

ASSOCIATION OF NYSTATIN AND SODIUM BENZOATE IN THE TREATMENT OF MACRORHABIDOSE IN COCKATIEL IN NORTHEAST BRAZIL

(Associação de nistatina e benzoato de sódio no tratamento de macrorbidose em calopsita no nordeste brasileiro)

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ABSTRACT

*Macrorhabdosis is an infection caused by *Macrorhabdus ornithogaster*, an opportunistic ascomycete yeast mainly found in the digestive system of birds. Macrorhabdosis presents high morbidity and variable mortality, with death reported more frequently in young and immunosuppressed birds. It can be treated with antifungals; however, resistance to these drugs has been described, which has driven the development of alternative treatments, such as sodium benzoate. This paper presents the first report of macrorhabdosis in cockatiel and describes the treatment protocol, which included nystatin and sodium benzoate and led to complete recovery. A cockatiel with a history of progressive weight loss was treated at a veterinary clinic, and examination showed that it was apathetic, thin, and presented signs of regurgitation. Samples of the ingluvial contents were collected for cytological examination, from which microorganisms compatible with *M. ornithogaster* were identified. Along with supportive therapy, nystatin-based treatment was administered; however, due to the lack of improvement in clinical conditions, sodium benzoate was added to the protocol. After 21 days of treatment, the bird recovered and a marked reduction of *M. ornithogaster* in the animal's microbiota was observed. The results suggest that the inclusion of sodium benzoate contributed to the treatment's success.*

Keywords: Avian clinic, gastrointestinal, alternative therapy.

RESUMO

A macrorhabidose é uma infecção causada por *Macrorhabdus ornithogaster*, levedura ascomiceto oportunista e responsável, principalmente, por sinais clínicos digestórios. A doença apresenta alta morbidade e mortalidade variável, sendo o óbito relatado com maior frequência em aves jovens e imunossuprimidas. O tratamento para a doença pode ser realizado com antifúngicos, no entanto, casos de resistência a essas drogas têm sido descritos, o que tem contribuído para o surgimento de tratamentos alternativos, como o benzoato de sódio. Desta forma, o presente trabalho descreve o primeiro relato de macrorhabidose em calopsita, bem como a terapêutica adotada associando nistatina e benzoato de sódio, levando à completa recuperação da ave. Uma calopsita, com histórico de emagrecimento progressivo, foi levada para atendimento clínico, no qual foi constatado que a ave estava apática, magra e com sinais de regurgitação. Foram coletadas amostras de conteúdo do inglúvio para exame citológico, a partir do qual foram identificados microrganismos compatíveis com *M. ornithogaster*. Foi instituído tratamento à base de nistatina, além da terapia de suporte, mas devido à ausência de melhoria no quadro clínico do paciente, foi realizada a associação de benzoato de sódio ao protocolo. Após 21 dias de tratamento, foi constatada a recuperação da ave e marcante redução de *M. ornithogaster* na microbiota do animal, revelando que a inclusão dessa substância contribuiu para o sucesso do tratamento e apresentou um ótimo custo-benefício.

Palavra-chave: Clínica de aves; gastrointestinal; terapia alternativa.

INTRODUCTION

Megabacteriosis, or “Light Going Syndrome,” is a condition caused by *Macrorhabdus ornithogaster* (CARVALHO *et al.*, 2011), an anamorphic ascomycete yeast that colonizes the

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isthmus, proventriculus, and ventricle, although it can also be found in superficial glands in birds (GRESPLAN and RASO, 2014). The newly proposed taxonomic classification led to a change in the disease name, and the terms macrorhabdiosis or macrorhabdomycosis are currently considered more appropriate (SILVA *et al.*, 2022).

M. ornithogaster is an opportunistic agent that is pathogenic in immunosuppressed individuals, where its clinical form is manifested. This fungus is associated with high morbidity and variable mortality, and it mainly affects young and immunosuppressed birds (SILVA *et al.*, 2022). Moreover, it has been reported in several countries and species, such as budgerigar (*Melopsittacus undulatus*), canary (*Serinus canarius*), lovebird (*Agapornis roseicollis*) (WERTHER *et al.*, 2000), and rhea (*Rhea americana*) (SEGABINAZI *et al.*, 2004).

M. ornithogaster is primarily transmitted horizontally through regurgitation feeding and the oral-fecal route, and its vertical transmission has not been reported (MOORE *et al.*, 2001). The disease is characterized by the involvement of digestive system organs (ALMEIDA *et al.*, 2019), with proventriculitis representing one of the main findings (MARTINS *et al.*, 2006; SILVA *et al.*, 2022); moreover, lesions in the koilin membrane that are observed in the isthmus (PÜSTOW *et al.*, 2017). The symptoms of macrorhabdiosis include undigested seeds in feces (ALMEIDA *et al.*, 2019), progressive weight loss, regurgitation, diarrhea, apathy, and proventriculus hemorrhages (MARTINS *et al.*, 2006; OZMEN *et al.*, 2013).

