INTERAÇÃO PARASITO-HOSPEDEIRO

2

Alana Maria Cerqueira de Oliveira
(Organizadora)

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APRESENTAÇÃO

A Obra "Interação parasito-hospedeiro 2", traz ao leitor cinco capítulos de relevada importância na área de Imunologia, Parasitologia e Genética. Entretanto, caracteriza-se como uma obra multidisciplinar que vai do estudo de parasitas de interesse humano a parasitas de interesses veterinário englobando os zoonóticos.

Os capítulos estão distribuídos em temáticas que abordam de forma categorizada e interdisciplinar a relação parasito-hospedeiro, as pesquisas englobam estudos de: polimorfismos genéticos, fases do ciclo de vida do parasita, expressão de citocinas, respostas imunológicas, técnicas de biologia molecular (extração de RNA, RT-PCR), técnicas de parasitologia, técnicas de imunologia, técnicas microbiológicas, transmissão zoonótica, doenças negligenciadas, virulência, patogenicidade, bioinseticida, Infecções oportunistas e resistência bacteriana.

A obra foi elaborada primordialmente com foco nos profissionais, pesquisadores e estudantes pertencentes às área de Parasitologia Médica e Veterinária e suas interfaces ou áreas afins. Entretanto, é uma leitura interessante para todos aqueles que de alguma forma se interessam pela área.

Cada capítulo foi elaborado com o propósito de transmitir a informação científica de maneira clara e efetiva, em português, inglês ou espanhol. Utilizando uma linguagem acessível, concisa e didática, atraindo a atenção do leitor, independente se seu interesse é acadêmico ou profissional.

O livro " Interação parasito-hospedeiro 2", traz publicações atuais e a Atena Editora traz uma plataforma que oferece uma estrutura adequada, propícia e confiável para a divulgação científica de diversas áreas de pesquisa.

Alana Maria Cerqueira de Oliveira

SUMÁRIO

CAPÍTULO 2

GIARDIA SPP. IN FREE-RANGING INTRODUCED MONK PARAKEETS AND ITS DISTRIBUTION IN SANTIAGO METROPOLIS, CHILE

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RESUMO: O periquito-monge (Myiopsitta monachus) é uma espécie de psitacídeo que, devido aos seus interesses como animal de estimação, tornou-se popular no comércio global de animais e atualmente invade cerca de 20 países em todo o mundo. Nos últimos cinquenta anos, os periquitos-monge se desenvolveram como uma ave invasora na metrópole de Santiago, Chile. Pouco se sabe sobre seu impacto, mas evidências recentes sugerem que seu papel como engenheiro de ecossistemas é relevante, em particular no que diz respeito a parasitas. Giardia spp. é um parasita gastrointestinal cosmopolita cujos hospedeiros potenciais incluem pássaros e humanos. Aqui, relatamos a presença desse patógeno em periquitos-monges da área urbana de Santiago. Durante os verões austral de 2017 e 2018, 207 filhotes de periquito-monge foram capturados e o conteúdo fecal estudado por meio de análises microscópicas. Dados ambientais relacionados às áreas onde os filhotes foram capturados foram analisados para estabelecer a existência de agrupamentos de infecção. Foram exploradas

associações entre agrupamentos espaciais, variáveis ambientais e a presença ou ausência desse patógeno. Em suma, 25 (12%) amostras foram positivas para Giardia spp. cistos. Os resultados sugerem que as árvores podadas podem ser um fator protetor contra a infecção por esse parasita. Este estudo contribui para a compreensão das implicações para a saúde da invasão do periquito-monge e seu papel potencial como reservatório e disseminador de parasitas em ambientes urbanos.
PALAVRAS-CHAVE: Giardia; invasive species; monk parakeet; Myiopsitta monachus; One Health; parasites; protozoa; synanthropic species; zoonoses

ABSTRACT: The monk parakeet (*Myiopsitta monachus*) is a psittacid species that due its interests as a pet, became popular in global animal trade and currently invades some 20 countries globally. Over the last fifty years monk parakeets have thrived as an invasive bird in Santiago metropolis, Chile. Little is known about their impact, but recent evidence suggests their role as ecosystem engineer is relevant, in particular in regard to parasites. *Giardia* spp. is a cosmopolitan gastrointestinal parasite whose potential hosts include birds and humans. Here, we report the presence of this pathogen in monk parakeets from urban Santiago. During the austral summers of 2017 and 2018, 207 monk parakeet nestlings were captured and fecal content studied via microscopical analyses. Environmental data related to areas were nestlings were captured was analyzed to establish the existence of infection clusters. Associations between spatial clusters, environmental variables and the presence or absence of this pathogen was explored. In sum, 25 (12%) samples were positive to *Giardia* spp. cysts. Results suggests that pruned trees may be a protective factor against infection with this parasite. This study contributes to understanding health implications of monk parakeet invasion, and their potential role as a reservoir and parasitic disseminator in urban environments.
KEYWORDS: Giardia; invasive species; monk parakeet; *Myiopsitta monachus*; One Health; parasites; protozoa; synanthropic species; zoonoses.

