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Impacts of seasons on intestinal helminthiasis in Columba livia (Columbiformes: Columbidae) in southern Brazil

Impactos das estações do ano sobre as helmintíases intestinais em *Columba livia* (Columbiformes: Columbidae) no sul do Brasil

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Abstract
Objective: *this study aimed to analyze intestinal helminthic infections in pigeons Columba livia Gmelin, 1789 (Columbiformes: Columbidae) collected in three seasons in southern Brazil.* ***Methodology:*** *ninety birds (hosts) were captured in the urban area in Pelotas, Rio Grande do Sul (RS) state, Brazil, in autumn, winter and spring in 2018 and 2019.* ***Results:*** *helminth species and their prevalences were: Ascaridia columbae (33.33%), Baruscapillaria obsignata (23.33%) (Nematoda), Brachylaima mazzantii (3.33%) (Digenea), Skrjabinia sp. (11.11%) and Killigrewia sp. (3.33%) (Cestoda). Prevalence and mean intensity of infection in the three collection periods did not show any significant difference, except for A. columbae, which was more prevalent in autumn than spring.* ***Conclusion:*** *helminths parasitizing C. livia were found in southern Brazil in three seasons (autumn, winter and spring), with no discrepancies among periods. Therefore, abiotic and environmental conditions of the southern region enabled the development of intestinal helminths associated with the host species.*
Keywords*: Pigeons; nematoda; digenea; cestoda; seasonal*

Resumo
Objetivo: o objetivo deste estudo foi analisar as infecções helmínticas intestinais em pombos *Columba livia* Gmelin, 1789 (Columbiformes: Columbidae) coletados em três estações diferentes no sul do Brasil. **Metodologia:** noventa aves (hospedeiros) foram capturadas na área urbana de Pelotas, Rio Grande do Sul, Brasil, no outono, inverno e primavera de 2018 e 2019. **Resultados:** as espécies de helmintos e suas prevalências foram: *Ascaridia columbae* (33,33%), *Baruscapillaria obsignata* (23,33%) (Nematoda), *Brachylaima mazzantii* (3,33%) (Digenea), *Skrjabinia* sp. (11,11%) e *Killigrewia* sp. (3,33%) (Cestoda). A prevalência e intensidade média de infecção nos três períodos de coleta não apresentaram diferença significativa, exceto *A. columbae*, que foi mais prevalente no outono do que na primavera. **Conclusão:** foram encontrados helmintos parasitando *C.livia* no sul do Brasil nas três estações do ano (outono, inverno e primavera), sem discrepâncias entre os períodos. Portanto, as condições abióticas e ambientais da região sul possibilitaram o desenvolvimento de helmintos intestinais associados às espécies hospedeiras.
Palavras-chave: Pombos; nematoda; digenea; cestoda; sazonal.

INTRODUCTION

Columba livia Gmelin, 1789 is a cosmopolitan bird native to Southern Europe, North Africa and South Asia. This species was introduced in various parts of the world, especially in urbanized regions, where it has found a favourable environment for colonization, probably because of shelter and food availability[1]. Even though C. livia was introduced as a domestic bird in Brazil in the 16th century; it became partially wild[2].

Large pigeon populations in urban centers may cause several problems due to the accumulation of faeces, feathers and nest remnants, which may clog drainage systems, damage public buildings, monuments and houses and contaminate food[3]. In addition, they are potential carriers of bacterial, viral and parasitic diseases and may be sources of infection for humans and other animals, including endangered species[4].

Helminthological studies of C. livia have been conducted in different regions where the species is native[5-6] and in areas where it was introduced[4,7-8]. The diversity

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of intestinal helminths is mainly represented by species of Nematoda and Cestoda[8-9]. The influence of seasons on helminth infections in pigeons has been investigated in Turkey[10], Bangladesh[5], Egypt[6] and Nigeria[11], where intestinal helminths were found to be more prevalent in autumn[5,10]. However, seasonal variations depended on the helminth species, a fact that may be associated with their life cycles[12].

