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Study of the presence of *Salmonella* spp. and gastrointestinal parasites in excreta from ornamental birds from breeders in the city of Umuarama, Paraná

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Pets have been associated with emotional and physical benefits for their owners, and among these, ornamental birds can bring positive influences on quality of life. On the contrary, they harbor pathogenic micro-organisms of public health importance. Thus, this study aimed to investigate the presence of *Salmonella* spp. and intestinal parasites in 52 samples of excreta of passerines and parrots from breeders in the city of Umuarama, Paraná. Conventional procedures for isolation and identification of *Salmonella* spp. were used. Colonies suspected with the genus *Salmonella* were tested serologically for flagellar H antigen and somatic antigen O. Faecal samples were also subjected to sedimentation and centrifugal flotation with zinc sulfate techniques to identify gastrointestinal parasites. Out of 52 excreta samples examined, 7 samples revealed large number of coccidian oocysts per field, highlighting the need to implement preventive measures to reduce the number of oocysts eliminated in animals. All samples were negative for the presence of *Salmonella* spp. Despite the lack of positive results for *Salmonella* spp. in samples of excreta of the birds in this study, control measures should be taken so that they are not a source of risk to human health.

Key words: *Salmonella*, coccidia, passerines, psittacines, zoonosis.

INTRODUCTION

The handling of a pet has been associated with physical and emotional benefits to its owners, since they can have a positive influence on the quality of life of people. On the other hand, the popular practice of handling birds as pets is an important risk factor for public health. Despite the benefit generated by living with pet birds, the birds can also host agents for infecto-parasitic diseases that can be transmitted to human beings (Grespan, 2009).

The zoonotic potential of pet birds is not limited to the direct contact with them, but can be associated with

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activities performed in the environment where they live, such as gardening and tree pruning both in urban and in rural environments (Fenga et al., 2007).

The identification of etiological agents and risk they represent for keeping the animals in captivity becomes extremely useful, since it allows interference on the factors involved, thus lowering the chance of diseases arising, and also contributing towards the preservation of threatened species (Saidenberg, 2008), as well as bringing benefits to public health.

Psiitacines are birds that occupy the whole world, from tropical areas to cold regions (Godoy, 2006), and are represented by cockatoos, macaws, parrots, parakeets, among others. According to the same author, from data collected in the world census of endangered Psittaciformes performed by Bird Life International in 1994, 86 (26%) of 332 psiitacine species in the world are threatened with extinction, and from these, 36 of them are very close to being extinct, which shows the importance of studying the prevalence of diseases that jeopardize the life of these birds.

Grahan and Grahan (1978) reported that Gram-negative bacteria are not part of psiitacine microbiota. However, Godoy (2006) reported that the normal intestinal biota of psiitacines is made of approximately 80% Gram-positive bacilli, 20% Gram-positive cocci, and might have rare Gram-negative bacilli and yeasts.

Salmonella is a very important bacterium for the breeding of ornamental birds. According to the new classification for *Salmonella*, based on the similarities of bacterial DNA, only two species have been recognized, *Salmonella bongori* and *Salmonella enterica*. Several *Salmonella* spp. serotypes are pathogenic to birds and men. In countries with specific surveillance service for salmonellas, the two microorganisms that are most frequently isolated are Salmonella enterica serovariety Typhimurium (*Salmonella* Typhimurium) and *Salmonella* enterica serovariety Enteritidis (*Salmonella* Enteritidis), which are potentially pathogenic to humans (Langoni, 2006).

The most common agent of salmonellosis in birds is *Salmonella* Typhimurium (Guimarães, 2006), and they can serve as infection reservoirs for other birds, mammals and humans, showing its importance in terms of public health. In psiitacine, the most frequent species include, as well as *Salmonella* Typhimurium, *Salmonella* Enteritidis, with virulent and non-virulent strains, which can be associated with the same host (Reavill, 1996).

Among the numerous sanitary issues affecting the wild and exotic birds kept in captivity, the parasitic diseases are one of the most frequent, and the effect they produce range from sub clinical infections to death (Carneiro et al., 2011). Among the parasitic diseases, coccidiosis, the intestinal protozoan disease, caused by *Eimeria* and *Isospora* species are important in ornamental birds, whereas *Isospora* are the most common species in Psittaciformes (Greiner and Ritchie, 1994). Parasitic diseases predispose the captive birds to secondary infections in addition to their interference in behavior and reproductive performance of birds (Freitas et al., 2002), emphasizing the importance of control of parasites in captive birds.

