

Periodicity of oocysts shedding of *Eimeria* spp. by Japanese quails *Coturnix japonica* Temminck, Schlegel experimentally infected

Bruno do Bomfim Lopes | Bruno Pereira Berto | Helcio Resende Borba | Viviane Moreira de Lima | Caroline Sousa do Carmo | Carlos Wilson Gomes Lopes

Submetido em 11.10.2013

Aceito em 22.10.2013

Abstract Lopes BB, Berto BP, Borba HR, Lima VM, Carmo CS, Lopes CWG. 2013. **Periodicity of oocysts shedding of *Eimeria* spp. by Japanese quails *Coturnix japonica* Temminck, Schlegel experimentally infected.** [Periodicidade de eliminação de oocistos de *Eimeria* spp. em codornas japonesas *Coturnix japonica* Temminck, Schlegel experimentalmente infectadas] *Coccidia* 1, 17-20. Departamento de Biologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro. BR-465 km 7, 23897-970 Seropédica, RJ, Brasil. E-mail: bertobp@ufrj.br

In coccidia of wild or captivity passerines there is a pattern in dynamic of oocysts shedding, with a predominance of shedding at late afternoon. The most common hypothesis suggest that this condition is an adaptation to prevent desiccation and ultraviolet radiation, reducing the mortality of oocysts. In this context, this study aimed to quantify oocysts shedding of three *Eimeria* spp. from Japanese quails *Coturnix japonica* Temminck, Schlegel experimentally infected, in two times of day: morning and late afternoon. The results suggested that the circadian rhythm of oocysts shedding, which occurs in *Isospora* spp. of passerines, not occurs in *Eimeria* spp. from Japanese quails. Whereas the predominant oocysts shedding at late afternoon is an evolutionary adaptation to the coccidia of wild animals, could be that the domestication of Japanese quails from the eleventh century, has not afforded the emergence of this evolutionary adaptation or it may have been lost.

Keywords OoPG, oocysts, coccidia, poultry, Galliformes

Resumo Em coccídios de pássaros silvestres ou de cativo há um padrão na dinâmica de eliminação de oocistos, com uma predominância de eliminação ao entardecer. A hipótese mais comum sugere que esta condição seja uma adaptação para prevenir a dessecação e a radiação UV, reduzindo a mortalidade dos oocistos. Neste contexto, o presente estudo teve como objetivo quantificar a eliminação de oocistos de três *Eimeria* spp. de codornas japonesas *Coturnix japonica* Temminck, Schlegel, 1849 experimentalmente infectadas, em dois momentos do dia: amanhecer e entardecer. Os resultados sugeriram que o ritmo circadiano de eliminação de oocistos, o qual ocorre em *Isospora* spp. de passeriformes, não ocorre em *Eimeria* spp. de codornas japonesas. Considerando que o predomínio de eliminação de oocistos ao entardecer seja uma a-

BB Lopes

Biólogo Autônomo associado ao Laboratório de Coccídios e Coccidioses, Universidade Federal Rural do Rio de Janeiro (UFRRJ).
 E-mail: biolopesbb@hotmail.com

BP Berto ✉ | HR Borba | VM Lima

Departamento de Biologia Animal, Instituto de Biologia, UFRRJ, BR-465 km 7, 23897-970 Seropédica, RJ, Brasil.
 E-mail: bertobp@ufrj.br
 borba@ufrj.br
 vilima@ufrj.br

CS Carmo

Curso de Graduação em Farmácia, UFRRJ.
 E-mail: carolinesousa@ufrj.br

CWG Lopes

Departamento de Parasitologia Animal, Instituto de Veterinária, UFRRJ. CNPq fellowship.
 E-mail: lopescw@ufrj.br

daptação evolutiva para coccídios de aves silvestres, pode ser que a domesticação de codornas japonesas desde o século XI não tenha proporcionado o surgimento dessa adaptação evolutiva ou esta pode ter sido perdida.

Palavras-chave OoPG, oocistos, coccidia, aves de produção, Galliformes

Introduction

Often in *Isospora* spp. of passerines in the wild or captive is observed a pattern in the dynamic of oocysts shedding. Pioneering, the French research group of Grulet et al. (1982, 1986a, b, c) described 12 *Isospora* spp., besides noting some aspects of the biological cycles of these species, including the fact that its development followed a circadian rhythm, which, during the summer, resulted in abundant oocysts shedding during the late afternoon.