In the Neotropical region, there is no information on the influence of seasons on intestinal helminthic infections in C. livia. In addition, helminthological studies of pigeons in southern Brazil are scarce, even though they are essential to understanding parasitic infections, mainly in areas where the species was introduced. Parasites brought by introduced hosts play an important role, not only because they affect the establishment of their host in new areas but also because they may be able to spread to native species and affect biodiversity[13].

Therefore, this study aimed to analyze intestinal helminthic infections in C. livia collected in three seasons in southern Brazil.

METHODOLOGY

Ninety free-living pigeons were captured in different places, such as buildings and squares, in the urban area in Pelotas, Rio Grande do Sul (RS), Brazil (31°46'11.0"S, 52°20'27.6"W). Adult pigeon specimens were captured in casting nets, while young ones were picked up in their nests.

The birds were captured in autumn (from April to June 2018) (n= 24 adults; 12 immatures), winter (from July to August 2018) (n= 10 adults; 19 immatures) and spring (in October 2018 and October 2019) (n= 12 adults; 13 immatures). The climate in the region is humid subtropical, according to the Köppen classification[14]. Mean temperatures and precipitation in the collection periods were 16.5°C and 67.7mm (autumn 2018), 12.4°C and 204.8mm (winter 2018), 17.5°C and 118.7mm (spring 2018) and 20.6 and 264.9mm (spring 2019)[15].

All animals were individually placed in cages, identified and transported to the Parasitology Laboratory of the Universidade Federal de Pelotas (UFPel), Campus Capão do Leão, RS, Brazil, where they were euthanised. Euthanasia followed recommendations issued by the National Council for Animal Experimentation Control[16]. Bird capture, transportation and euthanasia were licensed by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio no. 61235-1) and approved by the Ethics Committee for Animal Experimentation (CEEA/UFPel no. 12860/2018).

Small and large intestines were individualised in the necropsy, opened and rinsed under running water in a 150 µm-mesh sieve. A saline solution was added to the intestinal contents, and examination under a stereomicroscope was carried out again.

Nematodes were fixed and preserved in 70% ethanol. Cestoda and Digenea were fixed in AFA (70% ethanol, 37% formalin and glacial acetic acid) for 24 hours and preserved in 70% ethanol[17]. For morphological identification, helminths from Nematoda were clarified in Amann's lactophenol, studied in temporary preparations and identified in agreement with D'Ávila, Bessa, Rodrigues[8] (2017b), Kajerova, Barus, Literak[19] (2004), Moravec[20] (1982) and Wehr, Hwang[21] (1964). Digenea and Cestoda helminths were stained with Langeron Carmine or Delafiled Hematoxylin and individually mounted in Canada balsam[17]. Digenea and Cestoda were identified in agreement with Marques et al.[22] (2017) and Khalil, Jones, and Bray [23] (1994), respectively. Vouchers were deposited in the helminth collection at the Laboratório de Parasitologia de Animais Silvestres (CHLAPASIL) at UFPel (no. 931-952).

Parasitological parameters of prevalence (P%), mean abundance (MA) and mean intensity of infection (MII) were calculated in agreement with Bush et al.[24] (1997). Infections (P% and MII) found in the three sampling periods were compared two by two for all intestinal helminths and for each species that co-occurred in at least two seasons and whose prevalence was higher or equal to 10% in every period under analysis. The prevalence of coinfections (occurrence of more than one taxon at the same host) in the three sampling periods was compared. Infections (P% and MII) of adults and immatures, regardless of the sampling period, were compared to verify if maturity influenced seasonal analyses. Comparisons of P% were performed by the Fisher's Exact test ($p \leq 0.05$), while the ones of MII were carried out by the bootstrap t-test ($p \leq 0.05$) in Quantitative Parasitology, QPweb[25].

The richness of intestinal helminths in relation to the number of hosts under examination was analyzed by a species accumulation curve. To evaluate the similarity of helminth infections in the three collection periods, the Morisita index for quantitative data was used. The analysis was performed by the Paleontological Statistics – PAST 2.17 program[26].

