43%; $p < 0.01$); while there was no statistically significant difference in the prevalence of the other

cSTH per season (Table 1). Moreover, the risk and odds ratio analysis showed that there is 5 times greater risk of finding *Toxocara* spp. in dog fecal samples in the autumn compared to the other three seasons (RR = 5.38, 95% CI 2.14–13.5; OR = 5.58, 95% CI: 2.17–14.32). Using simple linear regression, the TMI significantly predicted *Toxocara* spp. prevalence ($R^2 = 0.67$, $F(1, 10) = 23.2$, $p < 0.01$), with the following fitted regression model: Δ Observed-Expected Value = $0.54 + 0.81 \cdot (\text{TMI})$. Figure 2 shows the

mean TMI during 2021 in four of the sampled areas. TMI of the entire study area of San Juan City (San Juan, Argentina) during 2021 is shown in [Supplementary Figure 1](#).

Additionally, the observation of the distribution of the three cSTH found in the different sampled areas, shows that they are heterogeneously and not homogeneously distributed ([Figure 3A](#)). This figure shows those areas where the presence of the parasites is either higher (red) or lower (green) than expected. The presence of *T. vulpis* ([Figure 3B](#)) was detected in 19 out of 32 (59.4%) of the parks and squares sampled; its presence was higher than expected in 5 of them. Although *T. leonina* was detected in 12 of the sites sampled (37.5%), its presence was higher than expected in 6 of these ([Figure 3C](#)). Like

T. leonina, *Toxocara* spp. was found in 12 of the parks and squares samples and its presence was higher than expected in 6 of these sites ([Figure 3D](#)). The difference between observed and expected values, assuming a homogeneous distribution, for each sampled area, is presented in [Table 2](#).

3.3. Weather data analysis

As previously stated, given that the detection of *Toxocara* spp. eggs was significantly more frequent during the autumn, the weather data was explored to identify any characteristics that might be driving this