In recent decades, many authors confirmed the presence of circadian rhythms suggested by the French group, including studies with common starlings *Sturnus vulgaris* L., dark-eyed juncos *Junco hyemalis* L., european greenfinches *Carduelis chloris* L., eurasian blackbirds *Turdus merula* L., green-winged saltators *Saltator similis* d'Orbigny, Lafresnaye, lesser seed-finches *Oryzoborus angolensis* L. and great-billed seed-finches *Oryzoborus maximiliani* Cabanis (Brawner III & Hill 1999, Dolnik 1999, Hudman et al. 2000, McQuiston 2000, Brown et al. 2001, Misof 2004, Coelho et al. 2012a, b, 2013).

Therefore, it was considered that any study of prevalence of coccidia in passerines should be done considering the circadian rhythm of these parasites (López et al. 2007). In an attempt to justify himself this dynamic of oocysts shedding in passerines were proposed two hypothesis: First, the period of oocysts shedding correspond to the peak of food activity. As many individuals share the same food, it is assumed that the oocysts shed in the feeding site, were more susceptible to be ingested. However, two peaks of food activity are recognizable: morning and late afternoon (Dolnik 1999, McQuiston 2000, Misof 2004).

The other hypothesis is based on the resistance of the oocysts to environmental factors such as temperature and humidity. It is recog-

nized that drying can reduce the infectivity of the oocyst. In this regard, the oocysts shedding in the late afternoon could be a adaptation to prevent desiccation under natural conditions. By testing this last hypothesis using oocysts of *Isospora turdi* Schwalbach, 1959, parasite of *T. merula*, it was observed that the short exposure of the feces to natural sunlight reduced considerably the infectivity of the oocysts, suggesting that heat and ultraviolet radiation are the main factors that damage the oocysts (Martinaud et al. 2009).

These two observations suggested that the oocysts shedding in the late afternoon is actually an adaptation to prevent desiccation and ultraviolet radiation, reducing thus the mortality of oocysts in the environment (Martinaud et al. 2009).

In this context, this study aimed to quantify oocysts shedding of three *Eimeria* spp. from Japanese quails *Coturnix japonica* experimentally infected in two periods of the day: morning and late afternoon.

Materials and methods

Eight one-day-old Japanese quails *C. japonica* were obtained from a commercial rearing farm located in the Municipality of Seropédica in the State of Rio de Janeiro, Brazil. The chicks were transported to the Federal Rural University of Rio de Janeiro (UFRRJ), and were reared and fed a single cage without anticoccidial additives. Feed and water were administered *ad libitum*.

The adult Japanese quails from the commercial rearing farm used to provide the chicks were naturally parasitized by three species of *Eimeria*: *Eimeria bateri* Bhatia, Pandey, Pande, 1965, *Eimeria tsunodai* Tsutsumi, 1972 and *Eimeria uzura* Tsunoda, Muraki, 1971.

Thus, an inoculum for experimental infection was produced by recovering and isolating oocysts from fecal samples of positive Japanese quails by flotation in Sheather's sugar solution (S.G. 1.20) according to the protocol of Duszynski & Wilber (1997). The oocysts were preserved in a 2.5% (w/v) solution of $K_2Cr_2O_7$ to induce sporulation and maintained in a refrigerator (2–5 °C) until use. *Coturnix japonica* chicks were inoculated at the age of 7 days, using inocula, quantified using a

Neubauer chamber, containing approximately 6.5×10^4 sporulated oocysts per chick, at the approximate proportion of: 50% *E. bateri*; 25% *E. tsunodai*; and 25% *E. uzura*.

Enumeration of oocysts per gram of feces (OoPG) was performed according to Menezes & Lopes (1995), as modified by Cardozo et al. (2010). The OoPG was determined at morning and late afternoon, at 35, 37 and 42 days after infection (DAI). Sample processing and data analysis were conducted at the Laboratório de Coccídios e Coccidioses and in the Departamento de Biologia Animal (Biology area), both located at UFRRJ.

