The architectural design of urban space and its influence in the communities of parasites in two areas of Buenos Aires City with different circulation dynamic of companion animals

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RESUMEN

Las excretas de mascotas constituyen un factor de riesgo para la transmisión urbana de zoonosis parasitarias. La abundancia de excretas depende del número de animales, de la posibilidad de acceso y el uso de distintos espacios urbanos. En plazas y parques públicos los animales deambulan acompañados por personas; en parques y jardines privados, el acceso y circulación está restringido a aquellos animales cuyos dueños habitan en el predio. El objetivo de este trabajo fue determinar si las barreras artificiales antropogénicas, condicionantes del tránsito de animales, limitan la dispersión de enteroparásitos. En áreas con desplazamiento restringido (BPLA) y con circulación libre (PP), fueron colectadas heces (BPLA: n=39; PP: n=50) y suelo (BPLA: n=20; PP: n=20) y procesadas por métodos convencionales. La frecuencia de especies en cada ambiente y para cada tipo de muestra fue comparada por el test de Diferencia de Proporciones. Las diferencias entre el número de especies parasitarias en cada matriz ambiental fueron establecidas con el test de Mann-Whitney (alfa=0,05). La similitud de las comunidades fue determinada con el Índice cualitativo de Sorensen (ICS). La proporción de muestras positivas en BPLA fue mayor que en PP (Tierra: 1,0 vs. 0,70; $p<0,05$

y Heces 0,56 vs. 0,32; p<0,05). En suelo, no se detectaron helmintos; el número de especies por unidad de muestreo fue mayor en BPLA que en PP (Mann-Whitney: U=300; p<0,01) así como la frecuencia relativa de *Cryptosporidium* sp. (1,0 vs. 0,56; p=0,0006); la riqueza de especies fue similar (ICS=0,8). El confinamiento de animales domésticos urbanos y la restricción de su desplazamiento al espacio verde cercado de su peridomicilio determinan el grado de contaminación fecal del suelo, agravado por el mal hábito higiénico de los propietarios. En estas situaciones, el suelo concentraría algunas formas infectivas y facilitaría su transmisión, realimentando los ciclos de infección y reinfección parasitaria.
PALABRAS CLAVE: parásitos intestinales, arquitectura urbana, movilidad del hospedador, comunidad de parásitos, muestras ambientales.

ABSTRACT

The presence of canine and feline faeces is a known risk factor for the transmission of zoonotic parasitoses. Their dispersion is tightly linked to the mobility of its hosts and their capability of contaminating such environments with faecal matter, and the latter will be clearly influenced by the ability of entering and exiting such area. Different uses of the urban space where the hosts inhabit may affect precisely that aptitude. It was hypothesized that certain artificial barriers created by urban settings, such as walls and fences, could limit the dispersion of parasitoses as they would limit the mobility of their hosts. The objective of this study was to determine if there were any differences between the communities of intestinal parasites found in excreta and soil samples within two bordering areas, one with restricted circulation (BPLA) and another one without such restrictions (PP). Faecal (BPLA: n=39; PP: n=50) and soil (BPLA: n=20; PP: n=20) samples were collected, processed according to the Willis and the Bacigalupo–Rivero techniques, and diagnosed by optical microscopy of fresh smears, lugol, Kinyoun and modified Ziehl-Neelsen stain. In order to establish statistical differences between the frequencies for all parasite species found in either environment for each sample type, the two populations' Difference in Proportions Test was performed. To assess communities' similarity, the Sorensen index was utilized and to determine if there were differences between the number of different species per sample the Mann–Whitney test was applied. Statistical differences were found in the total frequency of faeces and soil samples positive for any parasitic form, being higher in BPLA in both cases (p<0, 05). On the other hand, there were no statistical differences for any particular species in any of the two sample types studied, with the exception of *Cryptosporidium* sp. that proved to be higher in BPLA soil samples (p<0,01). The Sorensen index to compare the similarity among faecal and soil

communities found in each urban environment were both 0,8. However, the Mann-Whitney test showed that there were a statistically major number of species per soil sample in BPLA than in PP. When taking these results into consideration, it could be inferred that urban architectonic barriers restricting pet displacements tend toward a raise of faecal contamination, thus increasing the chance of transmission of the studied pathogens, and accelerating cycles of transmission and reinfection.
KEY WORDS: intestinal parasites, environmental samples, host mobility, parasite communities, urban architecture.

