

***Isospora bocamontensis* (Protozoa: Apicomplexa) in captive yellow cardinal *Gubernatrix cristata* (Passeriformes: Emberezidae)¹**

Larissa Quinto Pereira^{2*}, Isadora Mainieri O. Corrêa², Gustavo Henrique Schneiders³, Marcella Teixeira Linhares³, Dario Trevisan Almeida⁴ and Maristela Lovato³

ABSTRACT.- Pereira L.Q., Corrêa I.M.O., Schneiders G.H., Linhares M.T., Almeida D.T. & Lovato M. 2013. *Isospora bocamontensis* (Protozoa: Apicomplexa) in captive yellow cardinal *Gubernatrix cristata* (Passeriformes: Emberezidae). *Pesquisa Veterinária Brasileira* 33(3):384-388. Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria. Santa Maria, RS 97105-900, Brazil. E-mail: sissavet@yahoo.com.br

The yellow cardinal (*Gubernatrix cristata*) is a passerine found in southern Brazil, especially along the border with Uruguay and Argentina. It is an endangered species and its population is decreasing. Among the parasites that affect passerines, the genus *Isospora* is the most easily found in both captive and free-living birds. This parasite commonly causes injury to the intestinal tissue and could occasionally affect other organs. In this work we examined the occurrence of coccidiosis in captive yellow cardinals and its association with factors such as sex, use of parasiticides, type of enclosure, contact with feces, type of food and cleaning frequency. We collected fecal samples of 45 yellow cardinals, healthy and kept in captivity, in late afternoon at the end of the reproductive period. The examination showed parasitic infection by *Isospora bocamontensis* in 44.5% of the birds. This infection is not influenced by the sex of birds, but is significantly affected by the type of enclosure, contact with the feces, use of parasiticides, type of food and cleaning frequency. The results indicate that to keep yellow cardinals captive, these factors must be observed.

INDEX TERMS: *Isospora bocamontensis*, Apicomplexa, yellow cardinal, *Gubernatrix cristata*, Emberezidae, protozoa, extinction, passerine, captivity.

RESUMO.- [*Isospora bocamontensis* (Protozoa: Apicomplexa) em cardeais-amarelo *Gubernatrix cristata* (Passeriformes: Emberezidae) mantidos em cativeiro.] O cardeal-amarelo (*Gubernatrix cristata*) é um pássaro que ocorre no sul do Brasil, principalmente na fronteira com Uruguai e Argentina. É uma ave ameaçada de extinção e sua população está decrescendo. Dentre, os parasitas que afetam a ordem

Passeriformes, o gênero *Isospora* está entre o mais encontrado, tanto em aves de cativeiro quanto em aves de vida-livre. Comumente causam injúrias no tecido intestinal, podendo ocasionalmente afetar outros órgãos. Neste trabalho examinamos a ocorrência de coccidiose em cardeais mantidos em cativeiro e verificamos sua associação com fatores como sexo, uso de produtos parasiticidas, tipo de recinto, contato com fezes, tipo de alimentação e frequência de limpeza. Foram coletadas amostras de fezes, ao entardecer, de 45 cardeais-amarelos, hígidos, mantidos em cativeiro, no final do período reprodutivo. O exame coproparasitológico revelou infecção parasitária por *Isospora bocamontensis*, em 44,5% das aves. Esta infecção não é influenciada pelo sexo das aves, mas é significativamente afetada pelo tipo de recinto, contato com as fezes, uso de parasiticidas, tipo de alimentação e frequência de limpeza. Indicando que para a manutenção em cativeiro estes fatores devem ser observados.

TERMOS DE INDEXAÇÃO: *Isospora bocamontensis*, Apicomplexa, cardeal-amarelo, *Gubernatrix cristata*, Emberezidae, protozoários, extinção, Passeriformes, cativeiro.

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² Programa de Pós-Graduação em Medicina Veterinária, Laboratório Central de Diagnóstico de Patologias Aviárias, Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria (UFSM), Av. Roraima 1000. Prédio 44, sala 5152, Santa Maria, RS 97105-900, Brazil. *Corresponding author: sissavet@yahoo.com.br

³ Laboratório Central de Diagnóstico de Patologias Aviárias, Departamento de Medicina Veterinária Preventiva, UFSM, Av. Roraima 1000. Prédio 44, sala 5152, Santa Maria, RS.

