

Infectious Evolution, from Megabacteriosis to Escherichia coli and salmonellosis in Sayaca Tanagers (Thraupis sayaca): a Case Report

Evolução Infecçiosa, da Megabacteriose à Escherichia coli e salmonelose em Sanhaços-Cinzentos (Thraupis sayaca): Relato de Caso

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Abstract

This study documents the presence of megabacteriosis (*Macrorhabdus ornithogaster*) in two sayaca tanagers (*Thraupis sayaca*) received at the Wildlife Rehabilitation Center (CRAS) in Araras, São Paulo - Brazil. Additionally, the subsequent presence of *Escherichia coli* and *Salmonella* spp. bacteria were identified during veterinary follow-up. Megabacteriosis and the presence of the identified bacteria pose significant challenges to wild birds in urban environments. The former is a gastrointestinal fungal infection that can weaken birds, leading to symptoms such as emaciation and feeding difficulties. Urbanization can increase stress in birds, predisposing them to this condition. Furthermore, environmental contamination, scarcity of natural food sources, and exposure to pathogenic microorganisms like *E. coli* and *Salmonella* spp. exacerbate the situation. These bacteria can be transmitted through water, food, or direct contact (secretions, especially feces, food, respiratory secretions, water), proliferating in urban areas with a high population density of birds. Infected wild birds can exhibit a variety of symptoms, from diarrhea to respiratory problems. These infections compromise the animals' health, affect their ecological interactions, and even pose risks to public health when there is close human contact. The coexistence of wild birds with complex urban environments and their health challenges demands a multifaceted approach, including monitoring, public education, and management measures to preserve the health of these birds and harmony between wildlife and urban areas.

Keywords: Avian Pathology. Bacteriosis. Conservation. Conservation Institute. Wildlife.

Resumo

Este estudo documenta a presença de megabacteriose (*Macrorhabdus ornithogaster*) em dois sanhaços-cinzentos (*Thraupis sayaca*) recebidos no Centro de Reabilitação de Animais Silvestres (CRAS) em Araras, São Paulo - Brasil. Além disso, foi identificada a presença subsequente das bactérias *Escherichia coli* e *Salmonella* spp. durante o acompanhamento veterinário. A megabacteriose e a presença das bactérias identificadas representam desafios significativos para aves silvestres em ambientes urbanos. A primeira patologia é uma infecção fúngica gastrointestinal que pode debilitar aves, levando a sintomas como emaciação e dificuldades alimentares. A urbanização pode aumentar o estresse nas aves, predispondo-as a manifestação dessa enfermidade. Além disso, a contaminação ambiental, a escassez de alimentos naturais e a exposição a microrganismos patogênicos como *E. coli* e *Salmonella* spp. agravam a situação. Essas bactérias podem ser transmitidas por água, alimentos ou contato direto (secreções, especialmente fezes, ração, secreções respiratórias, água), proliferando em áreas urbanas com alta densidade populacional de aves. As aves silvestres infectadas podem apresentar sintomas variados, desde diarreia até problemas respiratórios. Essas infecções comprometem a saúde dos animais, afetam suas interações ecológicas e até representam riscos à saúde pública quando há contato humano próximo. A coexistência das aves silvestres com ambientes urbanos complexos e seus desafios sanitários exige uma abordagem multifacetada, incluindo monitoramento, educação pública e medidas de manejo para preservar a saúde dessas aves e a harmonia entre a fauna selvagem e as áreas urbanas.

Palavras-chave: Ornitopatologia. Bacteriose. Conservação. Instituto de Preservação. Animais Silvestres.