INTRODUCTION

The presence of canine and feline faeces in public spaces constitutes by itself a public health issue, as they can contain diverse parasitic stages (eggs, oocysts, larvae) which are readily adapted to withstand adverse environmental conditions and maintain its infective capacity throughout several years [1, 2]. Daily faecal excretion of a single dog reaches, on average, 100 grams of faeces which can be easily found in sidewalks, parks, residential gardens and streets, among others common spaces [3, 4]. As a consequence of the bad habit of some pet owners, not picked-up faeces may be spread by rain water, wind or diverse anthropogenic actions, favoring the potential dispersion of viable pathogens [3-5]. The census of companion animals performed in the Ciudad Autónoma de Buenos Aires (CABA) by the Zoonosis Institute Luis Pasteur among 1994 and 2003, estimated a total of 429.615 dogs inhabiting within the city, which deposit roughly 43 tons of faeces throughout all public and residential spaces [6]. These numbers clearly show the seriousness involved in the dispersal of potentially zoonotic parasites, which may result in a major health concern for animal and human populations. In addition, the ineffective education regarding health and hygiene, the lack of interest of pet owners in collecting droppings, the limited extent of veterinary sanitary programs, the elevated number of stray dogs and the presence of diverse zoonotic agents constitute the main risk factors, that, associated with the cohabitation with dogs and cats, favor the transmission of enteric parasitoses [7]. Therefore, it is essential to ascertain the richness of the parasitic species community which infects domestic animals. The variety and abundance of certain species could depend on environmental factors (temperature, pH, humidity, soil), availability of intermediate and definitive hosts, the parasite's infective capability and intrinsic characteristics of their hosts [8]. However, the effect of the urban architectonic design should also be considered. Whenever a fast and growing urbanization process is accompanied by a reduction of green areas, transmission of zoonotic parasitoses could be restricted because of the environmental unsuitability to support the survival and development of infective parasite stages [9, 10].

Therefore, it comes into question whether those artificial barriers could affect the richness of species and the frequency of any given intestinal parasite. The aim of the present work was to compare the communities of intestinal parasites of two bordering areas with different restrictions on access and movement for companion animals, potential hosts of parasitic species.

MATHERIALS AND METHODS

Study area

Barrio Parque Los Andes (from now on mentioned as BPLA) is a middle class residential complex located in the neighborhood La Chacarita, CABA, Argentina (34°35′27.4″S 58°26′58.5″W) (Figures 1 and 2). Seventeen buildings of apartments are distributed on the edges of the aforementioned city block (surface 1.3 ha). A common area in-between consists of circulation lanes, which are either asphalted or tiled, and several patches of grass with abundant vegetation and trees that provide shade. Data of a census of companion animals conducted by the homeowners association in early 2010 revealed that 28 domestic animals, all cats and dogs, lived in the premises of that complex.

BPLA is partially isolated from its surroundings by 1 m height brick walls with fences on top, doors and gates with iron bars, all around the perimeter of the block. These barriers would prevent the medium and big sized animals inhabiting the area to circulate outside of it, and also its counterparts from the outer area to enter the premises.

In the vicinity there are two public parks named Los Andes (which will be called PP from now on), surrounded by a fence with gates always open. The parks consist of a series of tile paths which cross the parks in several directions, two playing grounds and several grass areas, altogether covering an area of approximately 4,5 ha (Figure 1).

The remaining suspension was split in two and concentrated by the Bacigalupo–Rivero method. At least two fresh preparations from each tube were examined microscopically so that the diagnosis of each sample required the whole observation of six independent slides. When necessary, parasitic stages were measured with the aid of a calibrated micrometer eye-piece.