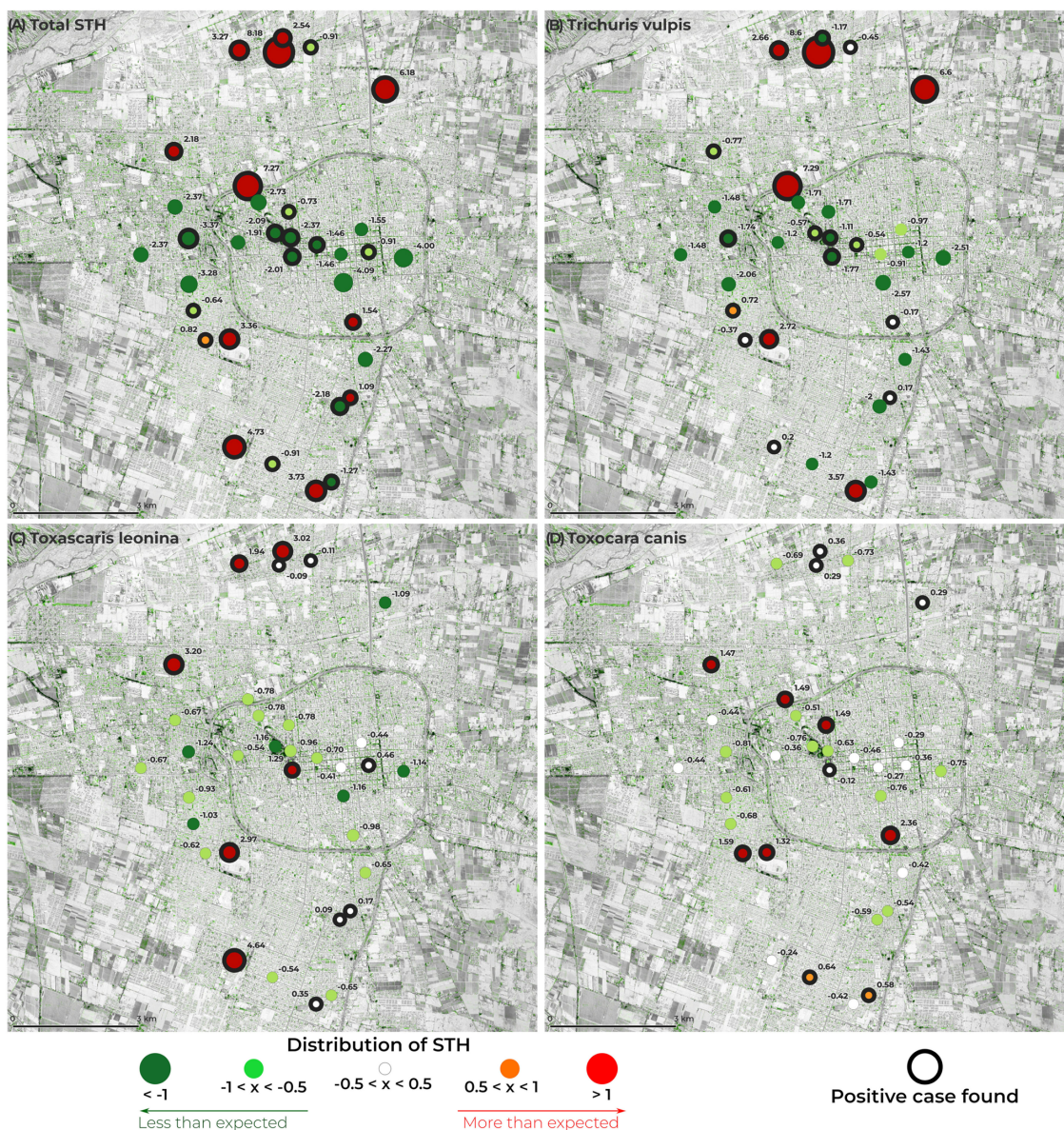


FIGURE 3
 Distribution of canine soil-transmitted helminths (STH) in the study area of the city of San Juan (San Juan, Argentina). (A) Canine STH as a group. (B) *Trichuris vulpis*. (C) *Toxascaris leonina*. (D) *Toxocara* spp. Circles in red represent positive values where observed cases were higher than expected. Circles in green represent positive values where observed cases were less than the expected. In white, the difference between observed and expected values was not different. The circles with a black border represent those sites where positive samples were detected.

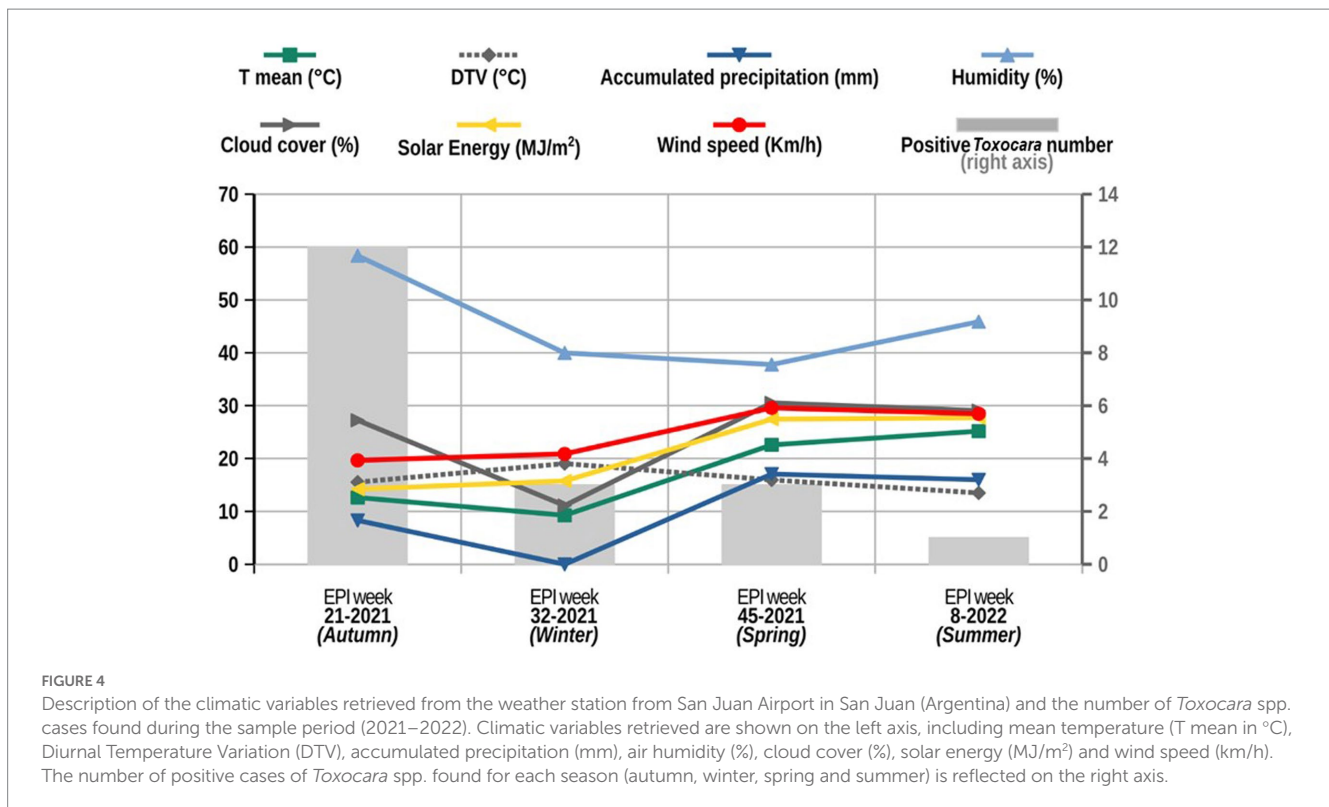
TABLE 2 The difference between observed and expected values, assuming a homogeneous distribution, of positive cases of canine soil-transmitted helminths (cSTHs) in canine environmental feces collected from different squares and parks from San Juan City (San Juan Province, Argentina).