1 I INTRODUCTION

Biological invasions are recognized as a major cause of biodiversity loss (Dunn and Hatcher, 2015; Mooney and Cleland, 2001; Vitousek *et al.*, 1997). They have been linked to the emergence of diseases in native populations and, recently, acknowledged as relevant factors contributing to the spread of zoonoses (Briceño *et al.*, 2017; Briceño et al., 2021; Estrada-Peña *et al.*, 2014; Hulme, 2014).

Monk parakeets (*Myiopsitta monachus*) are gregarious medium-sized psittacids naturally distributed in Paraguay, Uruguay, Bolivia, southern Brazil, and northern and central Argentina. However, due to the international pet trade this bird became invasive in some 20 countries of Africa, Asia, Europe, and America (Eberhard, 1998; Edelaar *et al.*, 2015; GISD, 2020; South and Pruett-Jones, 2000).

Monk parakeets are considered one of the most successful invasive bird species given their particular behaviour and ecology (Sol *et al.*, 1997; Strubbe and Matthysen, 2009;

Van Ham *et al.,* 2013). Their great reproductive capacity and ability to prosper in novel environments can be attributed to their reproductive, nesting, and dietary habits (Borray-Escalante *et al*., 2020; Bucher and Aramburú, 2014; Eberhard, 1998; Sol *et al*., 1997; Senar *et al*., 2019; South and Pruett-Jones, 2000; Strubbe and Matthysen, 2009; Viana *et al*., 2016). This includes their unique ability among psittacids to entwine their own communal nests with twigs and branches, and their condition of ecosystem engineers (Briceño *et al*., 2019; Spreyer and Bucher, 1998).

Several negative impacts are associated to the presence of monk parakeets, both in countries where they are native and in countries they have invaded (Bucher and Aramburú, 2014; Viana *et al*., 2016). These impacts are often associated to economic losses derived from monk parakeet's activity (Conroy and Senar, 2009). Parakeets may utilize human structures such as electric posts to build their nests, which can lead to power outages (Viana *et al*., 2016). Their generalist diet often results in considerable economical costs for agricultural fields (Van Ham *et al*., 2013). For instance, in Argentina these birds cause losses of up to 1 billion dollars every year due to crop damage (Iriarte *et al*., 2005). Studies on monk parakeets have mainly focused on its economic impact or ecological traits, though their sanitary condition has been less concerned (Briceño *et al*. 2017). It is only recently that the presence of pathogens such as the Beak and Feather Disease Virus and the parasite Leucocytozoon have been described in monk parakeets from southern Spain (Martínez-de la Puente *et al*., 2020; Morinha *et al*., 2020). Further, zoonotic parasite *Ornithonyssus bursa* has been detected in monk parakeets, in their native and introduced distributions (Ancillotto *et al*., 2018; Aramburú *et* al., 2002; Briceño *et al*., 2021).

Monk parakeets were introduced in Chile in the early 1970's, when parakeets were released in the north-east area of Santiago, from where they started to spread to the rest of the territory (Briceño *et al*., 2019). Nowadays, monk parakeets are considered to be one of the most harmful species of invasive birds in Chile, due to their negative impact associated to agricultural, fruit and ornamental trees (Briceño *et al*., 2017). Despite this, not much research has been conducted to establish the real impact of monk parakeets as an invasive species in Chile (Briceño *et al*., 2017).