⁴ Departamento de Estatística, UFSM, Av. Roraima 1000, Santa Maria, RS. E-mail dos outros autores: isamainieri@yahoo.com.br; schneiders.gustavo@gmail.com; mah_tl@hotmail.com; edcsdn@terra.com.br; lfmaristela@gmail.com

INTRODUCTION

The yellow cardinal (*Gubernatrix cristata*) is found in southern Brazil and is locally recorded in the southeastern mountains and along the border with Uruguay. Their habitat includes open areas with scattered trees, hedges and the Espinilho State Park (Belton 1994). The species is also found in Argentine and Uruguay; in Paraguay it is probably extinct (Collar et al. 1992). This passerine is considered a member of the family Emberizidae (Sick 1997). It is the only representative of the genus *Gubernatrix* (Collar et al. 1992, Pessino & Tittarelli 2006). Locally the species is considered endangered (Marques et al. 2002), and in extinction worldwide, with declining population (Birdlife International 2009). Raising yellow cardinals as a cage bird is highly appreciated because it is a songbird, and the capture of individuals in nature has contributed to the decrease of wild populations, as well as the loss and degradation of their habitat (Collar et al. 1992, Pessino & Tittarelli 2006). Besides the capture and the habitat loss mainly to farming and livestock, the lack of scientific information on population size, reproductive success and environmental requirements are also issues that prevent the development of appropriate management measures for the species preservation.

The only alternative to the massive capture and loss of habitat of some species has been captive breeding, but often performed without knowledge or information on how to keep and raise them in captivity or in the wild (Silveira et al. 2004, Olmos 2005). Wild birds already in captivity may represent an important source of genetic material, particularly the endangered species. Knowing how birds are affected by parasites and infections is an useful way to prevent the losses in captive management.

Studies show that the occurrence of parasites ranges in between 10% to 66%, and the endoparasites mostly found in birds are coccidia (Santos et al. 2008, Córdon et al. 2009, Marietto-Gonçalves et al. 2009, Dolnik & Hoi 2010, Godoy & Matushima 2010) and among Passeriformes, the genus *Isoospora* (Page & Haddad 1995, Cubas 1996, Lopez et al. 2007) are the most prevailing. *Isoospora* are generally associated with enteric infections, but may also affect other organs such as kidneys and liver (Friend & Franson 1999, Greiner 2008). Clinical signs will depend on the damage caused to the intestinal cells by juvenile parasites, which will result in decreased feeding, nutrients absorption and digestion process, besides increasing susceptibility to other agents (Friend & Franson 1999).

We know the importance of coccidiosis in domestic birds, but because of the protozoa ability to have preference for the host species, and most infections are self-limiting in nature, coccidiosis in wild birds has not received due importance. However, the habitat loss, the concentration of the population in smaller areas, added to the release of captive individuals into nature can raise the incidence of coccidiosis among the wild birds (Friend & Franson 1999). Except for the morphology of the oocysts, little is known about the impact of *Isoospora* infections in free-living birds (Greiner 2008). The identification of the parasites that have been found helps develop prophylactic, bio-safety and bio-security measures, either regarding captive birds or free-

-living individuals that are received and are in conditions to be relocated. In this work we examine the occurrence of coccidiosis in captive cardinals in the region of Santa Maria, Rio Grande do Sul.

MATERIALS AND METHODS

Fecal samples were collected from 45 healthy yellow cardinals (*Gubernatrix cristata* Vieillot, 1817) randomly chosen from seven amateur breeders registered at SISPASS (MMA 2003), located in Santa Maria, Rio Grande do Sul, Brazil (29°41'29S; 53°48'2W), at the end of the reproductive period. The material was taken from all individuals of the species kept in aviaries. To collect the feces we used sheets of clean brown paper placed on the bottom of the cage or aviary at the end of the afternoon and removed early in the morning the next day. The paper sheets containing the samples were placed in individual containers previously cleaned and transported under refrigeration to the laboratory where they were analyzed soon thereafter. Information was collected about sex, use of parasiticides, kind of enclosure, contact with feces, kind of food and the enclosures cleaning frequency.

