

Periodicity and intensity of oocysts of the genus *Isoospora* Schneider, 1881 shedding by passerines birds from wildlife trafficking and held in quarantine*

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ABSTRACT. Coelho C.D., Berto B.P., Neves D.M. & Lopes C.W.G. **Periodicity and intensity of oocysts of the genus *Isoospora* Schneider, 1881 shedding by passerines birds from wildlife trafficking and held in quarantine.** [Periodicidade e intensidade de oocistos do gênero *Isoospora* Schneider, 1881 eliminados nas fezes de passeriformes oriundos do tráfico de animais silvestres e mantidos em quarentena.] *Revista Brasileira de Medicina Veterinária*, 38(supl. 3):75-79, 2016. Programa de Pós-Graduação Ciência, Tecnologia e Inovação Agropecuária, Pró-Reitora de Pesquisa e Pós-Graduação. Universidade Federal Rural do Rio de Janeiro. BR 465 Km 7. *Campus* Seropédica, RJ 23.897-970, Brasil. E-mail: domingues.cleide@yahoo.com.br

Diurnal frequency has been observed in species of intestinal parasites, especially in the genus *Isoospora* Schneider, 1881 in wild birds, which shedding oocysts more often in the late afternoon. This study aimed to evaluate the periodicity and shedding oocysts by passerines birds maintained at the Center for Triage of Wild Animals, Seropédica, Rio de Janeiro, Brazil. Samples of one droplet of feces were collected from 602 passerine birds, in the morning between 9h and 12h and in the afternoon between 15h and 17h, from May to November 2010. Samples were diluted in potassium dichromate 2.5% 1:6 (v/v) and incubated at room temperature for seven days. Of each sample was also determined an OoPD (number of oocysts per defecation). Fecal samples collected in the morning had a lower number of positive samples (12/2%) in comparison with those collected in the later afternoon that they had a greater number of positive samples (136/23%), likewise OoPDs from birds of the families Cardinalidae, Emberizidae and Thraupidae were also different in intensity. Both results indicated that periodicity and intensity of oocysts shedding by passerine birds were extremely significant ($p < 0.0001$) when they were determined by using late samples.

KEY-WORDS. *Isoospora*, oocysts, passerines, periodicity.

RESUMO. Frequência diurna tem sido observada em espécies de parasitos intestinais, especialmente no gênero *Isoospora* Schneider, 1881 em aves selvagens, que eliminam oocistos nas fezes mais frequentemente no final da tarde. Este estudo teve

como objetivo avaliar a periodicidade e a eliminação de oocistos por pássaros mantidos no Centro de Triagem de Animais Silvestres, Seropédica, Rio de Janeiro, Brasil triagem. Amostras de uma defecação foram coletadas de 602 pássaros, no período

* Received on July 21, 2016.

Accepted for publication on November 17, 2016.

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da manhã entre 9 e 12h e à tarde entre 15 e 17h, de maio a novembro de 2010. As amostras foram diluídas em dicromato de potássio a 2,5% 1: 6 (v/v) e incubou-se à temperatura ambiente durante sete dias. De cada amostra foi também determinada a OoPD (número de oocistos por defecação), onde observou-se que as amostras fecais coletadas na parte da manhã tiveram um número menor de amostras positivas (12/2%) em comparação com aquelas coletadas no fim da tarde os quais apresentaram um maior número de amostras positivas (136/23%), da mesma forma, que os OoPDs de pássaros das famílias Cardinalidae, Emberizidae e Thraupidae também foram diferentes em intensidade. Ambos os resultados indicaram que a periodicidade e a intensidade de oocistos em passeriformes foram extremamente significativas ($p < 0,0001$), quando eles foram determinados usando amostras coletadas no período da tarde.

PALAVRAS-CHAVE. *Isospora*, coccidiose, pássaros, periodicidade.