Recently, the presence of *Cryptosporidium* spp. in fecal samples from Monk Parakeets captured in Northeast Santiago, Chile, was reported (Briceño *et al*., 2017). A second report described interactions between monk parakeets and other bird species in Santiago, Chile, revealing that parakeets and other species of birds can coexist pacifically (Briceño *et al*., 2019). This study showed that, as observed in other countries (Di Santo *et al*., 2017), other bird species make use of monk parakeet nests for breeding, evidencing the role of monk parakeets as ecosystem engineers (Briceño *et al*., 2019). *Cryptosporidium* spp. often coexists with *Giardia* spp., another gastrointestinal parasite. Both parasites are transmitted via orofecal route by ingestion of water or food contaminated with (oo) cysts (Han *et al*., 2020; Ludwig and Marques, 2011; Xiao and Fayer, 2008). Although infections with these parasites can be asymptomatic or produce imperceptible clinical signs, immunocompromised individuals can eventually become sick and exhibit symptoms such as diarrhea, abdominal pain, nausea, vomit, and fever (Domingos et al., 2017; Ravish *et al.*, 2014). *Giardia* spp. can be detected and isolated in the environment, being contaminated water sources one of the main sources of contagion (Han *et al.*, 2020; Xiao and Fayer, 2008). *Giardia* spp. has also been detected in soil samples from public parks in Madrid, Spain (Dado *et al.*, 2012a).

Giardiasis is considered to be a neglected disease by the World Health Organization (Savioli *et al.*, 2006). This is of importance since *Giardia* spp. infections primarily occur in developing countries where infection outcomes can be influenced by factors such as poverty and lack of access to appropriate resources. Disease by this pathogen can impede the proper physical and socio-economic development of those affected by them (Savioli *et al.*, 2006). It is important from a one health perspective, due to the interdependence of human, animal and environmental health (Osburn *et al.*, 2009; Zinsstag *et al.*, 2011).

Considering that biological invasions can be a source of pathogens, some of them zoonotic, and can thus, eventually affect the health of people (Estrada-Peña *et al.*, 2014; Hatcher *et al.*, 2012; Keesing *et al.*, 2010; Thompson, 2013), and that monk parakeets are synanthropic birds, keeping a close contact with human populations by building their nests within urban public squares and parks (Briceño *et al.*, 2019; Di Santo *et al.*, 2017), investigating the sanitary state of these invasive birds becomes important. Therefore, the aim of this study was to perform a thorough inquest into the occurrence of *Giardia* spp. within free ranging monk parakeets located in Santiago. We additionally sought to determine the existence of infection clusters within the city, and to study the correlation between environmental variables associated to the trees in which monk parakeets nest and positive rates of infection.

2 | MATERIALS AND METHODS

2.1 Study area

The sampling area compromised twenty-one municipalities of Santiago (33º27’ S; 70º38’ W), the capital city of Chile. Santiago is located in the Metropolitan Region in Central Chile, a Mediterranean bioclimatic zone defined by dry summers, wet winters, and interannual variability caused by the El Niño-Southern Oscillation phenomenon (Amigo and Ramírez, 1998). Mean annual temperature is 13.2 ºC and mean annual precipitation is 531 mm (Schulz *et al.*, 2010). Vegetation occurs in an assortment of *Acacia caven* shrubland on lower hillslopes, and evergreen sclerophyllous forest on watersheds and south-facing slopes (Badano *et al.*, 2005; Schulz *et al.*, 2010). The Metropolitan Region is currently divided into 52 municipalities and is the highest densely populated region in the country, concentrating 40% of the national population with 7,112,808 inhabitants and a density of 462 people/km^2 (INE, 2021). Due to its accelerated and unorganized expansion, Santiago is characterized by urban sprawl and deep environmental degradation (Silva, 2015). Green areas within the city are highly stratified, from a total of 3,825 ha, 62% (2,387 ha) are distributed in only 3% of the total green areas. These areas are associated to large public parks (Reyes and Figueroa, 2010).

2.2 Study species

Monk parakeets (*Myiopsitta monachus*) are medium-sized sexually monomorphic parrots originally distributed in the south-eastern area of South America, specifically in Paraguay, Uruguay, Bolivia, southern Brazil, and northern and central Argentina (Eberhard, 1998; Olrog, 1968). As a consequence of the international pet trade monk parakeets have been introduced into several countries, becoming an invasive species at least in 19 countries of Africa, Asia, Europe, and America, including Chile (Eberhard, 1998; Edelaar *et al*., 2015; GISD, 2020; South and Pruett-Jones, 2000). This invasive success can be attributed to several behavioral and ecological traits. More notably, monk parakeets are the only species among the order Psittaciformes that does not rely on preexisting cavities to nest, since they are able to build their own communal nests (Martin and Bucher, 1993; South and Pruett-Jones, 2000; Viana *et al*., 2016). Furthermore, they feed on a flexible diet, allowing them to exploit a wide variety of fruits and seeds (Borray-Escalante *et al*., 2020). Additionally, their gregarious behavior leads them to engage in communal parenting increasing their possibilities to avoid predatory attacks (Eberhard, 1998; South and Pruett-Jones, 2000; Viana *et al*., 2016). Ultimately, they display great tolerance to human presence and urban perturbations, often building their nests in public squares and parks (Briceño *et al*., 2019; South and Pruett-Jones, 2000; Sol *et al*., 1997; Strubbe and Matthysen, 2009). All these traits provide a great capacity to adapt to different environments and lead to an accelerated population growth (Bucher and Aramburú, 2014; Strubbe and Matthysen, 2009). Further, their reproductive success seems to be higher in introduced versus native distributions (Senar *et al*., 2019). Finally, their condition of ecosystem engineers places them in a particular invasive category of concern (Briceño *et al*., 2019).