A portion of the samples taken, of approximately 0.5 g, was standardized and set apart to search for endoparasites, and the rest was stored for further identification of the parasites found. The separated samples were processed according to the technique of Willis-Mollay modified. The technique consists of dissolving the feces in a hyper saturated salt solution, and after homogenization the content is filtered and deposited into a flask, completing it up to the brim with the solution to form the meniscus. A slide is placed for 10 minutes and then everted, and a cover slip is overlaid. The reading was made under an optical microscope (Olympus BX41, Melville, NY, USA), at magnifications of X100, X400 and X1000. When found coccidia, the portion set apart was placed in a thin layer of a solution of 2.5% of potassium dichromate ($K_2Cr_2O_7$) and incubated for 10 days or until 70% of the oocysts became sporulated. The sporulated oocysts were recovered using the Willis-Mollay technique described above, and the oocysts were examined under an optical microscope and compared the morphological and morphometric characteristics with data from literature for identification (Carvalho-Filho et al. 2005, Berto et al. 2009, Balthazar et al. 2009, Pereira et al. 2011).

The fecal samples were distributed by the intensity of infection, graded in five levels according to the average oocysts found in four fields, at the ends of the coverslip, viewed at magnification X100: negative (no oocyst), rare (1 to 30 oocysts), mild (31 to 99 oocysts), moderate (100 to 149 oocysts) or intense (above 150 oocysts). Regarding sex, the birds were divided into males, females and undefined. We classified as undefined all nestlings whose sexual dimorphism we were unable to identify for sure. The use of parasiticides was considered positive to those breeders who have used products containing substances that act against gastrointestinal helminthes at least once in the last six months. The enclosures were considered cages if they were up to 1 meter high and all other above this, were classified as aviaries. The location was not considered as external or internal. The contact with feces was classified as: with contact or without contact.

The type of food was grouped into: (A) mix of seeds for cardinals, (B) commercial food for Passeriformes, (C) fruits, eggs, vegetables and supplementary diet mix, (D) products for poultry (chicken and quail feed), (E) only one seed option, and each breeder marked the corresponding diet (more than one option could be marked). The cleaning frequency was divided into daily, almost daily, weekly or twice a month. The results obtained for the degrees of infection were compared to sex, the kind of enclosure, contact with feces and type of food, by means of the Kruskal-Wallis

test. The use of parasiticides was evaluated by the Mann-Whitney test. The cleaning frequency of the enclosures was compared to the level of infection by the test of Kruskal-Wallis when all intervals were considered and by the Mann-Whitney test when only daily or almost daily intervals were considered. The statistical analysis was conducted using the software SPSS Statistics, 17.0.1 version.

RESULTS

In the fecal samples, 55.5% of the samples were negative, and in 44.5% oocysts of *Isoospora bocamontensis* (Pereira et al. 2011) were found, and the infection intensity found is given in Table 1. No other parasite was found in the samples examined. The number of birds per breeder ranged in between 1 to 14. Of the 45 yellow cardinals, 17 were male, 15 female and 13 undefined. The birds' sex did not influence the infection level ($\chi^2 = 0.474$; $n-1=2$; $p=0.789$).

Parasiticides were used in 66% of the birds (Table 2) by five breeders, and its use influenced the increased susceptibility to infection by *Isoospora* in treated birds ($U=93.5$; $p<0.001$; $\eta=0.498$). Of the enclosures (Table 3) in which the birds lived, 27 were cages and 18 aviaries, and in 80% of the enclosures the birds were in contact with feces. When kept in cages, there was no significant variation in the level of infection due to contact with feces ($U=43.5$; $p<0.053$). By comparing cages (with and without contact) with aviaries (with contact), it was found that the birds kept in aviaries tend to have lower levels of infection by coccidia ($U=63.0$; $p<0.001$; $\eta=0.641$).

Table 1. Estimates (in %) for the population of captive yellow cardinals distributed according to the levels of infection by coccidia

Level of infection	Number of birds	
	Sample	Population
Negative	25	55,5%
Rare	12	26,7%
Mild	7	15,6%
Moderate	1	2,2%
Intense	0	0,0%
Total	45	100,0%

Table 2. Amount of captive yellow cardinals distributed according to the level of infestation by coccidia and the use of parasiticide

Use of parasiticide	Level of infection by coccidia				Total
	Negative	Rare	Mild	Moderate	
Yes	11	11	7	1	30
No	14	1	0	0	15
Total	25	12	7	1	45

Table 3. Amount of captive yellow cardinals distributed according to the level of infestation by coccidia and the enclosure/contact with feces