Considering the increasing contact between men and ornamental birds as pets, the present work aimed to research the presence of *Salmonella* spp. and intestinal parasites in 52 samples from passeriform and psiitacine excretion belonging to breeders in the city of Umuarama, Paraná.

MATERIAL AND METHODS

This paper was approved by the Ethics Committee and Research involving Animal Experimentation (*Comitê de Ética e Pesquisa Envolvendo Experimentação Animal* - CEPEEA) under protocol 21825/2012. A total of 52 fresh excrete samples were collected from bird cages (Psiitacide and Passeriform) with swab from five breeders in the city of Umuarama, Paraná (Table 1). These breeders raised their animals only recreationally or had them also for commercial reasons, keeping them in individual cages.

Analysis of the presence of *Salmonella* spp. in excretions

Excretion samples collected from cages were placed in vials containing 5 mL of sterile peptone water and were processed in the Laboratory of Preventive Veterinary Medicine and Public Health at Universidade Paranaense (UNIPAR) for the presence of *Salmonella* spp.

In the laboratory, the analytical procedure adopted was the conventional bacterial isolation, according to methodology described by Marietto-Gonçalves et al. (2010), with a few modifications. The samples were incubated for 18 to 24 h in an incubator at 37°C. Then, 1 mL from each sample was transfered to the selective broth Tetrathionate and selenite-cystine, and were incubated for 24 h. After the initial selective enrichment phase, an aliquot was taken for the sowing in Petri dishes in MacConkey (MC), Brilliant Green (AVB) and Xylose Lysine Deoxycholate (XLD) agars and reincubated for further 24 h. After this, the bacterial growth was read, and the presence of colonies that are characteristic of the bacterium was noticed. A biochemical screening was performed with the triple sugar iron (TSI) Agar, urea and lysine iron agar (LIA) and were later incubated at 37°C for 18 h. The colonies that presented biochemical behavior characteristic for the bacterium were submitted to the agglutination test in plates with anti-flagellar H and anti-somatic O polyvalent sera.

Analysis of the presence of gastrointestinal parasites in excretion

The faecal samples to be analyzed for the presence of gastrointestinal parasites were collected from the cages with the aid of spatulas and stored in falcon vials containing 10% formalin until usage. Initially, the technique by Hoffmann et al. (1934) was used to search for protozoan cysts and helminth eggs (spontaneous sedimentation). Further samples were subjected to the zinc sulphate centrifugal flotation technique (Craig et al., 1970) to specifically identify the protozoan cysts.

Table 1. Number of samples collected from excretions in relation to species (passeriformes and psiitaciforms), age and gender of ornamental birds from five breeders in the city of Umuarama, Paraná.

Birds	Species	Order	Number of samples	Age (months)	Gender
Canary	*Serinus canaria* (Linnaeus, 1758)	Passeriformes	10	4 to 132	04 male 06 SI
Hooded siskin	*Sporagra magellanica* (Vieillot, 1805)	Passeriformes	01	-	01 SI
Green-winged saltator	*Saltator similis (*d'Orbigny and Lafresnaye, 1837)	Passeriformes	16	12 to 84	06 female 10 male
Great-billed Seed-finch	*Sporophila maximiliani* (Cabanis, 1851)	Passeriformes	07	24 to 84	07 male
Creamy-bellied Thrush	*Turdus amaurochalinus* (Cabanis, 1850)	Passeriformes	01	-	01 male
White-throated Thrush	*Turdus albicollis* (Vieillot, 1818)	Passeriformes	02	12 to 60	02 male
Rufous-bellied Thrush	*Turdus rufiventris* (Vieillot, 1818)	Passeriformes	03	24 to 120	03 male
Chopi Blackbird	*Gnorimopsar chopi* (Vieillot, 1819)	Passeriformes	04	4 to 132	01 female 03 male
Chestnut-bellied seed-Finch	*Sporophila angolensis* (Linnaeus, 1766)	Passeriformes	03	48 to 72	02 female 01 male
Double-collared seedeater	*Sporophila caerulescens* (Vieillot, 1823)	Passeriformes	01	4	01 male
Cockatiel	*Nymphicus hollandicus* (Kerr, 1792)	Psiitaciformes	01	---	01 SI
Budgerigar	*Melopsittacus* undulatus (Shaw, 1805)	Psiitaciformes	02	---	01 SI
Ultramarine Grosbeak	*Cyanoloxia brissonii* (Lichtenstein, 1823)	Passeriformes	01	12	01 male

SI = No gender identification.