Coccidia were diagnosed in faecal and soil smears stained with a modified Ziehl-Neelsen stain. Briefly, smears were fixed with absolute methanol (60 sec), stained with 0.3% carbolfuchsine (5 min), rinsed with water, discolored with 1% H_2SO_4 in absolute ethanol (90 sec), rinsed again and counterstained with 1% methylene blue (90 sec). Two hundred microscopic fields (1000x magnification) were observed before registering a negative result for *Cryptosporidium* sp. or *Cyclospora* sp.

Data presentation and statistical analysis

Frequencies for each parasitic species as well as the richness of species were calculated in environmental matrices, soil and faeces, drawn from each study area, BPLA and PP.

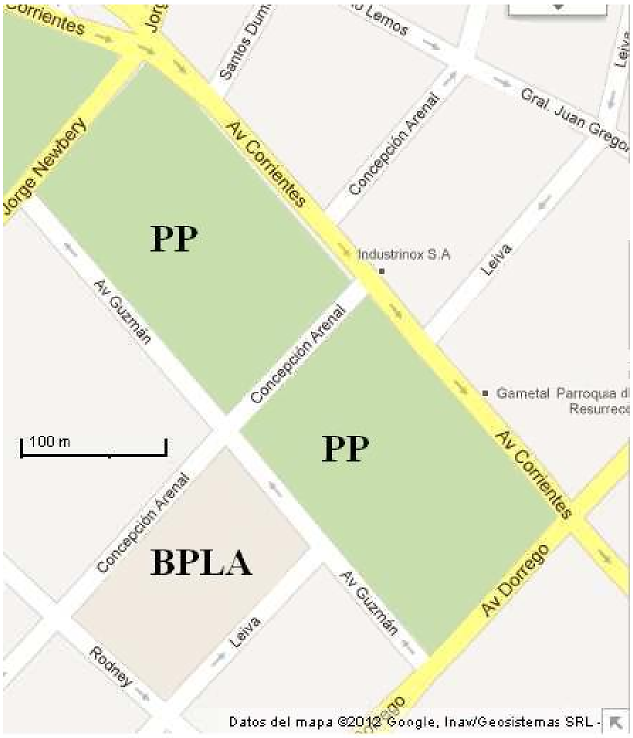

Figure 1. Map of the study area. The proximity of the residential complex “Barrio Parque Los Andes” (BPLA) and the public parks named “Los Andes” (PP) can be appreciated.

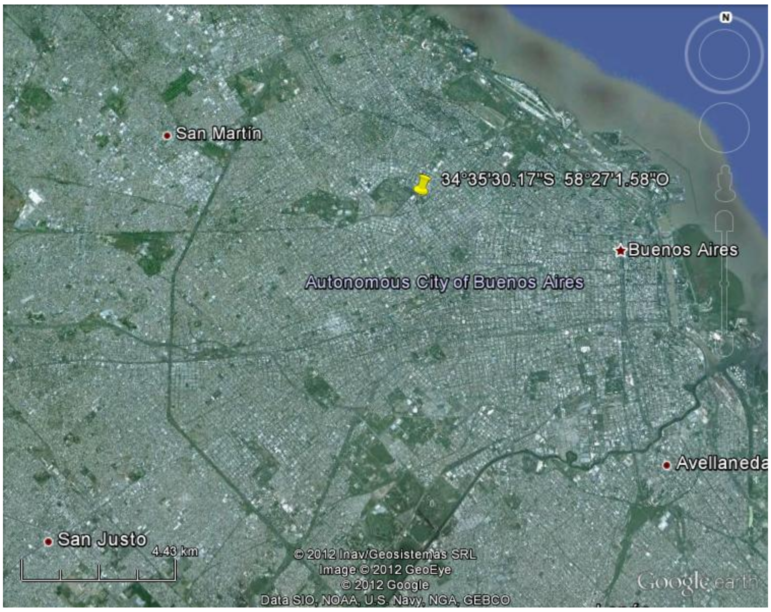

Figure 2. Map of the city showing the location of the study area. Source: Google Earth

Qualitative similarity in the intestinal parasite communities was explored with the Sorensen's index (SQI) by comparing species in a particular matrix (faeces or soil) from different zones (BPLA and PP) and different matrices (faeces and soil) form each zone (BPLA or PP) [12].