RESULTS

Among 90 specimens of C. livia under study, 48.89% were parasitized by intestinal helminths; the mean intensity of infection was 16.06 helminths/host. Nematoda occurred in 42.22% of them, followed by Cestoda (13.33%) and Digenea (3.33%). The following five taxa were recorded: Ascaridia columbae (Gmelin, 1790) (Ascaridiidae), Baruscapillaria obsignata (Madsen, 1945) (Capillariidae), Brachylaima mazzantii (Travassos, 1927) (Brachylaimidae), Skrjabinia sp. (Fuhrmann, 1920) (Davaineidae) and Killigrewia sp. Meggitt, 1927 (Anoplocephalidae). They parasitized the small intestine of hosts (Table 1). Ascaridia columbae was found at different developmental stages of its life cycle (L3, L4, L5 and adult).

The highest variation in intensity of infection was exhibited by B. mazzantii (69-178 helminths), which showed low prevalence and mean abundance. Cestoda occurred at low infection indices; the intensity of infection by Skrjabinia sp. was 1-14 helminths, while only three specimens of Killigrewia sp. were found in the samples (Table 1).

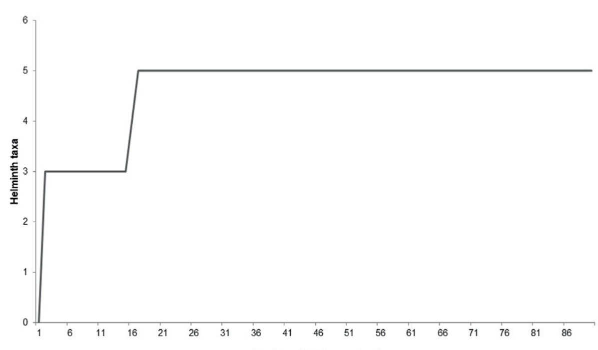
Table 1 – *Intestinal helminths found in Columba livia Gmelin, 1789 (Columbiformes: Columbidae) (n=90) in southern Brazil and parasitological indices of prevalence (P%), mean intensity of infection (MII ± SD), mean abundance (MA ± SD) and range (R)*

Helminths	P (%)	MII ± SD	MA ± SD	R
Nematoda				
Ascaridia columbae	33.33	3.97 ± 3.26	1.32 ± 2.65	1 – 14
Baruscapillaria obsignata	23.33	6.05 ± 8.44	1.41 ± 4.76	1 – 37
Digenea				
Brachylaima mazzantii	3.33	134.00 ± 57.45	± 4.47 ± 25.68	± 69 – 178
Cestoda				
Skrjabinia sp.	11.11	5.60 ± 5.72	0.62 ± 2.54	1 – 14
Killigrewia sp.	3.33	1.00	0.03 ± 0.18	1

SD: Standard deviation

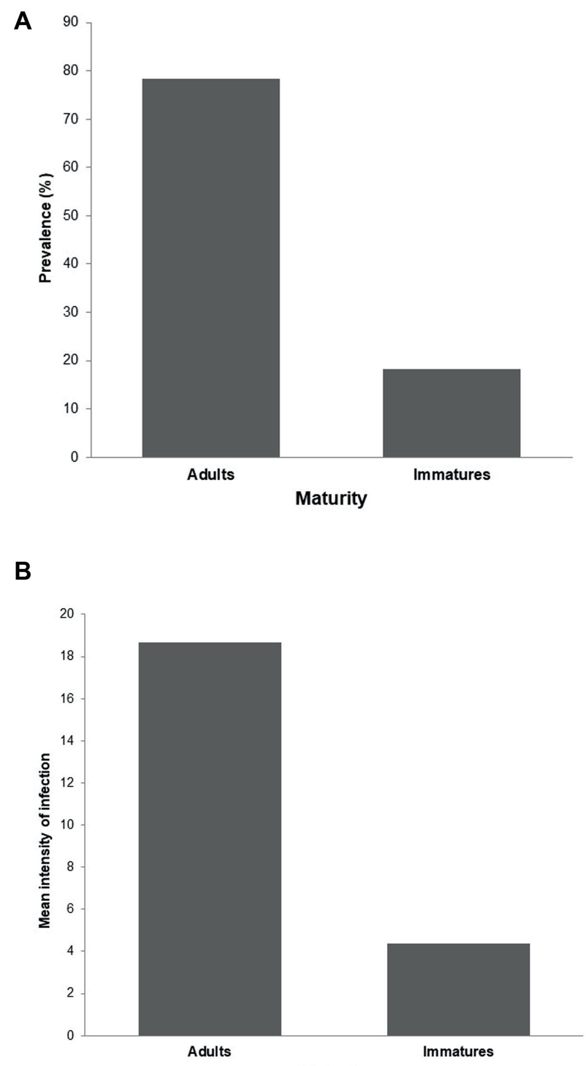
The species accumulation curve showed that the helminth species richness of C. livia was well represented in samples. After host 19, the number of helminth taxa did not increase (Figure 1).