INTRODUCTION

The phenomenon of daytime periodicity has been observed in species of intestinal parasites, especially of the genus *Isospora* Schneider, 1881 (Protozoa: Apicomplexa) in wild birds, which eliminate the oocysts more frequently in the late afternoon (Boughton 1937, Dolnik 1999, Dolnik 2006, Brown et al., 2001, Lopez et al., 2007, Martinaud et al., 2009). Many of the studies on periodicity in oocyst elimination were carried out on several passeriform species, such as: *Sylvia atricapilla* L., 1758; *Serinus canaria* L., 1758; *Sylvia Borin* Boddaert, 1783; *Turdus merula* L., 1758; *Carduelis chloris* L., 1758; *Carpodacus mexicanus* Müller, 1776; *Geospiza fuliginosa* Gould, 1837; *Xanthomyza phrygia* Shaw, 1794; *Passer domesticus* L., 1758, *Plectrophenax nivalis nivalis* L., 1758 (Box 1977, Brawner III & Hill 1999, Brown et al., 2001, Dolnik 2006, López et al., 2007, Martinaud et al., Dolnik et al., 2010, Morin Adeline et al., 2011, Pap et al., 2011, Dolnik et al., 2011). According to Silva et al. (2014), there are no studies related to the presence of *Isospora* oocysts for prolonged periods in feces of birds raised in captivity. Silva et al. (2010) reported that during breeding and moulting seasons, wild birds kept in captivity were more prone to infection by *Isospora* spp. Silva et al. (2014) observed that there is greater elimination of oocysts in the months corresponding to the beginning and peak of the reproductive period. However, an increase in the oocyst elimina-

tion was observed in the month of March, which corresponds to the end of the reproductive period and the beginning of the feathering period, and in July, probably due to the stress caused by low temperatures or by hormonal changes that precede the beginning of the reproductive period in August. Coelho et al. (2013) carried out a study on the periodicity in the elimination of oocysts of the genus *Isospora* in wild birds of the species *Saltator similis* d'Orbigny & Lafresnaye 1837, kept in captivity at the Wild Animals Triage Center (CETAS / IBAMA), Seropédica, Rio de Janeiro. In species of Passeriformes of the Brazilian avifauna, the reports of the diurnal periodicity in the elimination of oocysts in wild birds of free life or kept in captivity are scarce. The count of forms of parasite propagation in the feces, in this case, oocysts, can be subjective due to the great individual variation, due to factors such as: reproductive period of the host, season of the year, temperature, parasitic infection phase and time of collection sample. Thus, the determination of the effect of these variables on the elimination of oocysts and methods of sampling is fundamental for the correct evaluation of parasitism (Villanúa et al. 2006). In birds, for quantification of oocysts, when the samples are collected at an appropriate time of day, the sample can be used to characterize individual aspects of coccidia infection status. The time of day is a crucial factor when collecting samples of bird droppings because most of the species of coccidia that infect Passeriformes is of the genus *Isospora*. All stages of development of coccidia of this genus, including the elimination of oocysts, have a circadian rhythm in several Passeriformes families. The peak of oocyst elimination has been shown in the late afternoon, between 15h and 20h. It was also observed that when the collection time is chosen correctly, it is not necessary to collect all 24 - hour feces because among the defecations produced within one hour, the oocyst content does not vary much and then one of these samples can be collected for analysis. This allows the use of this method in field investigations, where often due to the fact that it is impossible to keep the birds trapped for long, only one defecation per bird may be available. Thus, this method can be applied successfully in wild populations of different bird species (Dolnik 2006).

The objective of this study was to evaluate the periodicity and intensity of the elimination of oocysts of the genus *Isospora* in fecal samples from passerines seized of illegal traffic and kept in captivity.