RESULTS AND DISCUSSION

All excrete samples were negative for the presence of *Salmonella* spp., which is in collaboration with Butron and Brightsmith (2010), who reported absence of *Salmonella* spp. in wild psiitacines in the National Reserve of Tambopata in Peru.

Marietto-Gonçalves et al. (2010) isolated *Salmonella* spp. in one sample (blue-fronted Amazon sample) out of 89 cloacal swabs from three different species of Psiitacines from a rehabilitation program for wildlife. This bird was withdrawn from the rehabilitation program and isolated due to the dissemination risk, both at the place where the project took place and in the future, in the wild, due to the possibility of the bird becoming an asymptomatic carrier as the normal intestinal microbiota of psiitacines can present rare Gram-negative bacilli (Godoy, 2006).

Contrary, Schubert (2008) and Lopes (2011) isolated *Salmonella* spp. in Psittacines from a zoo in the city Sorocaba - SP (11 %) and in the metropolitan region of Fortaleza, CE (1.65%), respectively. The absence of *Salmonella* spp. in the present study could be due to the absence of large number of viable cells for the isolation of *Salmonella* spp. that are necessary in conventional bacterial isolation as it was also reported by Oliveira et al. (2002). Thirty seven samples were positive for *Salmonella* spp., out of 280 cloacal swab samples examined, collected from Psittacine by polymerase chain reaction (PCR) (Allgayer, 2003) indicating the sensitivity of molecular techniques.

Monitoring the presence of Gram negative bacteria in the enteric microbiota of Psittaciformes in captivity must be included in the routine of private breeders, zoo parks, veterinary hospitals and mainly in projects for the reintroduction of

Table 2. Passeriforme species positive for the presence of *Eimeria* oocysts related to gender and age from two breeders in the city of Umuarama, Paraná.

Birds	Species	Gender*	Age (month)
Great-billed Seed-finch	*Sporophila maximiliani* (Cabanis, 1851)	M	72
Chopi Blackbird	*Gnorimopsar chopi* (Vieillot, 1819)	M	132
Green-winged saltator	*Saltator similis (*d'Orbigny and Lafresnaye, 1837)	F	48
Green-winged saltator	*Saltator similis (*d'Orbigny and Lafresnaye, 1837)	M	36
Chopi Blackbird	*Gnorimopsar chopi* (Vieillot, 1819)	F	4
Green-winged saltator	*Saltator similis (*d'Orbigny and Lafresnaye, 1837)	F	42
Green-winged saltator	*Saltator similis (*d'Orbigny and Lafresnaye, 1837)	M	50

M = Male; F= female.

captivity birds into the wild, due to the risk of disseminating possible pathogens in allocation enclosures and their dispersion into the environment, contributing therefore to the epidemiological chain of several enteric diseases to men and other animals (Marietto-Gonçalves et al., 2010).

Despite the negative results for *Salmonella* spp. in Psittacines and Passeriformes, there are more concerned due to their zoonotic potential while been used as pets. Parasitological examination of 5_2 excreta samples reveled coccidian oocysts in large number in seven samples. The species of positive birds, their age and gender are presented in Table 2.

Coccidiosis, caused by species of *Eimeria* and *Isospora* is the most common intestinal parasitosis in birds (López et al., 2007) and *Isospora* spp. is more frequent in Passeriformes, Psittaciformes and Piciformes, while *Eimeria* spp. is mostly found in Galliformes and Columbiformes (Greiner and Ritchie, 1994)

Similarly, coccidian oocysts were identified in the excreta of wild birds in the city of Seropédica, RJ (Costa et al., 2010) and also birds (green-winged saltators, seedeaters, cockattos, canaries and grosbeaks) from private breeders in the city of Alegre – ES (Carneiro et al., 2011). *Isospora* spp. oocysts were identified in 22 samples out of 42 faecal samples examined (Silva et al., 2013) from Passeriformes in Wild Animal Screening Center (Centro de Triagem de Animais Silvestres – CETAS).

Although no positive results were found for important parasites in terms of public health, it is important to stress the need for seeking voluntary assistance by the breeders, mainly regarding the appropriate usage of medications, sanitary handling and performance of parasitological examination in their breeding animals.

Conclusion

Considering the literature that the birds infected by *Salmonella* spp. as a source of risk for man, the absence of negative results does not justify the option of not monitoring them. New research with bacterial DNA would be pivotal for the real knowledge of infections by *Salmonella* spp. The positive results for coccidia in the excrete samples show the need of adopting preventive measures to reduce the elimination of oocysts.

Conflict of interest

The authors declare that there is no conflict of interest.

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