1 Introduction

The sayaca tanagers (*Thraupis sayaca*) belong to the *Thraupidae* family, with their bodies predominantly covered in gray feathers. They have a relatively short and conical beak, adapted for feeding primarily on fruits, nectar, and insects. Their legs are adapted for arboreal life, with claws that allow them to traverse branches and move skillfully among tree foliage (Batisteli *et al.*, 2019; Santos *et al.*, 2019). These birds are native to tropical and subtropical regions of the Americas and are frequently found in wooded environments, forest edges, and dense vegetation areas (Willis; Oniki, 2003). Even

within urban environments, areas with trees and vegetation exist, so sayaca tanagers can be found in urban parks and gardens, as they are attracted to the presence of trees and shrubs that provide shelter and food. In addition to their aesthetic beauty, these birds are also appreciated for their melodious songs that resonate through the landscape (Zima; Perella; Francisco, 2019).

As a frugivorous and insectivorous species, the sayaca tanager plays a crucial role in seed dispersal (Previatto; De Dainezi; Posso, 2016). While feeding on fruits and nectar, these birds transport seeds through their digestive system to distant locations, promoting the colonization of new vegetative

areas and contributing to biodiversity (Da Silva *et al.*, 2013). Their diet also includes insects and small invertebrates, making them a natural ally in controlling pest populations, thus maintaining ecosystem balance (Vitorino *et al.*, 2014).

The presence of sayaca tanagers in urban areas faces challenges, especially concerning microbiological issues. Urbanization can lead to alterations in the natural habitats of these birds, exposing them to environments contaminated by pollutants and pathogenic microorganisms from urban waste (Murgui; Hedblom, 2017). Proximity to landfills, polluted water, and contact with contaminated surfaces can increase the risk of exposure to pathogens, including bacteria, viruses, and parasites (Marques *et al.*, 2019). Furthermore, the concentration of birds in urban areas can facilitate disease transmission among individuals and species, rendering the health of sayaca tanagers vulnerable to infections and negative population impacts (Hamer *et al.*, 2012; Van Heezik; Seddon, 2017). Understanding and mitigating these microbiological issues are crucial to ensure the survival and health of sayaca tanagers in urban environments.

Megabacteriosis, also known as “Going Light Syndrome,” is a rod-shaped fungus called *Macrorhabdus ornithogaster*, typically found in the normal flora of a bird’s “stomach” (proventriculus) (Werther *et al.*, 2000), primarily affecting the gastrointestinal system (Almeida *et al.*, 2019; Queirós; Carvalho; Pita, 2011). Fungal infections are usually associated with immunodeficiency, stress, malnutrition, and poor hygiene conditions, as it is the case with megabacteriosis, which mainly occurs when birds ingest food or water contaminated with the fungus (Almeida *et al.*, 2019). Diagnosis involves laboratory tests to identify the agent in the bird’s feces or regurgitation (Queirós; Carvalho; Pita, 2011). Treatment may include antifungal medications such as amphotericin B, nystatin or ketoconazole, along with dietary and management adjustments (Almeida *et al.*, 2019; Speer *et al.*, 2004).

In the context of sayaca tanagers, megabacteriosis can become a concern when these birds are found in urban areas. The clustering of birds in urban environments can facilitate the spread of the disease, especially if there are inadequate hygiene conditions (Madi *et al.*, 2023). Therefore, health monitoring and the implementation of preventive measures are essential to ensure the health of this species in urban areas and the preservation of their populations.

The presence of *Escherichia coli* and *Salmonella* spp. in wild birds inhabiting urban environments is a common phenomenon due to environmental contamination, including water and food sources (Lopes *et al.*, 2016). Regarding the evolution of these agents when the immune system is combating other pathogens, such as a fungus, for example, a complex interaction may occur (De Moraes; Ribeiro; Listoni, 2019). A compromised immune system can make birds more susceptible to secondary bacterial infections (Murer *et al.*, 2018), and immunological weakening due to battling a

pathogen, as *Macrorhabdus ornithogaster* can open doors to additional bacterial infections, expanding the clinical signs and increasing the risk of complications. Therefore, the evolution of these agents in birds during periods of immunological stress underscores the importance of holistic health management approaches, considering not only individual pathogens but also interactions among them to ensure the overall health of the bird population.