Figure 1 – *Accumulation curve of intestinal helminthic species found in Columba livia Gmelin, 1789 (Columbiformes: Columbidae) (n=90) in southern Brazil*



The prevalence of intestinal helminths in adult birds (78.26%) was higher than the one found in immature ones (18.18%) (p <0.0001). Mean intensities of infection in adult and immature birds were 18.67 helminths/host and 4.38 helminths/host, respectively. However, values did not show any significant difference (Figure 2). All helminth species were recorded in adults, while in immatures, only A. columbae, B. obignata and Skrjabinia sp. were found at low infection indices.

Figure 2 – *(A) Prevalence and (B) mean intensity of infection of intestinal helminths in Columba livia Gmelin, 1789 (Columbiformes: Columbidae) adults (n=46) and immatures (n=44) in southern Brazil.*



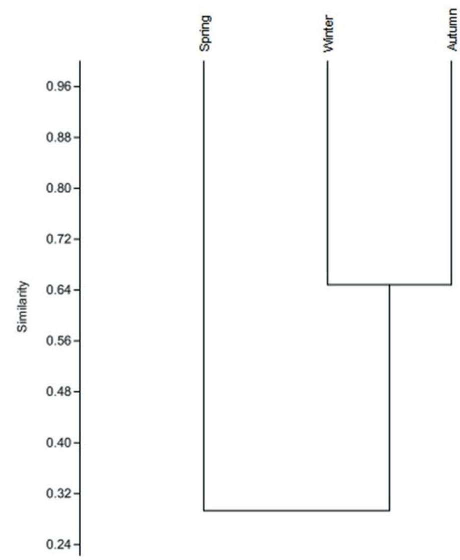
Regarding the three sampling periods, there were no significant differences in prevalence and mean intensity of infection, considering all helminths found in C. livia. However, the highest variation in intensity of infection (range) was found in winter (Table 2).

Table 2 – *Prevalence (P%), mean intensity of infection (MII ± SD), mean abundance (MA ± SD) and range (R) of intestinal helminthic parasites in Columba livia Gmelin, 1789 (Columbiformes: Columbidae) in three collection periods in southern Brazil*

Seasons	P (%)	MII ± SD	MA ± SD	R
Autumn (n= 36)	58.33	10.90 ± 18.05	6.36 ± 14.70	1 – 81
Winter (n=29)	41.38	35.62 ± 66.65	14.76 ± 45.44	1 – 183
Spring (n=25)	44.00	4.55 ± 5.84	2.00 ± 4.42	1 – 20

Based on the Morisita similarity index, helminth infections had higher similarity in winter and autumn (Figure 3).

Figure 3 – Clustering analysis of intestinal helminthic parasites in *Columba livia* Gmelin, 1789 (Columbiformes: Columbidae) (n=90) by the Morisita index in three collection periods in southern Brazil



The analysis of every taxon in relation to the sampling periods showed that prevalence of A. columbae was significantly higher in autumn (44.44%) than in spring (16%). The other species occurred at similar indices in the three periods (Table 3).

Concerning positive birds (n=44), 58.82% showed infection caused by a single species, while mixed infections were found in 43.18% of hosts. Sixteen birds (36.36%) were coinfected by two species, while 4.55% were coinfected by three species. Coinfection by four species occurred in a single host (Table 4).