2.3 Nestling sampling and processing

Sampling took place during the Austral monk parakeet's breeding season; November and December of 2017 and 2018. Twenty-one municipalities of Santiago, Chile were opportunistically sampled for the study (Figure 1). Monk parakeet nestlings were considered as the sample unit and were assumed to represent the nest inhabitants' sanitary condition. Nestlings were manually counted and captured from their nest and accessed through a hydraulic aerial platform. Only one individual was captured *per* nest and *per* tree.

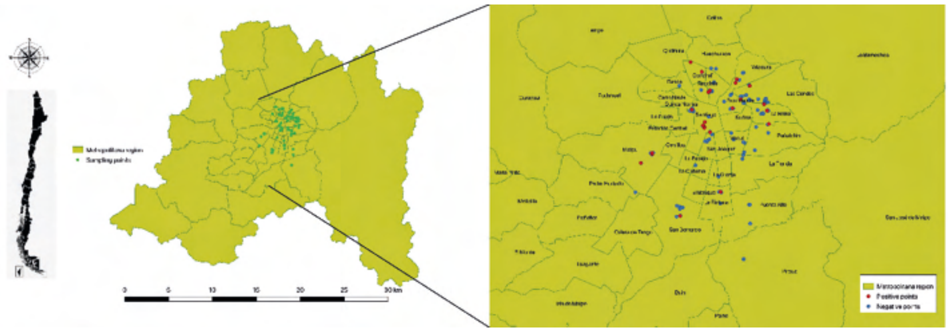

Figure 1. Sampled area within the city of Santiago metropolis, Chile. Red points represent positive samples and blue points negative samples.

Since parakeet sampling depended upon the number of trees with occupied nests *per* municipality, accessibility to nests, technical support provided by each municipality and daily possibilities to sample, among others, the sampling process was of convenience.

Nestlings were sacrificed via cervical dislocation following bioethical protocols. During the necropsy, individuals were aged and the distal portion of the digestive tract and its content was sectioned and preserved in 70% ethanol at 4°C, until further analyses. All procedures were conducted in compliance with national regulations established by Servicio Agrícola y Ganadero (SAG, Chilean Fish and Wildlife Service), under permit No. 716/2016, and bioethical and biosafety protocols issued by the Faculty of Animal Veterinary Sciences, University of Chile (Bioethical approval No. 19-2016 and Biosafety approval No. 82).

2.4 Protozoa detection

Fecal content was extracted from the preserved digestive tract for macroscopic and microscopic parasitological analyses. *Giardia* spp. cysts were detected by means of modified Telemann method using lugol's stain and 100 μL of sediment extended onto a 1 cm x 0,5 cm slide (Atías, 1998).

2.5 Environmental variables

For each nestling sample, the following environmental data related to the tree in which it was captured were recorded: municipality in which it was located, tree species, sanitary state of the tree (healthy/not healthy), tree management (pruned/not pruned), tree height, tree canopy diameter, diameter at breast height (DBH), number of nests per tree, nest size, nest height, number of chambers per nest, number of nestlings per nest, and GPS coordinates. Tree canopy diameter and DBH were measured using a 30 m. measuring tape while tree height and nest height were measured using a hypsometer. Health condition of trees was determined with the collaboration of academics at the Faculty of Forestry Sciences at the University of Chile.

