doi: 10.5216/rpt.v53i4.79323

ORIGINAL ARTICLE

PRESENCE OF *Cryptosporidium* SPP. IN CHILDREN AND PET DOGS IN TWO PERIURBAN AREAS IN NORTHEASTERN ARGENTINA

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ABSTRACT

Cryptosporidium is an intracellular coccidian parasite that has been found to infect humans and wild, domestic, and companion animals. This study aimed to examine the presence of *Cryptosporidium* spp. in children and their pet dogs in two periurban neighborhoods in Corrientes City, Argentina. We also evaluated the characteristics of humans, socio-environmental features, and hygiene practices associated with the presence of this protozoan. Socio-environmental variables and children's hygiene practices were assessed through semi-structured questionnaires, which were answered by every family. Samples were concentrated using the sedimentation-flotation technique and stained using the modified Ziehl-Neelsen technique. Fisher's exact test and Multiple Correspondence Analysis were used for data analysis. In total, stool samples of 88 children and 69 pet dogs from 57 households were analyzed. The study showed that 5.6% of children and 11.5% of dogs were parasitized. Simultaneous infection in children and their pet dogs in two households was detected. No statistically significant association was found between socio-environmental, hygiene practices, demographic variables, and *Cryptosporidium* spp. In conclusion, based on our results, the presence of this protozoan is reported in children and their pets, highlighting the need to implement molecular studies to determine the circulating species in the study area and the importance of adopting a One Health approach, because fragmented studies do not allow for a complete understanding of the parasite-host-environment system.

KEY WORDS: *Cryptosporidium* spp.; children and pet dogs; households; Argentina.

INTRODUCTION

Cryptosporidium spp. is an extracytoplasmic intracellular parasite with a worldwide distribution, inhabiting the intestines of humans and a wide variety of vertebrate hosts, among them wild, domestic, and companion

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Received for publication: 21/5/2024. Reviewed: 18/11/2024. Accepted: 21/11/2024.

animals (Fayer, 2010). Reports have indicated that the prevalence of cryptosporidiosis ranges from 0.1% to 73.3% in humans in different parts of the world (Berahmat et al., 2017; Odeniran & Ademola, 2019; Mohebali et al., 2020). Over 44 *Cryptosporidium* species have been identified to date, and most cases of human infections are due to the following species*: C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. mortiferum*, *C. cuniculus*, *C. ubiquitum*, *C. viatorum* and *C. ditrichi* (Beser et al., 2015; Feng et al., 2018; Beser et al., 2020; Ryan et al., 2021; Tumova et al., 2023).

Cryptosporidiosis affects principally immunocompetent and immunocompromised individuals and ranks second to rotavirus infection as a cause of death due to diarrhea in young children under five years in Asia and Africa (Kotloff et al., 2013). The most prominent clinical signs in animals are watery diarrhea, abdominal pain, and vomiting, which can be more severe in young pets (Cai et al., 2021; Li et al., 2021). Therefore, this pathogen represents a relevant problem for public and veterinary health (Zanaro & Garbossa, 2008; Sánchez-Ortega, 2017).

The transmission route of this protozoa is fecal-oral, occurring via ingestion of viable sporulated oocysts, which are highly infectious and resistant to harsh environmental conditions as well as to different disinfectants such as chlorine (Cacciò & Putignani, 2014). Human infection is commonly associated with poor sanitary conditions, consumption of food or water contaminated with fecal matter, and contact with infected persons or animals. Thus, infections can be acquired through several transmission routes, which can be direct, from human to human (anthroponotic transmission), animal to animal, from animal to human (zoonotic transmission), or indirect (waterborne and foodborne) pathways (Abu-Madi et al., 2011).

Direct contact with potentially infected animals is a significant factor in human infections of *Cryptosporidium* spp. (Bouzid et al., 2013). In this sense, pet owner behaviors, such as sleeping with pets, allowing pets to lick faces and hands, and insufficient/ infrequent handwashing can represent a risk in the transmission of this enteric protozoa (animal to human) (Cai et al., 2021; Meng et al., 2021). This hypothesis is supported by previous reports that using genotyping analysis, have suggested potential zoonotic transmissions between owners and pets (dogs and cats) from the same household or workplace (Xiao et al., 2007; Rojas-Lopez et al., 2020; Jiang et al., 2021).

