EVALUATION OF FECAL GRAM STAINS AND PREVALENCE OF ENDOPARASITES IN FREE-LIVING MACAWS

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ABSTRACT – Although Brazil has a great bird diversity, information on the health status of free living psittacine is scarce. The aim of this study was to determine the prevalence of endoparasites and the ratio of gram-negative versus gram-positive bacteria in the feces of free-living adult Hyacinth and Green-winged macaws. Fecal samples were collected placing camouflaged sheet covers on bird foraging areas. Fifty-five swabs were evaluated by gram staining technique and 101 fecal samples were analyzed using flotation and centrifugation methods. None of the samples presented parasites or ova. The presence of gram-negative bacteria was detected in 1.8% of the fecal samples. These results suggest that parasites and gram-negative bacteria may be transient in the gastrointestinal tract of healthy free-living psittacine birds. This situation is quite different from what is observed for birds in captivity, where stressful events and improper husbandry facilitate the dysbiosis and high parasite loads, with an imbalance in the host-parasite relationship.

Keywords: Endoparasites; gram negative bacteria; microflora; psittacines; wild birds.

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Avaliação de esfregaços corados pelo gram e prevalência de endoparasitas em araras de vida-livre

RESUMO - Embora o Brasil tenha uma grande diversidade de aves, as informações sobre o estado sanitário de psitacídeos de vida livre são escassas. O objetivo deste trabalho foi determinar a prevalência de endoparasitas e a relação entre bactérias gram-negativas e gram-positivas nas fezes de Arara-azuis e Araras-vermelhas-grandes adultas em vida-livre. Amostras de fezes foram coletadas empregando-se lonas camufladas onde as aves se reuniam para forragear. Cinquenta e cinco esfregaços foram avaliados pela técnica de gram e 101 amostras de fezes foram analisadas pelos métodos de flutuação e centrífugo-flutuação. Nenhuma amostra foi positiva para a presença de ovos ou formas adultas de parasitas. A presença de bactérias gram-negativas foi detectada em 1.8% das amostras fecais. Esses resultados sugerem que parasitas e bactérias gram-negativas podem ser apenas transientes no trato gastrointestinal de psitacídeos saudáveis de vida livre. Essa situação é bastante diversa do que ocorre em cativeiro, onde situações de estresse e manejo inadequado facilitam a disbiose e o aumento das cargas parasitárias, com um desequilíbrio na relação hospedeiro-parasita.

Palavras-chave: endoparasitas; bactéria gram negativa; microbiota; psitacídeos; aves selvagens.

INTRODUCTION

Most exploration of disease in wild birds is based on retrospective studies of mortality events, sampling individuals rather than populations (Spalding; Forrester 1993). Surveys in free-living animals are therefore important to establish a baseline for epidemiological studies and to better understand disease outbreaks (Stone et al. 2005).

The intestinal flora of healthy captive psittacines is essentially composed of gram-positive bacteria such as *Lactobacillus* spp., *Bacillus* spp., *Corynebacterium* spp., *Staphylococcus* spp., *Micrococcus* spp. and *Streptococcus* spp. (Bangert et al. 1988). In contrast, gram-negative bacteria

are a common cause of bacterial enteritis in captive parrots as either primary pathogens or opportunistic agents (Drewes; Flammer 1986).

Intestinal parasites are frequently found causing disease in captive parrots (Greiner; Ritchie 2004), and available reports on endoparasites of neotropic free-ranging parrots suggest that wild birds are seldom parasitized or, if found, the prevalence is extremely low (Gilardi et al. 1995, Stone et al. 2005, Masello et al. 2006, Allgayer et al. 2009).

This study explores the percentage of gram-negative versus gram-positive bacteria and prevalence of endoparasites in fecal samples of free-living adult Hyacinth macaws (*Anodorhynchus hyacinthinus*) and Green-winged macaws (*Ara chloropterus*), providing more information that can be used to further enhance husbandry standards and protocols for parrots maintained in captivity and/or intended for rehabilitation in rescue centers.

MATERIAL AND METHODS

The surveyed location in the Piauí state (northeastern Brazil) is one of the few remaining areas where large flocks of Hyacinth and Green-winged macaws gather for foraging and nesting on sandstone cliffs. This area, belonging to a farmer that embraces ecotourism as a form of income, comprises over 5.000 hectares of pristine savannah (cerrado vegetation) and includes vital feeding and nesting areas for these and many other wild animal species. Both studied species in their Northeastern range are severely affected by the destruction of habitat to give place to soybean plantations (Birdlife 2011).

During June 2009, about 15 *A. hyacinthinus* came to the foraging area every day (morning and afternoon) and about 30 to 40 *A. chloropterus* visited a nearby feeding area daily. In this ecotourism lodge, local wild food types are offered in front of carefully built blinds and the utmost care taken in order to not habituate birds to the human presence. Hyacinths typically feed on Piassava coconuts (*Attalea funifera*) and Green-winged feed on Pequi nuts (*Caryocar brasiliense*), among a variety of other native food sources according to the seasonality.