2.6 Statistical analyses

Descriptive statistic was performed to summarize each recorded variable. Considering the nature of the collected data (positivity to *Giardia* spp. cysts) a logistic regression model analysis was performed (Dohoo *et al*. 2009). Univariate logistic regression analyses were performed to assess the relationship between all recorded variables and the positivity to the pathogen. Spearman correlation, Pearson chi-square and Fisher's exact test were performed to assess for collinearity and potential confounding factors, setting significance on $p < 0.05$. Variables with a P-value ≤ 0.25 were included in a multivariable logistic regression model (liberal criteria). A stepwise backward elimination procedure was conducted, using the log Likelihood Ratio Test (LRT); the model with the lowest LRT was selected as the final model (in the three built models). Likewise, variables whose regression coefficients were not significant ($P > 0.05$) were removed from the multivariable logistic regression (Kleinbaum and Klein, 2010). Non-significant variables, which produced a change greater than 20% in the regression coefficients of the significant variables when removed, were retained in the model to adjust for confounding factors. All the biologically feasible interaction variables were included in the model design (Dohoo *et al*. 2009). The convergence of the models was set at a value of epsilon = e^{-16} in order to increase restrictions on models and to secure the representativity and power of results. Goodness-of-fit of the final model was evaluated using the Hosmer and Lemeshow Test (Hosmer *et al*., 1997).

Biologically logical interactions between variables that fulfilled the liberal criteria were also analyzed. All categorical variables were analyzed using the dummy variables approach (Kuiper, 2008). Local clustering of positive samples was assessed by means of the Bernoulli model of the spatial scan statistic, considering a purely spatial cluster analysis (Kulldorf, 1997; Kulldorf and Nagarwalla, 1995). Analyses were conducted using the RStudio and the statistical software R 3.3.1 (R Core Team, 2016) plus 'lme4', 'ggplot2' and 'gcookbook' packages, and SatScan software version 9.4.2. (Kulldorff, 2009). Odds ratio, 95% confidence interval and P-value were computed.

Finally, statistically significant differences between sampling years were determined by calculating the prevalence difference, equivalent to an attributable risk or excess risk, and 95% confidence intervals, based on approximation and null hypothesis testing (prevalence difference equals to 0) (Rothman, 2012). This analysis was performed using R version 3.6.1 (R Core Team, 2016) and "fmsb" package (Nakazawa, 2019).

3 I RESULTS

3.1 Protozoa detection

A total of 207 monk parakeet nestlings were captured during the sampling period, 98 in 2017 and 109 in 2018, and a subsequent total of 207 stool samples were analyzed.

Total number of positive samples *per* parasite and per municipality can be seen in Table 1. Out of the 207 stool samples 25 were positive to the presence of *Giardia* spp. cysts (Figure 2). Positivity to *Giardia* spp. was of 10.2% in 2017 (10/98) and 13.8% in 2018 (15/109). Overall, individuals that were positive to *Giardia* spp. presented one to five cysts detected by microscopy per 100 μL of analyzed sample.

The presence of other parasites such as helminths and other protozoa was discarded via microscopical observation during the modified Telemann exam.

Municipality	**Total number of analyzed samples**	***Giardia* spp. positive samples**
Conchalí	5	3
Huechuraba	4	0
Recoleta	20	2
Vitacura	8	2
Las Condes	22	1
Providencia	12	2
La Reina	37	2
Macul	5	0
San Bernardo	12	1
Peñalolen	13	1
Maipú	13	3
Puente Alto	5	0
La Granja	4	0
La Cisterna	1	0
Santiago	24	7
La Florida	3	0
San Miguel	9	0
La Pintana	5	1
Independencia	1	0
Pirque	3	0
Renca	1	0
TOTAL	207	**25**

Table 1. Number of positive parakeets to *Giardia* spp. per municipality.

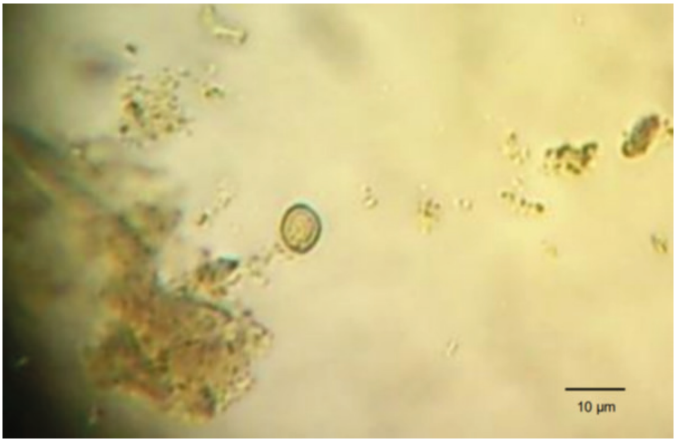

Figure 2. Microscopical image of intestinal content of monk parakeets: *Giardia* spp. cyst detected via Telemann method and stained using an iodine solution (400X).