Number	Difference from expected				
	Name	Total cSTH	<i>Trichuris vulpis</i>	<i>Toxascaris leonina</i>	<i>Toxocara</i> spp.
1	Plaza B° Ramos	2.54	-1.17	3.02	0.36
2	Plaza B° Chimbas	8.18	8.6	-0.09	0.29
3	Plaza Dep.Chimbas	-0.91	-0.45	-0.11	-0.73
4	Plaza B° Güemes	3.27	2.66	1.94	-0.69
5	Plaza B° Los Andes	6.18	6.6	-1.09	0.29
6	Plaza Barrio Aramburu	2.18	-0.77	3.2	1.47
7	Plaza Barrio del Carmen	7.27	7.29	-0.78	1.49
8	Plaza Ejército Argentino	-2.73	-1.71	-0.78	-0.51
9	Plaza Cementerio	-0.73	-1.71	-0.78	1.49
10	Parque de Mayo	-2.09	-0.57	-1.16	-0.76
11	Plaza España	-2.37	-1.11	-0.96	-0.63
12	Centro Cívico - Teatro	-2.01	-1.77	1.29	-0.12
13	Plaza Laprida	-1.46	-0.54	-0.7	-0.46
14	Plaza 25 de Mayo	-1.46	-0.91	-0.41	-0.27
15	Plaza Gertrudis Funes	-1.55	-0.97	-0.44	-0.29
16	Plaza Aberastain	-0.91	-1.2	0.46	-0.36
17	Zona Terminal	-4	-2.51	-1.14	-0.75
18	Plaza Hipólito Yrigoyen	-4.09	-2.57	-1.16	-0.76
19	Plaza Italia	-1.91	-1.2	-0.54	-0.36
20	Plaza Manuel Belgrano	-3.37	-1.74	-1.24	-0.81
21	Plaza Desamparados	-2.37	-1.48	-0.67	-0.44
22	Plaza Villa San Roque	-2.37	-1.48	-0.67	-0.44
23	Plaza Barrio Bancario	-3.28	-2.06	-0.93	-0.61
24	Pza. Barrio Foeva	-0.64	0.72	-1.03	-0.68
25	Pza. 2 Jardín Policial	0.82	-0.37	-0.62	1.59
26	Pza. Villa Sta. Anita	3.36	2.72	2.97	1.32
27	Plza Barrio La Estación	4.73	0.2	4.64	-0.24
28	Plaza Centenario	-0.91	-1.2	-0.54	0.64
29	Plaza B° San Ricardo	3.73	3.57	0.35	-0.42
30	Plaza Grillo	-1.27	-1.43	-0.65	0.58
31	Plaza Villa Fleuri	-2.18	-2	0.09	-0.59
32	Plaza Villa Lerga	1.09	0.17	0.17	-0.54
33	Plaza Echeagaray	-2.27	-1.43	-0.65	-0.42
34	Plaza Almirante Brown	1.54	-0.17	-0.98	2.36

difference. The analysis of the different climatic variables (Figure 4) showed that for EW 21/2021, the air humidity was notably higher, while the wind speed and solar energy were somewhat lower. These weather features might be involved in the higher prevalence of *Toxocara* spp. eggs observed during the autumn given that high humidity, low wind speed and low solar radiation are a good combination of weather factors for the survival of *Toxocara* spp. eggs in the soil (28–32).

4. Discussion and conclusion

Through the sampling of fecal canine samples collected from 34 of the main urban parks and squares of the City of San Juan (San Juan, Argentina), the presence of different cSTH species was detected, including *Toxocara* spp., which is a zoonotic parasite that poses a risk to humans. The overall prevalence of cSTH found in this study was 8.9%, which is lower than the prevalence reported in other studies



from urban areas in Argentina (33–36), and similar to the prevalence reported in Ushuaia (37) (Supplementary Table 1). Herein, the most prevalent cSTH was *T. vulpis*, while in other studies the most prevalent species found was *A. caninum* (33, 35, 38–40). The same pattern was observed in other countries such as Australia and Nigeria, where this hookworm species was the most prevalent (41, 42). In some of these studies, infection by protozoan species was higher than helminth infections (37); in this study, protozoan parasites were not detected, although the modified Ziehl-Neelsen technique (43), which is more sensitive, was not used.