This study reports the occurrence of megabacteriosis and subsequent detection of *E. coli* and *Salmonella* spp. through veterinary monitoring in two sayaca tanagers attended by the Wildlife Rehabilitation Center, Centro de Reabilitação de Animais Silvestres in Portuguese (CRAS) located in the city of Araras, São Paulo - Brazil.

2 Material and Methods

The Wildlife Rehabilitation Center (CRAS) - “Pró Arara Raul de Barros Winter,” established on July 27th, 2014, primarily focuses on the recovery and repopulation of blue-and-yellow macaws (*Ara ararauna*), the symbol of the city of Araras, São Paulo, Brazil. However, the institution also rehabilitates other wildlife, including birds and small mammals (Barros; Catojo, 2019). The facility provides care for wild birds found in the region, including orphaned chicks, victims of accidents, wildfires, fractures, and other illnesses, as well as those apprehended by the Environmental Military Police and kept illegally as pets. The animals receive veterinary care, undergo quarantine and, after health, nutrition, and behavior assessments, are housed in appropriate enclosures for eventual release.

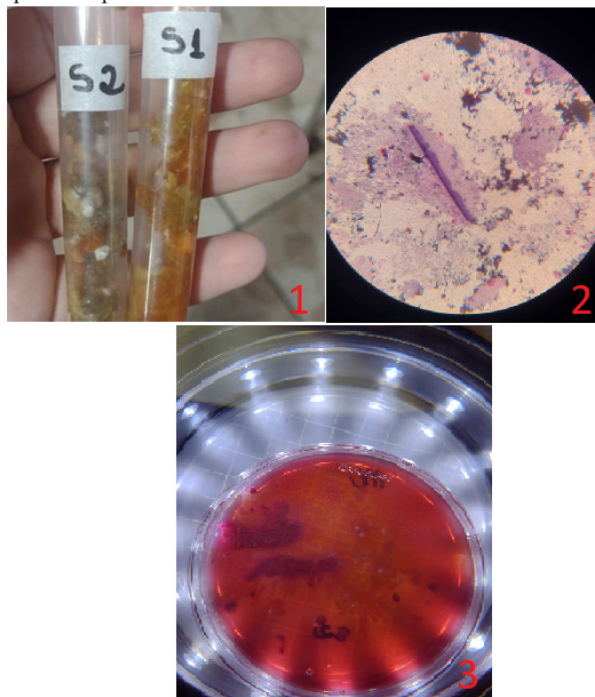
Two fledgling sayaca tanagers were rescued by local residents and brought to CRAS on separate occasions. The first fledgling (patient 1) arrived at the end of October 2022 and underwent the standard protocol for neonatal passerines’ care. It was fed a specific formula and housed in the Bird Treatment Unit (UTA) for warmth until it learned to self-feed, avoiding human contact. However, a setback in its development was noted, with feathers that did not grow or acquire the expected coloration, absence of singing, low activity, polyphagia and large, poorly digested feces. The second fledgling (patient 2), rescued in mid-December 2022, also underwent the same care protocol and was placed in the same enclosure as the first bird. Although it initially showed similar progress to the first sayaca tanager in the first seven days, identical signs of regression began to emerge. In January 2023, fecal samples from both animals were sent to the Veterinary Diagnostic Center (Cedivet) in Araras, where they underwent quantitative polymerase chain reaction (qPCR) tests, developed using fluorogenic hydrolysis probes (Taqman®), to provide a more detailed diagnosis of their health conditions, confirming the presence of *Macrorhabdus ornithogaster*.

After confirming the laboratory diagnosis, the animals were placed in a quarantine area and kept separate. Treatment

was initiated for both animals, with each receiving 0,1 mL of cinnamon-flavored nystatin 100.000 IU/mL twice a day (BID) for a duration of 60 days. Additionally, two drops of a multivitamin (Avitrim®) were added to their water dispenser.