3.2 Environmental variable analyses

Sample records for each nestling were analyzed. Models for *Giardia* spp. show a significant role of pruning trees as a protective factor (OR = 0.25, 95% CI LL = 0.09 and UL = 0.71; *P* = 0.01). Nest height also showed a borderline significance (*P* = 0.07), but the OR and 95% CI show a non-causal association (Table 2). Finally, the number of nestlings per nest exhibited a significant positive association to *Giardia* spp. infection (OR = 1.59, 95% CI LL = 1.17 and UL = 2.17; *P* = 0.003). Spearman correlation, Chi-square and Fisher's exact test results shows no collinearity and potential confounding factors among the variables included in the multivariable logistic regression models (p > 0.05).

Variable	***P*-value**	**OR**	**95% CI**	
			Lower limit	**Upper limit**
(Intercept)	<0.001	-	-	-
Number of nestlings per nest	0.003	1.59	1.17	2.17
Nest height	0.07	0.99	0.99	1.00
Pruning	0.01	0.25	0.09	0.71

Table 2. Results from the multivariate logistic regression analysis. Categories, *P*-value, odds ratio (OR) and 95% confidence interval (CI) lower and upper limit are reported for the variables that were retained in the model showing an association or relevance on the positivity to *Giardia* spp. on monk parakeet samples.

3.3 Spatial analyses

All 25 *Giardia* spp. positive samples were distributed throughout the study area. The Bernoulli model of the spatial scan statistic detected several clusters, none of them significant. Only one of them, a cluster for *Giardia* spp., presented borderline significance (*P* = 0.06). This cluster included nine sample points in a radius of 1.26 km, with a relative risk of 6.95, indicating a high risk of positivity at this site, located at Santiago, San Miguel and San Joaquin municipalities.

3.4 Sampling season analysis

Prevalence values showed no statistically significant differences ($p < 0.05$) for *Giardia* spp. in regard to sampling years (Table 3).

Parasite	2017		2018		*P-value*	95% Difference CI	
	Positive samples	n	Positive samples	n		Lower	Upper
Giardia spp.	10	98	15	109	0.568	-0.133	0.062

Table 3. Number of positive samples, p-value and 95% CI of prevalence differences to *Giardia* spp. between sampling seasons, obtained from introduced monk parakeets in Santiago, Chile.

4 I DISCUSSION

Even though introduced monk parakeets have been inhabiting Chile for almost 50 years, not much is known about the local impact of this invasive bird (Briceño *et al*., 2017; Briceño *et al*., 2019). This study detected the presence of *Giardia* spp. in intestinal samples of monk parakeets from urban Santiago, and identified a possible protective factor associated to the absence of infection with this parasite.

Monk parakeet nestlings rely completely on their parents for feeding and do not leave the nest until they reach approximately 40 days of age (Aramburú, 1997). Considering that G*iardia* spp. is an orofecally transmitted parasite (Ludwig and Marques, 2011), that monk parakeets are gregarious animals that live in communal nests where they maintain close contact and practice collaborative parenting, and that they defecate within these nests (Eberhard, 1998; South and Pruett-Jones, 2000; Viana *et al*., 2016), it is highly likely that nestlings acquired this pathogen by being in close contact with infected adult individuals or through their feces.

Detection of *Giardia* spp. in monk parakeet's fecal samples is of relevance, as this widely distributed gastrointestinal parasite, possesses great zoonotic potential exhibiting a wide range and variety of potential hosts that include mammals, fish, amphibians, reptiles, and birds (Berrilli *et al*., 2011; Evans, 2011; Fialho *et al*., 2008; Ludwig and Marques, 2011; Molina *et al*., 2006; Nakamura and Meireles, 2015; Zhang *et al*., 2012).

When studying the association between presence of this pathogen and environmental variables recorded at monk parakeet's nesting sites, it was possible to observe a negative association between parasite detection and pruned trees. This could indicate that pruned managed trees might constitute a protective factor against *Giardia* spp. infections. Considering that pruning is of great importance when it comes to maintenance of tree health (Badrulhisham and Othman, 2016), it is possible that this activity helps creating a less suitable environment for the survival of cysts in parakeet nests and surroundings, and therefore reducing the risk of infection. One way pruning trees could be contributing to lower infection rates is by allowing for higher U.V. light penetration towards nests. Another factor that could potentially be influencing this finding is pruning season. If trees are pruned during the winter season and some nests are destroyed during the process, it could be possible that some of the nests sampled during Monk Parakeet's breading season (spring and summer) corresponded to recently built structures. Due their novelty, new nests could present lower loads of accumulated cyst, which could translate into lower risk of infection.