Enclosure/Contact with feces	Level of infection by coccidia				Total
	Negative	Rare	Mild	Moderate	
Cage/without contact	1	3	4	1	9
Cage/with contact	6	9	3	0	18
Aviaries/with contact	18	0	0	0	18
Total	25	12	7	1	45

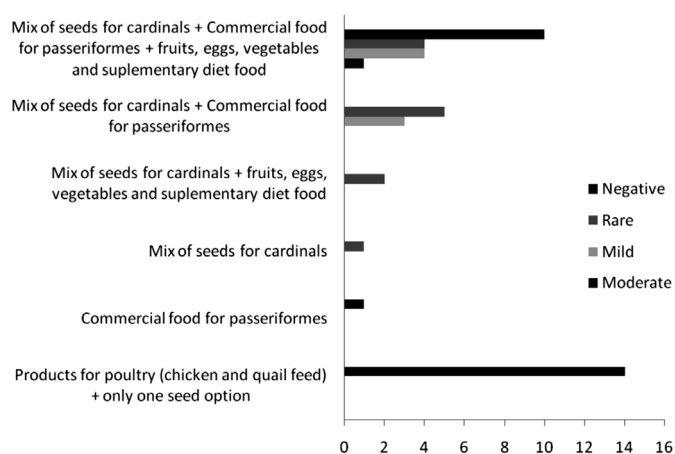


Fig.1. Distribution of the sampled birds according to the level of infection by coccidia and the type of food.

The birds' food was distributed into six groups formed according to the alternative marked by the breeders (Fig. 1). The type of food influenced the infection level, some of them in lower degree ($\chi^2 = 24.012$; $n-1=5$; $p<0.001$; $\eta=0.610$).

In the breeders studied, all intervals of cleaning frequency were found ranging from daily to twice a month. Daily or almost daily cleaning was present in 64% of the breeders (Table 4). When all intervals were compared, they had impact on the level of infection and this association was inverse and of moderate intensity. Thus, when cleaning interval decreased, the level of infection by coccidia increased ($\chi^2 = 20.469$; $n-1=3$; $p<0.001$ e $\Gamma=-0.553$; $p<0.003$). By comparing only the daily or almost daily intervals, the association was direct and very intense. Therefore, as the cleaning interval decreased, the level of infection decreased too ($\chi^2 = 23.000$; $n-1=1$; $p<0.032$; $\alpha=5\%$ e $\Gamma=+0.860$; $p<0.014$).

Table 4. Amount of captive yellow cardinals distributed according to the level of infestation by coccidia and the enclosure cleaning frequency

Frequency of cleaning	Level of infection by coccidia				Total
	Negative	Rare	Mild	Moderate	
Daily	4	1	0	0	5
Almost daily	6	10	7	1	24
Weekly	1	1	0	0	2
Twice a month	14	0	0	0	14
Total	25	12	7	1	45

DISCUSSION AND CONCLUSION

The occurrence of *Isoospora* in passerines of the family Emberezidae has been reported worldwide (Pereira et al. 2011). Coccidiosis is reported both in captive birds and in free-living birds (Santos et al. 2008, Lindström et al. 2009, Dolnik et al. 2010), and the prevalence of infection in free-living passerines can reach 100% of the birds (Schwalbach 1960). The only noninvasive method to determine the presence of coccidia is by counting the oocysts in the host's feces, and because of the variation found in the amount of *Isoospora* sp. oocysts dropped by birds, the samples should be collected preferably at late afternoon, when the release of oocysts is higher (Lopez et al. 2007, Lindström et al.

2009). This is because of oocysts' adaptation to prevent dissection and increase the likelihood of survival in the environment (Filipiak 2009).

In most wild birds, *Isoospora* sp. is widely spread (Dolnik 2010). In the Americas, the birds of the families Embereziidae and Thraupidae are affected by about 30 different species of this protozoon (Pereira et al. 2011).

The species found in the samples was *Isoospora bocamontensis*, the same described in Boca do Monte (Pereira et al. 2011), a district of Santa Maria, where the samples were taken. The sampled breeders were located in different sites of the town, which indicates that the cardinals in this region are likely affected by a single species.