In particular, pet dogs are acknowledged as one of the most frequent hosts of *C. canis.* According to a comprehensive and recently reviewed study, the overall mean prevalence in these pets has been reported at 8.0% using microscopic detection, 7.0% using coproantigen detection, and 6.0% using molecular diagnostic methods (Taghipour et al., 2020; Barbosa et al., 2023). Dogs can also be infected with other species, such as *C. parvum*, which is a more significant public health risk. Zoonotic transmission of *C. parvum*

from dogs has been suggested, however, if dogs are mechanically carrying *C. parvum* due to coprophagy of human or cattle feces or infected (Neira et al., 2010; Gharieb et al., 2018).

South America is considered to have ideal conditions for this microorganism, such as increased rates of poverty, inadequate sanitation, and lack of potable water in many regions (Rosado-Garcia et al., 2017; Sánchez-Ortega, 2017). In Argentina, this protozoan has been previously reported in environmental samples (water for consumption) with a prevalence from 1.3% to 24.6% in children and 1.0% to 20.0% in dogs (Salomon et al., 2007; Soriano et al., 2010; Cerezuela et al., 2017). Particularly, Corrientes province (Northeastern Argentina) has socio-environmental and housing characteristics that could favor the proliferation of this and other parasitosis (INDEC, 2019). In this sense, a recent study has reported high prevalences of gastrointestinal parasites in children and domestic animals in the study area (Alegre et al., 2023). However, the understanding of cryptosporidiosis is limited, making the province an area with great potential for study to assess the presence of this parasite.

This study aimed to investigate the presence of *Cryptosporidium* spp. in children and pet dogs living with them in two periurban neighborhoods in northeastern Argentina. In addition, some characteristics of children (gender and age), socio-environmental features, and hygiene practices are evaluated as possible risk factors for parasitic infection. The results of this study provide valuable information that government agencies can use to design control and prevention strategies for *Cryptosporidium* spp. infection, health policies, and educational programs aimed at reducing its transmission in humans and animals in these areas.

MATERIAL AND METHODS

Study design

This was a cross-sectional, observational, and descriptive study. Contact with the population was made during 2018–2021 through elementary school. All household units whose inhabitants agreed to participate were included. Each household evaluated was considered a unit of analysis (Milano et al., 2007; Cociancic et al., 2018). This work is part of a broader study on intestinal protozoa and helminths in children and domestic animals in the study area (Alegre et al., 2023).

Study area

The study was conducted in two suburban neighborhoods (A and B) in the Capital Department of Corrientes Province (27º28´00” S, 58º50´00” W),

Northeastern Argentina (Figure 1). These neighborhoods are characterized by poor sanitary conditions such as precarious housing, lack of sewer services, inadequate supply of potable water, and high levels of contact with domestic animals. Most of the dogs analyzed had access both to inside the households and their peridomicile. Also, they circulated outside the peridomicile and maintained contact with other pet dogs and animals like cats, horses, pigs, and ducks. Neighborhood A is located on elevated, non-floodable terrain, while neighborhood B is situated along the Paraná River and is prone to flooding.

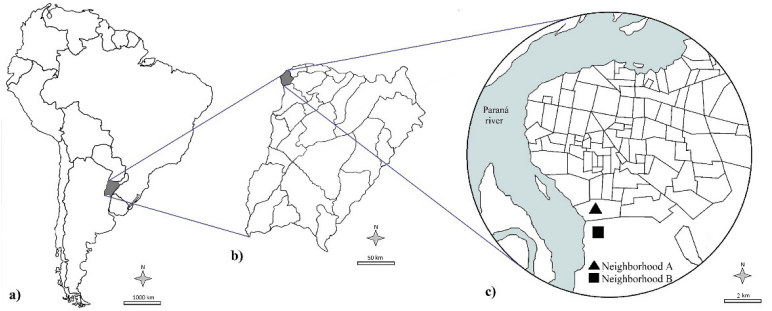

Figure 1. The geographical location of the Province of Corrientes in northeastern Argentina (a), Capital Department (b), and the periurban neighborhoods analyzed (c).