Interestingly, a statistically significant positive association between the number of nestlings per nest and presence of *Giardia* spp. was observed. Further investigation is warranted to determine if these findings are associated to specific environmental variables or are casual results, consequence of the sampling limitations of the present study.

Due to the lack of statistical significance associated to the existence of infection clusters, it was not possible to confirm an association between the occurrence of infection with this agent and a specific geographic area within the study site. Nonetheless, a borderline significant cluster was detected in the central area of the city, compromising the municipalities of Santiago, San Miguel and San Joaquin. Further analyses would be required to confirm the existence of this cluster and to determine the factors underlying its existence.

Monk parakeets were introduced in Chile during the early 1970s as fueled by the international pet trade market (Iriarte *et al*., 2005). It is unknown if parakeets carried this pathogen upon their arrival, or whether they acquired them in Chile. No reports of this agent existing within Monk Parakeets in their original distribution range have been issued and although several endoparasitic studies have been performed on different Chilean birds (Fernández *et al*., 1996; González-Acuña *et al*., 2007; González-Acuña *et al*., 2011a; González-Acuña *et al*., 2011b; González-Hein, 2006), and more specifically on birds located in the city of Santiago (Toro *et al*., 1999), only *Cryptosporidium* spp. has so far been reported in Monk Parakeets (Briceño *et al*., 2017). As previously mentioned, nestlings most likely became infected via contact with adult individuals, which in turn may have acquired the pathogens from the surrounding environment. Infection with *Giardia* spp. is most commonly associated to the consumption of water contaminated with cysts (Han *et al*., 2020; Ludwig and Marques, 2011; Xiao and Fayer, 2008). Further studies should evaluate the quality of water sources present in public parks and squares. Interestingly, *Giardia* spp. was detected in soil samples from public parks in Spain (Dado *et al*., 2012a), evidencing the importance of this substrate as a potential additional source of pathogens.

A different factor that could possibly be contributing to the presence of this parasite in monk parakeets relates to the coexistence of these birds and other domestic animals in public squares or parks. A study performed in 2012 detected the presence of *Giardia* spp. in fecal samples of dogs that were collected in public squares of the city of Santiago, Chile (Pastenes, 2015). A similar study conducted in Spain also detected the presence of *Giardia* spp. in fecal samples collected in public parks of Madrid (Dado *et al.*, 2012a). A second investigation, also in Madrid (Spain), detected the presence of *Giardia duodenalis* in dogs from an animal shelter (Dado *et al.*, 2012b). This is a relevant finding since *G. duodenalis* has been previously described in aquatic birds (Plutzer and Tomor, 2009) and has been isolated from a parrot (McDonnell *et al.*, 2003). Considering that in Santiago monk parakeets make use of same type of environments to nest than dogs and, most importantly, to forage (Briceño *et al.*, 2019), the possibility of this agent being transmitted back and forth between parakeets and other species is possible. This also opens the possibility of monk parakeets being infected with other species of endoparasites and would also explain the general ubiquity of these protozoa in the studied area. This possibility is supported by the previous detection of *Cryptosporidium* spp. oocyst in monk parakeets from Northeast Santiago, Chile (Briceño *et al.*, 2017). Lastly, the possibility of Monk Parakeets contracting these pathogens from other bird species with which they share a common niche should also be considered, as it has been reported that monk parakeets in Santiago are able to peacefully forage and share space with other birds (Briceño *et al.*, 2019).

Interestingly, other species of birds make use of parakeet's nests (Briceño *et al.*, 2019; Di Santo et al., 2017). Such occurrence was recently registered in Santiago of Chile, where nine different species of birds, including rock doves and seven native species, were detected nesting inside monk parakeet's nests (Briceño *et al.*, 2019). Monk parakeets often abandon their nest constructions in order to build new ones (Bucher *et al.*, 1991). Additionally, incidents in which other birds attack monk parakeets, expel them from their nest and later proceed to occupy it have been registered previously (Wagner, 2012), and instances in which other bird species have been observed using empty chambers of parakeet nests that are otherwise still being occupied by parakeets have also been documented in Chile and other countries (Briceño *et al.*, 2019; Eberhard, 1998). Regardless of whether monk parakeets abandon their nests voluntarily, are expelled from them, or share them, the sanitary implications of finding other birds inside these structures should not be overlooked. Coexistence in nesting sites, can eventually increase the transmission of parasites and diseases between different species (Myczko *et al.*, 2016). *Giardia* spp. is an orofecally transmitted parasite (Ludwig and Marques, 2011), and considering that monk parakeets defecate within their nests (Viana *et al.*, 2016), it is quite possible that bird species using abandoned parakeet's nests or making use of unoccupied chambers while parakeets still remain in the nest, could eventually acquire this, and other pathogenic agents, especially given that this protozoa may stay viable for weeks (Olson *et al.*, 1999). Monk parakeets could also be spreading this parasite to other animals when being consumed by predators. American kestrel (*Falco sparverius*) and Harri's hawk (*Parabuteo unicinctus*) have been reported as parakeet predators in Chile (Briceño *et al*., 2019; Celis-Diez, 2014). Recently, the role of monk parakeets as potential ecosystem engineers, providing a novel nesting resource in urban areas was determined (Briceño *et al*., 2019). The present report shows that their role as ecosystem engineers could extend beyond the addition of a new nesting resources and include the potential role of monk parakeets as pathogens disseminators. This is supported by recent findings in Spain where monk parakeets were found to be infected with the Beak and Feather Disease Virus and the parasite Leucocytozoon (Morinha *et al*., 2020; Martínez de la Puente *et al*., 2020).