The positivity rate of 44.5% for oocysts found in yellow cardinals is similar to that reported in other captive birds, like canaries, in which the level of infection by coccidia was of 50.5%. In this species, parasitism by *Isoospora* sp. is very common and may be associated with loss of weight, decreased reproduction and death. The absence of clinical signs may occur in birds that have acquired immunity during infection and despite this continue eliminating oocysts in the excreta (Freitas et al. 2002), causing subsequent chronic infection in the birds (Dolnik & Hoi 2010). The asymptomatic birds may develop clinical signs when the symbiosis between the parasite and the host is broken (Cubas 1996).

Infection by coccidia has influence on the most diverse aspects of the birds' behavior, such as hierarchy, male aggressiveness, choice of mates and nesting places (Dolnik & Hoi 2010). The samples were collected in the late period of reproduction, and this stage demands great efforts by the birds, affecting their immunity response and favoring parasitic infections (Norris 2000). The analyzed sample was homogeneous regarding the gender of the birds and has not influenced the level of infection. In some species, predisposition to infection is found in males, because a high level of testosterone can lead to increased infection by coccidia and diminishes the cell-mediated immunity (Mougeot et al. 2004). However, this may vary according to the host species, and the immune response may be similar between males and females (Filipiak et al. 2009).

The type of enclosure and the contact with feces had influence on the infection, and it was found that the captive birds in contact with feces had the lower levels of infection. It is believed that the contact with the oocysts stimulates the immune response in a compensatory way (Luchese et al. 2007), but it does not prevent re-infections, including self-infection (Dolnik & Hoi 2010). A primary infection can reduce significantly the oocysts excretion in a subsequent secondary infection. The birds infected naturally by contact have shown that even a low oocysts excretion may cause infections by contact, and the level of oocysts expelled will depend on the condition of acquired immunity (Velkers et al. 2010).

The constant exposure of the birds to the pathogens increases the risks of infections and diseases. Daily cleaning of the aviaries is recommendable, but in sites where reproduction occurs, frequent human interference may cause diminished reproductive rates (Cubas 1996). Most of the enclosures in this study were cleaned up every day or al-

most every day, but when comparing all samples reduced cleaning intervals contributed to an increase of the levels of infection. Complete elimination of the oocysts in the environment is difficult due to its high resistance to chemical destruction, but they are sensitive to high temperatures and dissection (Belli et al. 2006).

Considering only the daily or almost daily cleaning of the enclosures, present in most cases, it showed a strong correlation between the infection level and the frequency, and the shorter the cleaning interval the lower the infection level. The diet of the birds whose enclosures were cleaned up every two weeks may have interfered in the result of all samples.

The characteristics of the diet may affect the susceptibility to infections, once frequently subtle changes in the levels and kind of ingredients may affect the immune system (Hoer 2010). Considering the infection levels, the birds fed with the following groups consisting of: 1) mix of seeds for cardinals, commercial food for Passeriformes and fruits, eggs, vegetables and supplementary diet mix 2) commercial food for Passeriformes, and 3) poultry products (chicken and quails feed) and only one option of seed had shown the lower rates of infection by coccidia. The bird samples that received food of group 3 were all negative, and this may be associated with the presence of premix with coccidiostatic in the commercial formulations for poultry available in the market. To determine the appropriate diet for captive birds, other parameters not contemplated in this study should be evaluated.

The presence of coccidia was that expected for captive passerines, and the levels found were low, classified as rare, mild or moderate, assuming a chronic or recurrent infection, a characteristic of the protozoa of the genus *Isoospora* in Passeriformes. The birds kept in captivity provide information on reproductive, behavioral and health matters, and may be used in programs for the species preservation. After increasing the number of individuals able to reproduce in captivity, these birds may be released into protected areas. According to IBAMA (2005), 78% of the animals captured are released into nature without any criteria regarding sanitarian precautions. Although wild birds are considered to be asymptomatic carriers of coccidia, studies about the existing population at the sites of release should be made to determine the occurrence of parasites, thus avoiding that the entry of new birds may result in the introduction of extraneous pathogens to the population or contribute to an increased number of parasites.

According to the samples surveyed, the yellow cardinals in captivity showed parasitic infection by coccidia, *Isoospora bocamontensis*, in 44.5% of the birds. This infection is not influenced by the sex of the birds, but is significantly affected by the type of enclosure where they are kept, contact with feces, use of parasiticides, type of food and cleaning frequency. The findings of this study indicate that to keep these birds in captivity these aspects should be observed.

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