Differences between frequencies were proved at a significance level alpha = 0.05, by applying the test of Difference in Proportions (based on Fisher's exact test), whose null hypothesis states the equality of two population proportions for two independent groups. The Mann Whitney U-test was applied to assess differences between the numbers of species found per sample. Data analysis was performed employing the statistical software *Infostat* [13].

RESULTS

The visual inspection of the study areas showed evident differences. In BPLA, it was noted a marked predominance of land covered with grass, herbs and plants whereas in PP, most of the soil was stripped of vegetation and displayed obvious signs of erosion by anthropogenic action. Unlike BPLA gardens where no pets were viewed during the sample collection days, in PP always were sighted a few dogs and cats, be they stray or accompanied by their owners.

Notwithstanding, the density of stool samples was higher in BPLA than in PP. It was observed also, that faeces collected in the former area were fresher and those from the latter were drier and seemed to have been during more time in the environment.

The frequencies for all parasites found in stool and soil samples are shown in Table I.

No significant differences were found when comparing frequencies of single parasite species in fecal samples collected in any environment (BPLA and PP). On considering soil samples, *Cryptosporidium* sp. was the only species detected in every sample from BPLA (Frequency=1.0; n=20) and statistically different form the frequency found in PP (Frequency=0.56; n=50; p=0.0006). Interestingly, no helmith eggs or larvaes were observed in any soil sample studied.

On the other hand, statistical differences were observed when comparing the proportion of positive samples as a whole in both, faeces (Frequency-BPLA=0.56 versus Frequency-PP=0.32; p=0.0305) and soil (Frequency-BPLA=1.00 versus Frequency-PP=0.70; p=0.0101). It is also noteworthy that, no soil sample was negative for parasitic stages in BPLA.

Although 10.3% of stool in BPLA and 20.0% in PP had more than one parasite species, no significant differences were found between them (Difference in Proportions=0.0974; NS) nor when comparing the number of different species in each sample (Mann-Whitney U=1915; NS). In the same way, 40% BPLA and 20% PP soil samples harbored more than one parasite species (Difference in Proportions=0.0200; NS) although the number of different species proved to statistically differ (Mann Whitney U=300; $p<0.01$).

The Sorensen's qualitative index (Table II) showed the parasite species community to be highly similar in fecal (SQI=0.8) and soil samples (SQI=0.8). Interestingly, when studying fecal or soil communities from the same environment (BPLA or PP), the Sorensen's index evidenced limited similarity (Faeces vs. Soil: SQI=0.29 for BPLA and SQI=0.36 for PP, respectively).

CONCLUSSION AND DISCUSSION

The assessment of richness of parasitic communities in different environments is generally limited to the detection of parasitic stages in stool or even the body cavity of living organisms, such as fish [8, 14, 15]. Notwithstanding, the influence of the urban environment in parasitic communities has been poorly studied. In this context, it was interesting to explore the effect of artificial barriers, if any, on the richness of particular intestinal parasite species. In such a way, the communities of intestinal parasites of two bordering areas with different restrictions on access and movement of potential hosts for parasitic species were compared.

The BPLA fenced area with access restricted solely to pets inhabiting the property presented the highest level of environmental contamination. In fact, *Cryptosporidium* sp., coccidian with high zoonotic potential, was demonstrated in all soil samples and a higher proportion of excreta harboring parasites. Despite the above information, qualitative Sorensen index suggest great similarity between faecal and soil samples from both environmental areas.