In this study, *T. vulpis* was the most prevalent cSTH, this could be due to the longer survival time of eggs of this species in the soil; this might be increasing the chances for dogs that frequent the parks/squares to become reinfected (44). Moreover, *T. vulpis* has a prepatent period of 3 months, therefore antiparasitic treatment should be routinely repeated at monthly intervals to kill all the worms as they mature and prevent contamination of the environment (3). On the other hand, *Toxocara* spp. and *T. leonina* require only a few weeks to mature (1–2 months), and with a second dose of anthelmintic administered 2 or 3 weeks after the first one, the dogs would be free of all the worms. Considering that the samples analyzed herein are from the environment and that the status of each definitive host is unknown, we could assume that canine deworming is either not being performed or not given in periodic intervals.

Unfortunately, since fecal environmental samples were used, the association with characteristics of the dogs themselves (i.e., age, free-roaming or kept, underlying conditions, among others) (45) and with the conduct of care of the owners (i.e., antiparasitic treatment) (46) could not be considered. Moreover, the setting where this study was conducted was urban and the prevalence and variety of parasites

found might be greater in rural areas where there is also exposure to other animals (4, 47, 48).

Additionally, differences in prevalence could also be due to the climatic and soil conditions of San Juan, given that it is an area with very low precipitation and other studies have shown that the average amount of rainfall was found to be strongly associated with the environmental contamination of parks with cSTH (41). Through the analysis of the association between the presence of *Toxocara* spp. and environmental characteristics, in this study, the regression analysis revealed that shadow significantly contributes to the increased prevalence of the parasites as measured by the TMI, as previously observed in other studies (28–32). The presence of trees and their shadows, along with other factors like irrigation and management of the park (not considered in this study), could create an ecological urban niche for the parasites to develop in the soil regardless of the general dry environment of San Juan. The significantly higher prevalence of *Toxocara* spp. observed in the autumn coincides with increased air humidity, lower wind speed and sun radiation, these environmental conditions could potentially facilitate transmission of *Toxocara* spp. eggs. This was confirmed under laboratory conditions in a previous study (32). In addition, other studies have shown that Argentina and Brazil have optimal humidity conditions for the development of *Toxocara* spp. eggs (49–51). Nevertheless, extreme temperatures (high or low) are also important as they can lead to desiccation of eggs and larval stages or arrested development of infective stages in the environment (52).

The cSTH species found herein were not homogeneously spread throughout the city, and there were areas that had conditions that were more appropriate for the transmission from one dog to another. In general, the cSTH detected in this study were found in the areas surrounding the Capital Department. Nonetheless, when analyzing per

species, the prevalence of *Toxocara* spp. was greater than expected within the Capital Department, which is the most densely populated. These areas with the detection of a higher prevalence of *Toxocara* spp. than expected may be used to guide public health measures for screening of antibodies specific for *Toxocara* spp. in humans, especially children, given that Toxocariasis is a silent disease that could be acquired during infancy and have severe consequences (9). Further studies could be conducted to determine the possible risk factors associated with these areas (53). Unfortunately, evaluation of the egg's viability and ability to become infective (54) was not performed, future studies must be conducted to evaluate these, given its implications on the risk to public health.

The regular administration of anthelmintic treatments and the promotion of responsible dog ownership, including picking up dog feces and hand hygiene are important measures which need to be adopted to minimize environmental contamination with *Toxocara* spp. and other STHs (41). Multidisciplinary research, formulated under "one health approaches" can deliver reinforced tools for exploring zoonotic parasites, including cSTHs (46).

Due to the low number of public squares and parks studied herein ($N = 34$), future studies with a higher number of squares and public parks should be conducted to improve the correlations analysis.

This is the first study in San Juan, Argentina to describe the presence of cSTH parasite species in public areas. The specific localization of squares and parks infected with cSTH eggs aim to provide information to design strategies to lower the cSTH infection burden in dogs and to provide information to direct serological screening of the human population, specifically for *Toxocara* spp. Given the zoonotic nature of these cSTHs we hope this information will help to reinforce activities of control programs, focusing on the "One Health" approach.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

HA, VP, and MP: conceptualization. MP, HA, LS, PA, and VP: methodology. HA and MP: formal analysis. HA, PA, and SM: investigation. HA, LS, and MP: data curation. GG: statistical analysis. LS: spatial analysis. HA, GG, and LS: writing—original draft preparation. MP: writing—review and editing. VP and MP:

supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmed.2023.1102396/full#supplementary-material>

SUPPLEMENTARY FIGURE 1

Mean Tree Magnitude Index (TMI) of the study area of San Juan City (San 473 Juan, Argentina) during 2021. The squares and parks where the samples were collected are 474 marked in red.

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