As a synanthropic species, this may be of high health impact, in case this parasite is of zoonotic nature, representing a major risk upon urbanized metropolises as Santiago, were these parakeets seem to be successful invaders. Further, monk parakeet pathogen transmission risk is increased as this species tend to be favored by the public and are coveted as pets. In 2014, a domiciliary outbreak of psittacosis in the city of Dom Pedrito (Rio Grande do Sul State, Brazil) was attributed to monk parakeets, after eight members of a family presented psittacosis associated symptoms. Two parakeets illegally purchased by this family were identified as the source of *Chlamydophila* spp. (Freitas *et al*., 2014). Incidents such as this could eventually increase and include the transmission of other pathogens.

For the time being monk parakeets have remained confined to urban environments, but if they reach rural areas, they could eventually encounter native Chilean parrots and transmit pathogens to them or to other bird species (e.g., Beak and feather disease). This possibility is supported by a recent report issued in Spain that describes how monk parakeets from Madrid have spread into rural areas where they have been detected nesting in association to white storks (*Ciconia ciconia*). This type of behavior could allow parakeets to increase their invasive potential by avoiding biotic resistance in the form of predator pressure for example (Hernández-Brito *et al*., 2020). Although white storks are not present in Chile, parakeets could eventually benefit from the presence of other bird species and expand their range of distribution.

Current environmental variations associated to climate change and agricultural land use can, generally, be positively associated with higher risks of contagion with *Giardia* spp. (Lal *et al*., 2013). In addition to that, since infection can occur by consumption of contaminated water, control efforts for this pathogen usually focus on the sanitary management of water sources (Collinet-Adler and Ward, 2010). The present findings could contribute to highlight the importance of biological invasions in the emergence and control of diseases, especially those of urban densely populated areas.

It must be noted that, despite exhibiting a wide variety of potential hosts, the *Giardia* genus contain many different species and genotypes that differ in their ability to infect specific host species. This translates in a high level of species-specific infections. Since the identification of the parasites detected in this study was restricted to the genus identity of the pathogens, further analyses should be performed in order to determine the specific species infecting monk parakeets from the city of Santiago. Determining the species -and the genotypes- of the *Giardia* spp. cysts found in this study could help assessing the real potential of these birds as possible transmitters of zoonotic agents to human and animal populations.

It should also be considered that, even though the detection of *Giardia* spp. is important and the finding of pruned trees as a possible protective factor is interesting, further studies should be conducted in order to draw proper ecological and epidemiological conclusions about the presence of this pathogen and the environmental factors that influence their presence or absence. The sampling process carried out in this study was one of convenience, meaning that, several arbitrary factors influenced the selection of the sampled trees. These ranged from, nest accessibility, to time and resources availability, to permits granted by municipalities. For this reason, even though the statistical analyses performed in this work can reflect reality, they should still be considered with caution. Future studies on the sanitary state of monk parakeets should aim at a more thorough sampling design, ideally with a larger sample size or using a random sampling process, not influenced by the previously mentioned factors.

5 I CONCLUSIONS

This study reports presence of *Giardia* spp. in free-ranging parakeets from Santiago. Further studies focused on determining the specific identity of this protozoa, the presence of other pathogens in this invasive bird and the existence of environmental variables favoring or impeding infections are of great importance. As an ecosystem engineer this invader may by affecting the health of native birds in central Chile, but also represents a risk of pathogen transmission to human beings. Further studies should aim to assess these health risks at this Mediterranean ecosystem, one of the biodiversity hotspots for conservation priority (Myers *et al*., 2000).

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