Table 3 – *Prevalence (P%), mean intensity of infection (MII), mean abundance (MA) and range (R) of species of Nematoda, Digenea and Cestoda parasitizing Columba livia Gmelin, 1789 (Columbiformes: Columbidae) (n=90) in three collection periods in southern Brazil*

	Autumn (n= 36)				Winter (n=29)				Spring (n=35)			
Helminths	P (%)	MII ± SD	MA ± SD	R	P (%)	MII ± SD	MA ± SD	R	P (%)	MII ± SD	MA ± SD	R
Nematoda												
Ascaridia columbae	44.44*	4.62 ± 4.10	2.05 ± 3.55	1 – 14	34.48	3.30 ± 1.64	1.13 ± 1.85	1 – 6	16.00*	3.00 ± 2.45	0.48 ± 1.42	1 – 6
Baruscapillaria obsignata	33.33	5.67 ± 5.73	1.89 ± 4.20	1 – 20	20.68	8.50 ± 14.05	1.75 ± 6.90	1 – 37	12.00	2.67 ± 1.53	0.32 ±0.99	1 – 4
Digenea												
Brachylaima mazzantii	2.77	69.00	1.92 ± 11.50	69	6.89	116.50 ± 16.26	11.48 ± 43.05	155 – 178	0	0	0	0
Cestoda												
Skrjabinia sp.	11.11	4.25 ± 6.50	0.47 ± 2.34	1 – 14	3.45	11.00	0.38 ± 2.04	11	20.00	5.60 ± 5.73	1.12 ± 3.27	1-14
Killigrewia sp.	2.77	1.00	0.03 ± 0.17	1	0	0	0	0	8	1	0.08 ± 0.28	1

**significant difference among collection periods by the Fisher's exact test (p=0.02); SD: Standard deviation*

Table 4 – *Coinfections of intestinal helminthic parasites in Columba livia Gmelin, 1789 (Columbiformes: Columbidae) (n=44) in southern Brazil*

Helminth species	N	P (%)
Simple infections		
A. columbae	13	
B. obsignata	7	
Skrjabinia sp.	4	
Killigrewia sp.	1	
Subtotal	25	56.82
Coinfection by two species		
A. columbae and *B. obsignata*	10	
A. columbae and *B. mazzantii*	1	
A. columbae and *Skrjabinia* sp.	2	
A. columbae and *Killigrewia* sp.	1	
B. obsignata and *Skrjabinia* sp.	1	
Skrjabinia sp. and *Killigrewia* sp.	1	
Subtotal	16	36.36
Coinfection by three species		
A. columbae, B. obsignata and *B. mazzantii*	1	
A. columbae, B. obsignata and *Skrjabinia* sp.	1	
Subtotal	2	4.55
Coinfection by four species		
A. columbae, B. obsignata, B. mazzantti and *Skrjabinia* sp.	1	
Subtotal	1	2.27

n: number of parasitized birds in each period; P (%): prevalence

In autumn, 30.55% of birds were coinfected by at least two helminth species; in winter, the prevalence was 17.24%, and in spring, it was 12%. However, there was no significant difference in the prevalence of coinfections in the periods under investigation. Ascaridia columbae and Skrjabinia sp. coexisted with all recorded parasites. Brachylaima mazzanti was associated with the other species.

DISCUSSION

The helminth fauna in C. livia has been poorly studied in some regions, especially where the birds are not native. Helminthological studies of introduced species are necessary since parasites play a fundamental role in measuring impacts caused by these species[13]. In Brazil, research has focused on the southeastern region[4,8,27]. Some records have been made in other countries in the Neotropical region[4,7,28]. However, no study evaluated intestinal helminthic infections in C. livia in relation to seasons. Helminths differ in complexity in life cycles, and their dependence on biotic and abiotic factors is related to their free-living stages and interactions with the environment and host population[12]. Among abiotic factors, climatic variables may alter the population dynamics of parasites and affect infection rates and dissemination of helminth infections[29]. Species whose developmental stages occur in the environment may be affected by humidity and extreme temperatures since eggs and larvae are susceptible to desiccation in dry places and at high temperatures. On the other hand, low temperatures may also affect embryos and, consequently, the development of infective forms[30]. Climatic conditions may also influence the development and distribution of intermediate hosts and affect the heteroxenous life cycle of parasites and their abundance in the host[31].