Despite one of the animals undergoing a feather molt at the end of the treatment, both remained lethargic, apathetic, and showed an increase in the quantity of feces. As a result, fecal samples from both birds were sent to the Multicenter Laboratory of the Centro Universitário Anhanguera in Leme (UNIFIAN) for microbiological analysis and antibiotic susceptibility testing (antibiogram). The samples were homogenized in Brain Heart Infusion (BHI) broth, and subsequently, 1,0 g of each sample was taken (Brasil, 2003). Initially, a 1:10 dilution (1,0 g of sample and 10 mL of sterile saline) was prepared and homogenized by agitation in a capped tube with 25 inversions. The dilutions were plated on MacConkey agar and *Salmonella-Shigella* (SS) agar using the pour plate method with 10 µL from the dilution. After plating, the Petri dishes were inverted and incubated at 36°C ± 1 for 24 hours. After 24 hours, the colonies that grew were subjected to Gram staining, and for Gram-negative bacteria, a series of biochemical tests was performed (H₂S, pH, urease, gas production, tryptophan, indole – EPM, motility, lysine – Mili, citrate, lactose, raffinose, rhamnose, malonate, and ornithine). For Gram-positive bacteria, catalase, NaCl 6,5%, bacitracin and optochin tests were conducted to identify the species found (Figure 1). This confirmed the presence of *Escherichia coli* and *Salmonella* spp.

Figure 1 – 1. Stool samples taken for microbiological analysis and subsequent antibiogram, with S1 being the sample from patient 1 and S2 from patient 2; 2. *Macrorhabdus ornithogaster* found in a swab sample of patient 1’s stool, by GRAM staining; 3. *Escherichia coli* colony on MacConkey agar, derived from a sample from patient 2



Source: the authors.

Antimicrobial drug sensitivity tests were conducted with the isolated bacteria using the disk diffusion test, following internationally established standards based on the Kirby-Bauer technique (Wayne, 2008). Antibiotics commonly employed in the treatment of sayaca tanagers with *E. coli* and *Salmonella* spp. were utilized, including cefoxitin, chloramphenicol, doxycycline, enrofloxacin, erythromycin, gentamicin, neomycin, tetracycline, and vancomycin. Enrofloxacin and chloramphenicol showed the most effective results in the sensitivity test.

A second treatment protocol was devised, carried out in May 2023, which again involved the administration of 0.1 ml of nystatin twice daily for 40 days. Additionally, 0.03 ml of another vitamin complex (Glicopan® Pet) along with 1 drop of oral enrofloxacin in 20 ml of water was administered for a period of 14 days.

There was an improvement in the physical appearance, behavioral activity, and consistency of feces. However, patient 1 still did not show signs of feather molt and remained apathetic. Therefore, microbiological studies and antibiogram were conducted again using the previously mentioned methodology, and the same agents were found, with enrofloxacin and chloramphenicol still proving effective. So, in the third protocol, implemented in July, the approach was to administer 0.06 mL of enrofloxacin (diluted in saline solution) orally, once a day (SID), for a period of 14 days, in addition to 0.1 mL of nystatin BID for 40 days. Each attempt represented a unique treatment strategy aimed at addressing the observed symptoms in the animals and improving their health condition (Figure 2).

Figure 2 - A. Patient 1 newly arrived at CRAS; B. Patient 2 newly arrived at CRAS; C. Patient 1, showing responsive aspects and improvement following the treatment outlined in the third protocol; D. Patient 2 having received and completed all the prescribed treatments



Source: the authors.

3 Results and Discussion

CRAS plays a crucial role in the conservation and rehabilitation of wildlife, with a particular focus on the recovery of birds in urban environments. Its importance lies in promoting the health and well-being of animals in vulnerable situations, offering specialized veterinary care, rehabilitation, and specific treatments. Furthermore, the center serves as an educational role, raising awareness within the community about the importance of wildlife preservation and the protection of ecosystems (Barros; Catojo, 2019). By rehabilitating and releasing animals back into their natural habitat, CRAS contributes to local biodiversity and the maintenance of ecological balance, demonstrating its commitment to wildlife conservation and its positive impact on urban communities (Da Luz, 2021).