MATERIAL AND METHODS

For the evaluation of the periodicity and intensity of oocyst elimination, 602 fecal samples of various Passeriformes species (IUCN 2016) were collected from May to November 2010. These passerines were seized from wildlife trafficking and kept in the CETAS (Centro de Triagem de Animais Silvestres - Center for Triage of Wild Animals)/IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - Brazilian Institute of Environment and Natural Resources)/MMA (Ministério do Meio Ambiente - Ministry of Environment) (22° 43' 23.79" S and 43° 42' 36.94" W), located in the municipality of Seropédica, state of Rio de Janeiro, Brazil. The method described by Dolnik (2006), Dolnik et al. (2009) and Coelho et al. (2013), where fresh fecal samples, one defecation per bird, were individually collected from a sheet of paper towels previously placed on the bottom of the cages after adequate hygiene in the morning (between 9 and 12h) and in the afternoon (between 15 and 17h). Samples were stored in sterile plastic containers and sent to the LCC (Laboratório de Coccídios e Coccidioses - Coccidia and Coccidiosis Laboratory)/DPA (Departamento de Parasitologia Animal - Department of Animal Parasitology), located at Annex 1 in the UFRRJ, Seropédica *Campus*, RJ. Samples were stored in 2.5% potassium dichromate solution (K₂Cr₂O₇) 1: 6 (v/v) in Petri dishes and incubated at room temperature (around 23-28 °C) for several days at least 4 days, according to the methodology described by Dolnik (2006) or until 70% of the oocysts were sporulated. For the preparation of the slides for microscopy, the technique described by Duszynsky & Wilber (1997) and Dolnik (2006) with modifications were used, using the centrifugal-flotation technique with sugar (SG 1.20) (500 grams of sugar and 320 mL of distilled water) at 2000 rpm (= 447 x g) for five minutes. For the statistical analysis Fisher's Exact Test was used according to Campos (2016) and Sampaio (2002).

RESULTS AND DISCUSSION

The evaluation of the periodicity of the elimination of oocysts of *Isospora* species presented highly significant results ($p < 0.0001$) and it was observed that fecal samples collected in the morning (between 9 and 12 h) had a lower number of positive results (12/2%), unlike those collected in the afternoon (between 15 and 17 h), which had a greater number of positive samples (136/23%) (Table 1). The evaluation of the intensity of infection measured by OoPD (number of oocysts by defecation) in the different families of Passeriformes showed highly significant results ($p = 0.0001$) and it was observed that the fecal samples collected in the morning showed a lower OoPD in the morning and higher in the late afternoon. The OoPDs of the passerines of Cardinalidae, Emberizidae and Thraupidae were also different in intensity (Table 2). In the other fa-

Table 1. Periodicity of *Isospora* oocysts in Passeriformes fecal samples from CETAS/Seropédica, RJ.

Periods ^a	Oocysts	Total	p value ^b
Morning:	Positive	12 (2) ^c	301 (50)
	Negative	289 (48)	
Afternoon:	Positive	136 (23)	301 (50)
	Negative	165 (27)	
Total			602 (100)

^a Fecal samples collected in the morning, between 9 and 12h a.m. and in the afternoon, between 15 and 17h p.m.; ^b Highly significant to Fisher's Exact Test; ^c percentage in parentheses.

Table 2. Intensity of *Isospora* oocysts in Passeriformes fecal samples from CETAS/Seropédica, RJ.

Families	N ^a	OoPD ^b		p values ^c
		Morning	Afternoon	
Cardinalidae	122	0.041 (0.0-1.0)	102,344 (0.0 - 3,668)	0.0001
Emberizidae	142	0.035 (0.0-1.0)	42,014 (0.0 - 4,192)	0.0001
Thraupidae	21	0.048 (0.0-1.0)	168,480 (0.0 - 1,800)	0.0001

^a Number of samples used; ^b Mean and lower and upper limits of oocysts shed between 9 and 12h a.m. and between 3 and 5h p.m.; ^c Highly significant to the Wilcoxon test.

milies examined, Icteridae, Turdidae, Mimidae and Cotingidae had the same tendency to shed more oocysts in the late afternoon, between 15h and 17h, but were not significant given the small number of samples examined, 6, 3, 5 and 2 respectively. These results are similar to Boughton (1937, 1988), where was reported a peak of oocyst elimination between 15h and 21h, Dolnik (1999) between 16h and 20h, Brown et al. (2001) between 14h and 17h, Misof (2005) between 12h and 18h, Martinaud et al. (2009) between 16h and 19h, Filipiak et al. (2009) between 16h and 21h, Zinke et al. (2004) between 15h and 17h, Dolnik et al. (2010) between 16h and 18h, and Morin-Adeline et al. (2011) between 14h and 19h. Dolnik et al. (2011) observed that under the permanent light conditions of the Arctic summer, *Isospora plectrophenaxia*, a parasite of a passerine of Arctic, still maintains a 24h rhythm of oocyst production with peak elimination in afternoon, despite the absence of daytime periodicity of host activity. Birds kept in captivity may begin elimination earlier and end later when compared to free-living birds with a clearance peak ranging from 13h to 21h. In most birds kept in cages during the first day of captivity, the number of oocysts recovered in the late afternoon is significantly higher than during the next few days in captivity (Dolnik 1999). Based on this report, in the present work was collected whenever possible, on the first day of the arrival of the birds in the CETAS quarantine, as this would also guarantee a more reliable diagnosis, which was confir-