On the other hand, the richness found in different sample types for the same environment varied considerably, which could be attributed to the intrinsic characteristics of the sample types. The finding of only protozoarian species in soil samples from both study areas could be explained on the basis of the sensitivity of nematode's eggs and larvae to adverse environmental conditions. Those conditions range from climatic factors, such as temperature, pressure, relative humidity, rain and sunlight to the action of birds, fungus and insects among others [16]. However, findings could be contradictory, as viable parasitic forms could be dependant on pH but not on relative humidity, and also the inverse

Table I. Relative frequencies for all diagnosed species in both sample types and and environment with its statistical significance, calculated by applying the test of Difference in Proportions of the statistical software Infostat. nd: not detected

	Soil			Faeces		
	BPLA (n=20)	PP (n=20)	p-value	BPLA (n=39)	PP (n=50)	p-value
Cryptosporidium sp.	1,00	0,55	<0,001	0,28	0,22	NS
Cyclospora sp.	0,00	0,10	NS	nd	nd	
Endolimax nana	0,25	0,30	NS	0,05	0,04	NS
Entamoeba coli	0,15	0,05	NS	nd	nd	
Blastocystis sp.	0,10	0,05	NS	nd	nd	
Isospora sp.	0,15	0,00	NS	nd	nd	
Giardia sp.	nd	nd		0,05	0,12	NS
Chilomastix sp.	nd	nd		0,03	0,06	NS
Trichuris vulpis	nd	nd		0,08	0,02	NS
Toxocara canis	nd	nd		0,05	0,06	NS
Ancylostoma caninum	nd	nd		0,08	0,00	NS
Uncinaria stenocephala	nd	nd		0,03	0,00	NS
Filaroides sp.	nd	nd		0,03	0,00	NS
Total positive samples	1,00	0,70	<0,05	0,56	0,32	<0,05

Table II. Matrix of comparison for species found in each sample type and environment with the correspondent Sorensen index

	BPLA	**PP**	**Sorensen index (rows)**
Feces	*Cryptosporidium* sp. *Giardia* sp. *Chilomastix* sp. *E. nana,* *T. vulpis,* *T. canis,* *A. caninum,* *U. stenocephala* *Filaroides* sp.	*Cryptosporidium* sp. *Giardia* sp. *Chilomastix* sp. *E. nana,* *T. vulpis,* *T. canis*	0,8
Soil	*Cryptosporidium* sp. *E. nana,* *E. coli* *Isospora* sp. *Blastocystis* sp.	*Cryptosporidium* sp. *E. nana,* *E. coli* *Cyclospora* sp. *Blastocystis* sp.	0,8
Sorensen index (columns)	0,29	0,36	

relationship [5, 17]. In addition, the mobility of larvae through soil is extremely low (species with the most mobility do not exceed 50 centimeters of traveled distance), and that said maximum distance can be diminished by the same adverse conditions that affect the survival of larvae [16].

Therefore, it cannot be established linearly the relationship of any given variable, climatic, environmental or else, as a sole determinant of the viability of parasitic forms found in soil. It is clearly a model of multiple variables that influences on the survival of parasitic forms in soil samples, which, in turn, could potentially affect its detection.

In agreement with our results, Rinaldi et al. demonstrated a widespread urban distribution of canine faeces, being more contaminated the high density residential neighborhoods [4].

It could then be inferred, at least provisionally, that the urban artificial barriers generated by different uses of urban space do influence intestinal parasite's dispersion, enabling the increase of parasitic infection and environmental contamination in areas with restricted movement for potential hosts. This augmented contamination could, in effect, act as a positive feedback for transmission cycles of said parasites, resulting in increased possibility of infection and reinfection.

These results reinforce the need for a constant and wider educational campaign towards pet owners and general public in regards to maintaining an adequate environmental hygiene, the correct handling of animal stools and the potential for zoonotic transmission presented by unhygienic habits and environments. It could also be suggested that, in environments with similar characteristics to BPLA, recurring campaigns of massive deworming could discontinue the cycles of transmission generated in those areas, and, along with proper hygiene, it could be reduced the zoonotic transmission of this type of disease.

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