Study population and fecal specimen collection

The study encompassed children and pet dogs. Children aged between 0 and 14 years old of both genders and two age groups were considered: non-schooled children (3 years old and younger) and schooled children (4 years old and older). Each household received a child and pet dog collection kit containing 10% formaldehyde. Oral explanations were given and complemented with illustrated instructions included in the kit. Stool samples were collected by their parents for 5 - 6 consecutive days, while the dog stool samples were collected from animals by their owners or by the research team for 3 - 4 consecutive days. Owners were advised to collect animal feces samples immediately after defecation to prevent contamination.

Socio-environmental and demographic data collection

Data was collected through direct observation of the family environment and semi-structured questionnaires. The data obtained were related to different risk variables or exposure factors including hygiene practices.

Parasitological analysis

All fecal samples were examined at the Laboratory of Parasite Biology, FaCENA-UNNE (Corrientes, Argentina). The samples were processed using sedimentation and flotation concentration techniques (Sheather's solution) (Sheather, 1923). During the flotation technique, distilled water was added to the surface layer to remove excess sugar. The sediment obtained from both techniques was used to prepare a slide smear and perform modified Ziehl-Neelsen staining (Girard de Kaminsky, 2014). Samples were considered positive for *Cryptosporidium* spp. when oocysts were observed exhibiting morphology, optical properties, internal structure, and size consistent with Del Pozo-Perez et al. (2014) descriptions.

Statistical analysis

Overall prevalence was calculated as the number of positive units of analysis and positive parasitized hosts for at least one species divided by the total number of households evaluated and analyzed hosts, expressed in percentages. To compare the two neighborhoods and evaluate the relationship between age group, sex, and parasitosis (presence of *Cryptosporidium* spp.), a Fisher`s Exact Test was used. The relationships between socio-environmental characteristics and parasitosis and the impact of hygiene practices on parasitosis were first evaluated using Multiple Correspondence Analysis. This allows for an overall understanding of the relationships between the variables and subsequently verifying these associations (through a Chi-square test or Fisher`s exact test). Databases were entered and imported to R software (R Core Team, 2022).

Ethical aspects

The study was approved by the Science and Technology Secretariat of Universidad Nacional del Nordeste (PI 20F007), Argentina. This research was also conducted in compliance with Argentine laws. Sample collection was carried out during fieldwork under official permits according to the Universal Declaration of Human Rights of 1948, the ethical standards established by the Nuremberg Code of 1947, the Declaration of Helsinki of 1964, and successive amendments and the provisions of National Law 25,326 on the protection of personal data. All families received written results of the parasitological diagnosis of children. Likewise, a technical report with the results obtained from children and animals was delivered to the corresponding sanitary authorities.

RESULTS

Parasitological analysis

A total of 57 households, 31 in neighborhood A and 26 in neighborhood B, were analyzed. *Cryptosporidium* spp. was detected in 10 households (17.5%). The prevalence of 7.0% and 10.5% were registered in neighborhood A and neighborhood B, respectively. These neighborhood differences were not statistically significant ($p > 0.05$).

Stool samples from 88 children were analyzed, and five positives (5.6%) were found (Figure 2a). The differences observed between the demographic variables (gender and age group) of children were not significant ($p > 0.05$) (Table 1). Concerning pet dogs, 69 stool samples were analyzed, and a prevalence of 11.5% was registered for *Cryptosporidium* spp. (Figure 2b). In two households, simultaneous infections in both types of hosts (human-animal) were found.