Helminth infections in C. livia (considering all intestinal helminths) did not differ in the three periods under investigation. Mohammed et al.[11] (2019) reported higher helminth infection indices in captive pigeons in the wet season than in the dry season. In the region of the study reported by this paper, the distribution of precipitation is relatively even during the year[32]. It suggests that this factor may have contributed to similar occurrence of parasites in the periods under study. Although summer was not analyzed, it should be highlighted that infections observed in autumn may have been acquired in summer.

In contrast, infections acquired in spring may have remained throughout the other seasons. Ascaridia Dujardin, 1845 species, for example, can live up to a year in their host[33]. However, studies of the longevity of parasites in their definitive hosts are scarce; thus, it is important to trigger further discussions about abiotic influence on helminth infections.

Ascaridia columbae was more prevalent in autumn than in spring. It was similar to what was observed in native C. livia regions, where the parasite was more frequent in autumn and winter and less frequent in spring and summer[10] or even absent in these seasons[5] in free-living pigeons. This study's results may be related to the temperature since there is much variation in the study area[32]. Eggs of A. columbae eliminated in faeces require appropriate environmental conditions to become infective[33]. Therefore, mild autumn temperatures, associated with humidity that occurs all year round in the study, may provide adequate conditions to enable nematode eggs to develop.

Results of the analysis of seasons were represented by infections in adult pigeons since intestinal helminthic infections were more prevalent in this group. They corroborate investigations in free-living pigeons in other areas in the Neotropical region[7] and captivity in the Middle East[34]. In this study, immature showed low species diversity and low infection rates. This difference between the groups may be attributed to age and time of exposure to infective forms of parasites[11]. Immature birds were picked up in or close to their nests and had not developed the ability to fly. They depended on their parents to be fed, which may reduce exposure to infection.

The diversity of intestinal helminths found in this study was similar to that found in free-living pigeons in regions where the species was introduced[4,28]. In countries where the bird is native, a high diversity of helminth species has been observed in free-living and captive pigeons[5-6]. Introduced species may show a loss of diversity and lower infection rates than native ones[13]. Further studies are needed to expand knowledge about helminths associated *with invasive species and the potential implications of co-introduced parasites.*

Nematodes showed higher prevalence values than the other parasites, thus corroborating the results of other studies in the Neotropical region[7-8-9]. Ascaridia columbae and B. obsignata have direct life cycles[20-21], while Cestoda and Digenea have more complex ecological interactions and depend on intermediate hosts to transmit infective forms to definitive hosts. Furthermore, it should be highlighted that transmission of both Cestoda and Brachylaima Dujardin, 1843 species, depends on prey-predator interactions[33-35]. In this context, the feeding behaviour and living conditions of C. livia may disfavor the ingestion of invertebrates parasitized by Cestoda and Digenea larvae. Pigeons feed on several foods, such as grains, annelids, insects and mollusks[1]. However, it should be mentioned that it is common to see people feeding corn, rice and bread to pigeons in urban areas, a fact that may mitigate exposure to infective forms in the environment and to intermediate hosts[1].

Infections caused by only one helminth species per host were more common than mixed infections, as previously reported by studies in Brazil[4] and other countries[6,11]. Regarding mixed infections, the larger the number of helminth species per host, the more prevalence decreases. It may be related to several species' difficulty cohabiting in the same bird. Besides, food preference and availability may interfere in establishing either simple or mixed infections[11].

CONCLUSION

In general, intestinal helminthic infections in Columba livia did not vary due to seasons in southern Brazil, except for A. columbae, which was more prevalent in autumn than spring. Therefore, it is possible to conclude that the abiotic and environmental conditions of the region allowed the development of intestinal helminths associated with the host species. Additional studies are needed to understand the influence of abiotic and environmental conditions on the dynamics of helminth infections.

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