Results and discussion

Eimeria bateri and *E. tsunodai* were the most significant in the oocyst shedding on the total samples with 24,000 (40%) and 25,620 (42%) oocysts, respectively. *Eimeria uzura* was quantified with 10,980 (18%) oocysts. As can be observed in the graph of the Fig. 1 the lines that demonstrate the oocysts shedding at morning and late afternoon are close, demonstrating that there was no discernible difference in the quantification of oocysts between the two periods of the day. Besides, the lines intersect, demonstrating that there was reversal of the period which more oocysts were shed.

These results suggest that the circadian

rhythm of oocysts shedding which occurs in *Isospora* spp. from passerines not occurs in *Eimeria* spp. from Japanese quails.

As mentioned before, it was suggested that the predominance of oocysts shedding at late afternoon is an adaptation to prevent desiccation and ultraviolet radiation reducing the mortality of oocysts in the environment and thus becoming an evolutionary adaptation for coccidia of wild birds (Dolnik 1999, McQuisition 2000, Misof 2004, Martinaud et al. 2009). As the Japanese quail *C. japonica* were domesticated from the eleventh century being bred in captivity, it may be that this evolutionary adaptation has not developed or has been lost in these poultry.

Acknowledgements

This study was supported by grants from the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) to B. P. Berto (E-26/110.987/2013). We also thank the commercial rearing farm of Japanese quails at the Municipality of Seropédica in the State of Rio de Janeiro, Brazil, for permitting and assisting in the collection of material.

References

Brawner III WR, Hill GE. Temporal variation

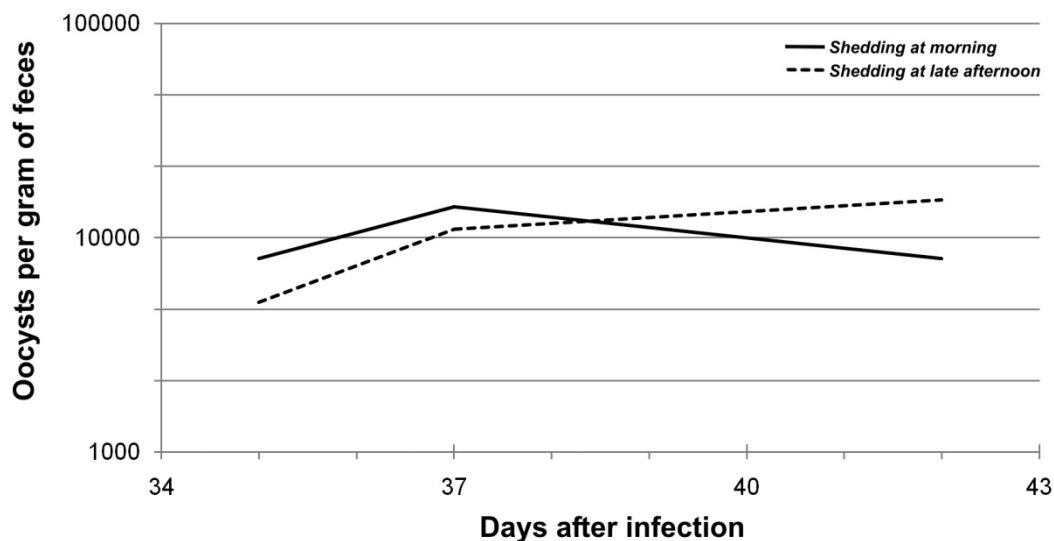


Fig. 1. Periodicity of oocyst shedding of *Eimeria* spp. from Japanese quails *Coturnix japonica* experimentally infected in two periods of the day (morning and late afternoon).