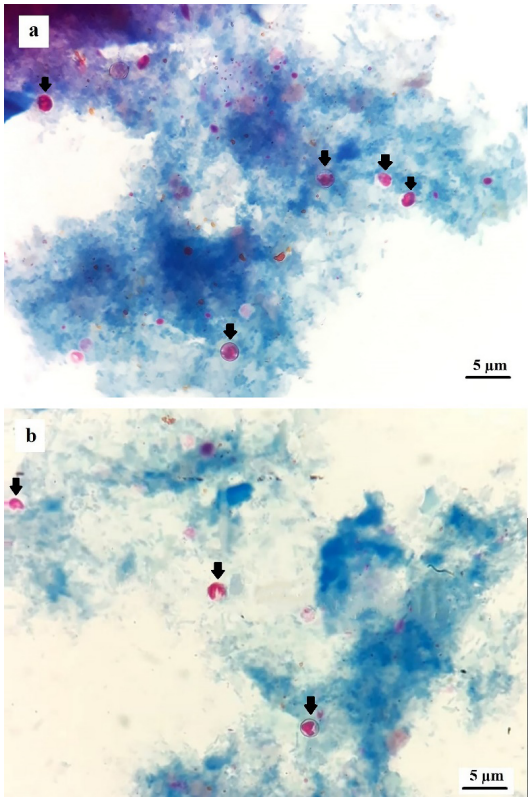

Figure 2. Optical microscope micrographs of *Cryptosporidium* spp. (a) human samples, and (b) pet dog samples. * Black arrows indicate the presence of *Cryptosporidium* spp.

Table 1. The presence of *Cryptosporidium* spp. in children (*n*= 88) and pet dogs (*n*= 69) in two periurban neighborhoods of the Capital Department, Corrientes Province.

Study population	Number of samples analyzed	Number of positive samples (%)
Children	88	5 (5.6)
Gender		
Girls	42	1 (1.1)
Boys	46	4 (4.5)*
Age group		
Non-schooled	22	1 (1.1)
Schooled	66	4 (4.5)*
Pet dogs	69	8 (11.5)*

* Simultaneous infection was identified in two school-aged boys and their pet dogs.

Socio-environmental and hygiene practices analysis

Socio-environmental data from 57 households and personal hygiene practices data from 88 children were obtained (Tables 2 and 3). Statistical associations between socio-environmental and hygiene practices and the presence of *Cryptosporidium* spp. were not recorded.

Table 2. Socio-environmental characteristics of the population in the periurban neighborhoods analyzed in Corrientes city.

Characteristics	Neighborhood A *n* (%)	Neighborhood B *n* (%)
Water supply		
Drilling	-	16 (61.5)
Community stream	30 (96.8)	8 (30.8)
No data	1 (3.2)	2 (7.7)
Flooding		
Never	14 (45.2)	22 (84.6)
Occasionally	16 (51.6)	2 (7.7)
No data	1 (3.2)	2 (7.7)
Disposal of human feces		
Latrine	8 (25.8)	14 (53.8)
Bathroom installed	22 (71.0)	11 (42.3)
No data	1 (3.2)	1 (3.9)
Overcrowding		
With overcrowding	15 (48.4)	16 (61.5)
No overcrowding	16 (51.6)	10 (38.5)

Domestic animals		
With domestic animals	31 (100.0)	26 (100.0)
No domestic animals	-	-
Wastewater disposal		
Cesspool	31 (100.0)	24 (92.3)
Sewer	-	-
No data	-	2 (7.7)
Disposal of animal feces		
Stays on the ground	11 (35.5)	8 (30.8)
Disposed of in bags	14 (45.1)	16 (61.5)
No data	6 (19.4)	2 (7.7)

*Frequency was estimated concerning the total number of houses with socio-environmental data (*n*= 57).

Table 3. Hygiene practices of the children's population in the periurban neighborhoods of Corrientes City.

Hygiene practices	Neighborhood A *n* (%)	Neighborhood B *n* (%)
Frequency of bathing		
More than once a week	4 (6.8)	4 (13.8)
Every day	53 (89.8)	24 (82.8)
No data	2 (3.4)	1 (3.4)
Washing hands before eating		
Occasionally	18 (30.5)	14 (48.3)
Always	36 (61.0)	15 (51.7)
Never	2 (3.4)	-
No data	3 (5.1)	-
Washing hands after going to the bathroom		
Occasionally	18 (30.5)	14 (48.3)
Always	36 (61.0)	15 (51.7)
Never	2 (3.4)	-
No data	3 (5.1)	-
Nail maintenance		
Short	45 (76.3)	27 (93.1)
Long	12 (20.3)	2 (6.9)
No data	2 (3.4)	-

*Frequency was estimated concerning the total number of children with hygiene practices data (*n*= 88).