Fecal samples were collected by spreading camouflaged sheets below preferred perching and feeding trees. Samples were collected twice daily (early morning and late afternoon) during three days at the Hyacinth area and three days at the Green-winged area. To minimize the likelihood of contamination, samples were collected within one hour of deposit on the sheets.

The upper part of the feces was collected using a sterile swab for gram staining and the other stored in formaldehyde solution for parasite detection, taking care to avoid contamination. If contamination was suspected, the sample for gram was discarded.

A total of 55 swabs (25 from Hyacinth macaws and 30 for Green-winged macaws) were subjected to gram staining. The slide was prepared by gently rolling each swab on a slide and heat fixing it. Three slides for each sample were obtained for more accuracy (n= 165). Gram staining was performed following manufacturer's instructions (Newprov©, Pinhais, PR, Brazil) and screened for bacteria morphology and staining with total numbers of bacterial population reported in percentage.

A total of 101 fecal samples for parasites detection (41 from Hyacinth macaws and 60 for Green-winged macaws) were processed according to standard methods using fecal flotation and zinc sulfate centrifugation techniques and screened for the presence of parasites and ova (Sloss; Kemp 1978).

RESULTS

Of the 55 samples (165 slides), an average of 1.8% of gram-negative bacteria were observed (gram-positives: 77% bacilli and 21.2% cocci). No budding yeasts or white blood cells were found and no differences between the two species were evident. The majority (162/165) of the fecal slides did not show a single gram-negative bacterium.

No parasites or ova were observed in the 101 tested fecal samples using the flotation or centrifugation techniques.

DISCUSSION

Even though the gram stain technique is still viewed with a degree of skepticism by some clinicians, it remains an inexpensive and straightforward technique which is able to reveal information that would otherwise be difficult or labor intensive to obtain, such as culturing anaerobic or fastidious microorganisms, as well as a useful technique to quantify bacteria in a given sample (Harrison; MacDonald 2006).

Abilleira et al. (2006) studied free-living Hyacinth Macaw nestlings and also found that gram-negative were present in a similarly low proportion (1.7%) of fecal samples from healthy chicks. In Australia, similar results were also obtained in samples from adult wild cockatoos where no gram-negative bacteria were detected on fecal slides (Harrison; MacDonald 2003), even though this family of parrots had higher amounts of gram-negative bacteria on fecal cultures in captivity, without showing any clinical signs at the time of the sampling (Flammer; Drewes 1988).

While individual identification of each bird was not possible and repeated samples may have been obtained in subsequent days, given our sampling regime, sample size, and number of birds visiting each day, it is likely that our results provide a representative picture of the intestinal microflora in these populations at this time of the year. In regard to the parasite shedding that would be more difficult to determine, working with large number of birds and sampling three days in a row for each species seems to reflect what has been found in other studies (Gilardi et al. 1995, Stone et al. 2005, Masello et al. 2006, Allgayer et al. 2009).

Even though Hyacinths frequently come to the ground to forage on palm nuts, including those previously digested by cattle (Munn et al. 1990) thus causing them to be in close contact with several microorganisms (especially Enterobacteriaceae), our results suggest that these organisms may be transient in their gastrointestinal tracts and may not be able to colonize a healthy bird. This has been shown to be true in experimentally infected budgerigars (*Melopsittacus undulatus*) where gram-negative bacteria were not able to colonize healthy individuals (Glünder 2002).

The opposite seems to be true in captivity were immunosuppressed birds, due to poor husbandry and improper nutrition, lack of physical and mental stimulus and socialization, sexual frustration and neglect, frequently develop health issues involving gram-negative bacteria overgrowth (Flammer 1998; Ness 2006).

The lack of parasites or ova in this study is similar to the findings in several species of neotropical parrots. Although there is still the possibility that they were not shedding at the time of the sampling, it appears that parrots, even when coming to forage on the ground or for geophagy purposes (Gilardi; Munn 1998), rarely come in contact with infective forms of enteric parasites in nature. This is contrary to the situation in captivity where organic material builds up in enclosures with access to the ground, and inappropriate husbandry, coupled with contact with other animal species and stressful events, facilitates high parasite loads and an imbalance in the host-parasite relationship (Greiner; Ritchie 1994). It has also been hypothesized that wild parrots ingest secondary compounds in their diets that function as natural parasiticides (Janzen 1978, Gilardi; Munn 1998).

This sampling method might represent a new approach of surveying birds in situations that would be extremely difficult or dangerous to be attempted (e.g. capturing large adult birds with mist nets) as a tool for epidemiological studies of populations. Although some contamination with environmental bacteria could occur, results were similar to previous works using a more controlled sampling (Abilleira et al. 2006).

A larger number of samples including other species would be important to study in the natural habitat further elucidating gram-negative and prevalence of endoparasites in parrot populations, especially those that are under threat of extinction.

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