- in shedding of coccidial oocysts: implications for sexual-selection studies. *Canadian Journal of Zoology*, 77, 347-350, 1999.
- Brown MA, Ball SJ, Holdman D. The periodicity of isosporan oocyst discharge in the greenfinch (*Carduelis chloris*). *Journal of Natural History*, 35, 945-948, 2001.
- Cardozo SV, Berto BP, Ferreira AMR, Macedo HW, Lopes CWG. Frequência de *Eimeria bateri* em codornas japonesas (*Coturnix japonica*) desafiadas com dose subletal de aflatoxina. *Revista Brasileira de Parasitologia Veterinária*, 32, 211-214, 2010.
- Coelho CD, Berto BP, Neves DM, Oliveira VMD, Flausino W, Lopes CWG. Diagnóstico e tratamento das coccidioses em trinca-ferros-verdadeiros *Saltator similis* d'Orbigny, Lafresnaye, 1837 mantidos em regime de quarentena. *Revista Brasileira de Medicina Veterinária*, 34, 46-54, 2012a.
- Coelho CD, Berto BP, Neves DM, Oliveira VMD, Flausino W, Lopes CWG. Tratamento da coccidiose causada por espécies do gênero *Isoospora* Schneider, 1881 em curióis *Sporophila angolensis* Linnaeus, 1766 e bicudos *Sporophila maximiliani* Cabanis, 1851 mantidos em regime de quarentena. *Revista Brasileira de Medicina Veterinária*, 34, 102-108, 2012b.
- Coelho CD, Berto BP, Neves DM, Oliveira VMD, Flausino W, Lopes CWG. Oocyst shedding by green-winged-saltator (*Saltator similis*) in the diagnostic of coccidiosis and *Isoospora similis* n. sp. (Apicomplexa: Eimeriidae). *Revista Brasileira de Parasitologia Veterinária*, 22, 64-70, 2013.
- Dolnik, O. V. Diurnal oocyst periodicity in *Isoospora dilatata* (Sporozoa: Eimeriidae) from the Common Starling (*Sturnus vulgaris*) in nature. *Parazitologija*, 33, 74-80, 1999.
- Duszynski DW, Wilber PG. A guideline for the preparation of species descriptions in the Eimeridae. *Journal of Parasitology*, 83, 333-336, 1997.
- Grulet O, Landau I, Baccam D. Les *Isoospora* du moineau domestique; multiplicite des especes. *Annales de Parasitologie humaine et Comparee*, 57, 209-233, 1982.
- Grulet O, Landau I, Millet P, Baccam D. Les *Isoospora* du moineau. I: Compléments à l'étude systématique = *Isoospora* of sparrows. I: Complementary data on the systematics. *Annales de Parasitologie humaine et Comparee*, 61, 155-160, 1986a.
- Grulet O, Landau I, Millet P, Baccam D. Les *Isoospora* du moineau. II: Etudes sur la biologie = *Isoospora* of sparrows. II: Studies on biology. *Annales de Parasitologie humaine et Comparee*, 61, 161-192, 1986b.
- Grulet O, Landau I, Millet P, Baccam D. Les *Isoospora* du moineau. III: Action élective de la primaquine sur les formes d'attente = *Isoospora* of sparrows. III: Elective action of primaquine on latent stages. *Annales de Parasitologie humaine et Comparee*, 61, 193-198, 1986c.
- Hudman SP, Ketterson ED, Nolan V. Effects of time of sampling on oocyst detection and effects of age and experimentally elevated testosterone on prevalence of coccidia in male dark-eyed juncos. *Auk*, 117, 1048-1051, 2000.
- López G, Figuerola J, Soriguer R. Time of day, age and feeding habits influence coccidian oocyst shedding in wild passerines. *International Journal for Parasitology*, 37, 559-564, 2007.
- Martinaud G, Billaudelle M, Moreau J. Circadian variation in shedding of the oocysts of *Isoospora turdi* (Apicomplexa) in blackbirds (*Turdus merula*): An adaptative trait against desiccation and ultraviolet radiation. *International Journal for Parasitology*, 39, 735-739, 2009.
- McQuiston TE. The prevalence of coccidian parasites in passerine birds from South America. *Transactions of the Illinois State Academy of Science*, 93, 221-227, 2000.
- Menezes RCAA, Lopes CWG. Epizootiologia de *Eimeria arloingi* em caprinos na microrregião Serrana Fluminense, Rio de Janeiro, Brasil. *Revista da Universidade Rural: Ciência e Vida*, 17, 5-12, 1995.
- Misof K. Diurnal cycle of *Isoospora* spp. oocyst shedding in Eurasian blackbirds (*Turdus merula*). *Canadian Journal of Zoology*, 82, 764-768, 2004.