DISCUSSION

In this study, 5.6% of the children were parasitized by *Cryptosporidium* spp. Previously, a low and slightly higher prevalence (1.3% and 7.7%, respectively) have been registered in children of the central region of Argentina (Mendoza and Buenos Aires) (Salomón et al., 2007; López-Arias et al., 2022). In contrast, Cerezuela et al. (2017) reported a higher prevalence (19.3%) in the northwest region of the country (La Rioja). In the same way, higher prevalences have been found in other Latin American countries such as Mexico (41.0%), Venezuela (10.9%), Perú (29.6%), and Colombia (40.0%) (Carreño et al., 2003; Sánchez-Vega et al., 2006; Cama et al., 2008; Devera et al., 2010). The fact that the population of children evaluated in these studies includes mostly oncological patients and/ or certain clinical manifestations could explain the discrepancy in these results.

As for pet dogs, in this research, *Cryptosporidium* spp. was observed to have a prevalence of 11.5%. In another study, La Sala et al. (2015) reported a slightly higher prevalence (14.7%) in domestic dogs also from Argentina. However, in another study area, the prevalence was lower (1.0%) in dog feces collected from streets, parks, and squares in urban locations, while conversely, in other provinces, prevalences of 20.0% in stray dogs and pet dogs were registered (Cerezuela et al., 2017; Enriquez et al., 2019). These findings evidence heterogeneity in the prevalence of this parasite in dogs from Argentina. Concerning other countries, in urban areas and dairy barns, prevalences of 9.0% and 30.0%, respectively, were registered in domestic dogs from Mexico. On the other hand, prevalences of 16.3% in urban zones from Colombia and 26.6% in rural zones from Brazil were registered also in domestic dogs (Rodríguez et al., 2009; Moreira et al., 2018; Vitela-Mendoza et al., 2019). These differences may be due to the origin of dogs. In this sense, studies have reported that stray dogs and those owned by rural communities have a higher frequency of infection than city dogs (Dubná et al., 2007).

In this work, in 10 households (17.5%), the presence of *Cryptosporidium* spp. and, particularly in two domestic units, simultaneous infection was found in schooled boys and their pet dogs. In another study using genotyping analysis, *C. canis* was identified in a 2.5-year-old child, her 6.5-year-old brother, and their dog in Peru (Xiao et al., 2007). In a recent study in Egypt, *C. canis* was identified in 12 domestic dog owners and six dog shelter workers (Khalifa et al., 2023).

Concerning statistical analyses, we did not identify an association between the presence of *Cryptosporidium* spp. and the demographic variables (age group and gender). Considering the age of children, in the Global Enteric Multicenter Study (GEMS), it has been demonstrated that this protozoan is significantly present in the first two years of life in children with moderate to severe diarrheal diseases (Kotloff et a., 2013). Moreover, the Global Burden

of Diseases, Injuries, and Risk Factors study (GBD) 2015 established its association with diarrhea mortality in children under five years old (Troeger et al., 2017). This suggests that the age of children becomes relevant in patients with a prior clinical condition associated with diarrheal diseases. On the other hand, we did not identify an association between the presence of *Cryptosporidium* spp. and the socio-environmental features or hygiene practices evaluated. In the same way, in the research conducted in the same study area, in which the relationship between parasitosis and parasitic species with socio-environmental features has also been evaluated, no association has been demonstrated with any of the variables (Alegre et al., 2023).

Despite the results obtained from the statistical analyses, we highlight the possible risk posed by specific environmental characteristics of the evaluated area, such as the absence of a sewer system. Intense rainfall and potential floods can resuspend fecal matter infected with oocysts into the surface, causing contamination through the dispersal of these infective forms (Garbossa et al., 2013; Lal et al., 2013). Similarly, the absence of potable water in some households reflects the need to implement studies that incorporate the analysis of the water source used by this community.

The study reports the presence of *Cryptosporidium* spp. in some children and their pet dogs; these results cannot be neglected, and further studies should be conducted to understand better the epidemiology of the protozoan in the region studied, with the use of molecular methodologies being relevant to demystify these findings. Also, it emphasizes the importance of adopting a One Health approach because fragmented studies do not allow for a complete understanding of the parasite-host-environment system.

ACKNOWLEDGEMENTS

We thank everyone who offered their households to carry out the sample collection and the school authorities, the teachers, and the students who participated in this research. We are grateful to Liliana Giménez for her professional assistance.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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