In urban settings, avian populations confront substantial health risks attributable to their close proximity to anthropogenic landscapes. Such environments pose threats by subjecting birds to pollutants, hazardous waste, and human-borne pathogens (Habib *et al.*, 2023). The dense aggregation of avifauna in urban locales fosters conditions conducive to the swift dissemination of diseases, facilitating transmission within and across species boundaries. This phenomenon is exacerbated by crowding, which fosters intimate contact and pathogen diffusion, thereby heightening the susceptibility to infection (Havliček *et al.*, 2021; Seress; Liker, 2015). Prolonged exposure to suboptimal nutrition and contaminated water, prevalent in urban ecosystems, can severely compromise avian immune systems, augmenting susceptibility to infectious agents. Consequently, the interplay among heightened avian population densities, resource scarcity, and sustained exposure to infection reservoirs presents a formidable challenge needing strategic management interventions and heightened public awareness to ameliorate adverse health outcomes among wild avian populations (Isaksson, 2018). So, after a detailed analysis of the case involving the two fledgling sayaca tanagers rescued and treated at CRAS, the complexity and challenges in rehabilitating these birds become apparent.

Megabacteriosis is caused by the fungus of the genus *Macrorhabdus*, specifically *Macrorhabdus ornithogaster* (De Paula *et al.*, 2017). It induces specific alterations in the intestinal cells of affected birds. There is colonization of the ventriculus and intestines' lining, resulting in chronic inflammation and irritation of the mucous membranes, which can lead to reduced nutrient absorption, impacting digestion and metabolism (Phalen, 2014). The presence of *M. ornithogaster* can lead to changes in intestinal villi, diminishing the animals' ability to absorb essential nutrients, contributing to weight loss and other associated symptoms such as regurgitation, abnormal feces, apathy, and lethargy (Martins *et al.*, 2006; Püstow; Krautwald-Junghanns, 2017). Diagnosis involves detecting the fungus in regurgitation, feces, or biopsies through microscopy techniques or molecular tests

like PCR, as performed in this study. The prognosis depends on the infection stage and response to treatment (Silva *et al.*, 2014). When diagnosed early and treated appropriately with antifungal medications, recovery can be favorable, as observed in both cases (Hannafusa *et al.*, 2007). Proper administration of medications, coupled with nutritional care and immune system support, is crucial to improving the chances of recovery in affected birds.

The effectiveness of quarantine, nystatin treatment and the administration of polyvitamins for birds can vary based on individual circumstances and the health of the birds in question (De Paula *et al.*, 2017). Quarantine is an important measure to prevent the spread of diseases, especially in new animals introduced to an existing group, allowing for observation and isolation of birds, helping to prevent the spread of potential pathogens (Püstow; Krautwald-Junghanns, 2017). Nystatin is a commonly used antifungal to treat fungal infections, including megabacteriosis (Baron *et al.*, 2019; Speer *et al.*, 2004). Its effectiveness depends on the correct dosage, treatment duration, and individual response of the animals. Initially, the birds in the study appeared to respond well to treatment, but further investigation was needed to explore the possibility of opportunistic agents, as clinical and behavioral signs remained unstable. It's worth noting that the administration of polyvitamins can be beneficial for these animals, especially birds that may be experiencing nutritional deficiencies due to illness or stress (Rutz *et al.*, 2002). Polyvitamin complexes for birds typically include essential vitamins like A, D, E, K, and various B complex vitamins, which play vital roles in ocular, bone, metabolic, and immune health (Lavor; De Sousa, 2008). However, vitamin supplementation should be used in moderation and preferably under veterinary guidance, complementing a nutritionally balanced diet.

The detection of *E. coli* and *Salmonella* bacteria in an animal undergoing treatment for megabacteriosis raises concerns about the complexity of interactions between different infectious agents and the bird's health. These bacteria, commonly associated with gastrointestinal diseases in birds and humans, can further worsen the animal's clinical condition, especially when the immune system is already compromised due to a previous infection (De Oliveira *et al.*, 2018; Lopes *et al.*, 2005). The coexistence of these pathogens can increase the risk of complications and make treatment more challenging, thus it is essential to adopt integrated veterinary approaches to address and manage the health of the animals, which includes specific protocols for each infectious agent, continuous monitoring and treatment adjustments as needed, aiming for successful recovery and the preservation of avian health (Díaz-Sánchez *et al.*, 2012).