med, since the largest number of positive samples. For the presence of oocysts of species of the genus *Isospora* was found in the late afternoon. In an experiment with house sparrows (*Passer domesticus*) was verified that the circadian rhythm of oocyst elimination does not depend on the feeding habit of the birds and is related to the photoperiod, since, after the reversal of the light in darkness, there is a reversion in the oocysts elimination. It has also been observed that birds have individual circadian rhythms of oocyst elimination and the rhythm exists for different species of *Isospora* spp. and their hosts. As this genus has a monoxenous cycle, a circadian rhythm cannot be an adaptation of the time of the activity of the intermediate host such as *Plasmodium* spp. (Dolnik 1999). It is known that oocyst elimination is controlled by host and physiological differences between eating habits are probably responsible for these differences. The variation in the amount of feces produced according to the time of day could influence this rhythm (López et al. 2007). In nature, birds have two peaks of food activity, one in the morning and the other in the evening. During this period, the birds are in the same territories to feed themselves. At the breeding season they feed on the same territory every day as well as during the migration season, many species gather in flocks to feed themselves. Therefore, the appearance of the parasites in the feces during this period would increase the concentration (Dolnik 1999) and this would offer a greater chance for coccidia to infect a new host of the same species (Dolnik 1999, Martinaud et al. 2009).

Oocysts are relatively resistant to environmental factors such as temperature and relative humidity and it has been reported that desiccation may reduce the infectivity of oocysts of *Eimeria* spp. In the birds of production and confirmed in an experiment with infection by *Isospora turdi* in *T. merula* in which it was observed that the release of oocysts at late afternoon is a form of adaptation to provide resistance to desiccation and ultraviolet radiation. If oocysts were released during the course of the day, most would be rapidly destroyed by the action of sunlight (Martinaud et al. 2009). In addition, in some species of birds which begin to migrate, the morning feeding peak disappears, but never the peak feeding at dusk (Dolnik 1999). In this way, these birds meet each other at least at one time of the day and the oocysts remain viable, increasing the chances of infection of new hosts. The described methodology provides faster and stress-free sample collection for birds, being practical and

can be used in rehabilitation centers for wild birds, commercial breeding and zoos where on many occasions treatment or control of coccidiosis has to be applied quickly. Mainly in the quarantines where there is entry and exit of birds constantly. Collection of fecal samples in the inadequate period of the day could lead to false diagnosis and erroneous treatment evaluations. Another advantage of this methodology is that it does not require expensive reagents or equipment, unlike other methods that do not fit into the reality of the wildlife screening centers of this country. The training of technicians, biologists and veterinarians working in these institutions could be the first step towards the release of birds with more discretion and safety both for those birds that are destined for release, and for those that often remain in the quarantine or nurseries waiting for the resolution of legal proceedings or transportation for the realization of releases in other municipalities and states. The results indicated that the late afternoon period between 15h and 17h offers greater reliability ($p < 0.0001$) for the prevalence and diagnosis studies of coccidiosis in Passeriformes kept in captivity and destined for release, as these wild birds maintain a rhythm circadian pattern of oocyst elimination very similar to those described in other species of endemic birds in other countries with different latitudes and longitudes.

Acknowledgments. This study was supported by CAPES and FAPERJ). The authorizations to carry out the studies were SISBIO/ICMBio (License # 45200-1) and CEUA/UFRRJ (# 036/2014 and FAPERJ # E-26/101-365/2014). We are grateful to the staff of the Center (CE-TAS/IBAMA), Seropédica, RJ, mainly to the Veterinarian, Dr. Daniel Marchesi Neves.

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