The presence of the bacterium *E. coli* in wild birds can primarily cause gastrointestinal infections (Knöbl *et al.*, 2008). Although it is normally found in the intestines of healthy birds, pathogenic strains can pose problems, especially under stress, compromised immune systems, or

challenging hygiene conditions (Lopes *et al.*, 2016). This results in symptoms such as diarrhea, weight loss, and lethargy (Padiál *et al.*, 2020). *Salmonella*, on the other hand, is transmitted through contaminated food, water or contact with infected feces, and birds can be asymptomatic carriers; when symptomatic, they may experience diarrhea, lethargy, weight loss, and respiratory problems (Murer *et al.*, 2008). Spread occurs among birds, posing risks to human health (Lopes *et al.*, 2005; Islam *et al.*, 2021). It is also noted that these bacteria affect the avian cellular system in distinct ways. *E. coli* adheres to intestinal cells, produces toxins, and causes inflammation, impairing membranes and nutrient absorption (Padiál *et al.*, 2020); while *Salmonella* invades and replicates within intestinal cells, potentially causing systemic infections (Lugosz *et al.*, 2015). Both compromise health and trigger immune responses, affecting avian well-being.

To treat infections caused by *E. coli* and *Salmonella* in birds, antibiotics such as enrofloxacin, ciprofloxacin, and sulfonamides like trimethoprim-sulfamethoxazole are prominent (Halder *et al.*, 2022). Enrofloxacin and ciprofloxacin, belonging to the quinolone class, disrupt bacterial DNA replication by inhibiting the enzyme DNA gyrase, resulting in bacterial death (Halder *et al.*, 2022; Karim *et al.*, 2020). On the other hand, sulfonamides like trimethoprim-sulfamethoxazole block the synthesis of folic acid in bacteria, halting their growth (Troxler *et al.*, 2017). Enrofloxacin and ciprofloxacin distribute well in tissues, especially in the gastrointestinal tract, while sulfonamides have broad distribution (Li *et al.*, 2017). In the study, enrofloxacin was widely used as it not only showed good results in the antibiogram tests but was also the financially viable antibiotic of choice for the institution.

The proposed feeding regimen and implemented biosafety measures in the case of the sayaca tanagers showcase a meticulous approach to their care and recovery. The administration of specific formulas tailored to the birds' nutritional needs, alongside vitamin supplementation, aimed to support their overall health and immune function during the treatment period. Additionally, the use of nystatin and enrofloxacin, alongside careful monitoring of water quality, exemplifies the commitment to ensuring the birds' safety and minimizing the risk of cross-contamination. Furthermore, the implementation of quarantine measures and separation of the animals during treatment underscored the importance of biosafety protocols in preventing the spread of infectious agents. Overall, the combination of tailored nutrition and stringent biosafety measures reflects a comprehensive strategy aimed at optimizing the birds' health outcomes and mitigating potential risks during their rehabilitation process. It is worth mentioning that at the end of the last treatment and recovery of both animals, patient 2 was released, and patient 1 is still in the growth, feathering, and weight recovery phase for future release.

4 Conclusion

Initially affected by megabacteriosis, the sayaca tanagers exhibited symptoms associated with gastrointestinal dysfunction and overall health. However, the progression of infections led to the identification of *E. coli* and salmonellosis, indicating a possible interaction between these pathogens. This evolution highlights the importance of continuous avian health surveillance, especially in urban environments where factors like exposure to pollutants and stress can contribute to susceptibility to various infections. Understanding this progression of infections is crucial for the implementation of effective prevention, diagnosis, and treatment measures, aiming to preserve the health and well-being of gray tanagers and the maintenance of ecosystem balance.

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