Macrorhabdiosis treatment involves using antifungals agents, such as nystatin, amphotericin B, or ketoconazole (CARVALHO *et al.*, 2011). Successful alternative treatments using sodium benzoate have been reported in budgerigars (*Melopsittacus undulatus*) (MADANI, GHORBANI and ARABKHAZAEI, 2014), although such treatments have not been reported for cockatiels. Thus, this study reports a case of macrorhabdiosis in a cockatiel.

PATIENT CARE

In August 2021, a nine-month-old, nonsexed cockatiel weighing 85 g that had a history of regurgitation, apathy, and weight loss was taken to the Veterinary Hospital of Universidade Federal Rural do Semi-Árido (UFERSA) in Mossoró, Rio Grande do Norte, Brazil. Anamnesis revealed that the bird was raised freely in a home environment and its diet was based on extruded feed supplemented with vegetables (corn, carrots, and parsley). Less than a month prior to the examination, a new baby cockatiel was introduced into the house. Physical examination revealed that the animal was thin (body score of 2) and apathetic, and baby food was observed on the feathers surrounding the gnathotheca, suggesting regurgitation.

Owing to the clinical signs presented by the bird and the recent introduction of a new individual of the same species, macrorhabdiosis was suspected. For confirmatory diagnosis, a swab was used to collect the content from the ingluvium, and this content was smeared on a slide and Gram stained for a cytological analysis and bacterial and fungal investigation using a conventional light microscope under immersion with a 100x objective. The microscopy analysis revealed a significant increase in the number of gram-positive microorganisms with a rectilinear shape, rounded end, and long stem measuring 1–5 µm in width and 20–80µm in length, which is compatible with *M. ornithogaster* (Fig. 01.A).

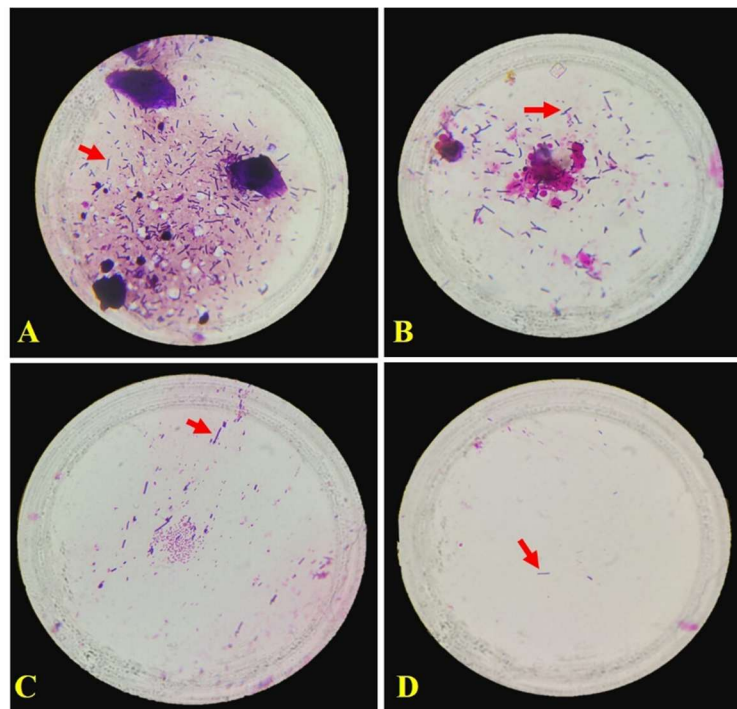


Figure 01: *Macrorhabdus ornithogaster* observed during a cytological examination of the contents of a cockatiel (*Nymphicus hollandicus*) with macrorhabdosis submitted to treatment for 21 days.

Obs.: Gram stain, 100× objective. A = Examination performed before the start of treatment demonstrated long-stemmed Gram-positive microorganisms (red arrow) compatible with *M. ornithogaster*; B = Persistence of *M. ornithogaster* after seven days of treatment using nystatin alone; C = Marked reduction of *M. ornithogaster* at 14 days after the initial treatment and only seven days after the administration of nystatin and sodium benzoate; D = Few microorganisms compatible with *M. ornithogaster* were observed, with a balance of the microbiota observed 21 days after the start of treatment and 14 days after the administration of nystatin and sodium benzoate.

Once the diagnosis was confirmed, treatment with nystatin suspension (500,000 IU/kg, orally by gavage, BID, provided initially for 14 days), metoclopramide (1mg/kg, orally by gavage, BID, used as an antiemetic and prokinetic, provided for 12 days), apple cider vinegar diluted in water (acetic acid 4%, provided over 21 days of treatment), baby food (3mL, provided four times a day until the animal returned to spontaneous feeding after 14 days of treatment), and fluid therapy with lactated ringer (50mL/kg/day, SC, provided for seven days). After seven days of treatment, the animal showed no apparent regression of clinical signs and the condition progressed to worsened apathy and anorexia, with significant weight loss. A new sample collection from the ingluvium was collected using a swab, a new slide was produced for cytological evaluation by Gram staining, and numerous structures compatible with *M. ornithogaster* were visualized again (Fig. 01.B). The use of nystatin, metoclopramide, and baby food was continued, and sodium benzoate (500mg/L of drinking water provided ad libitum for 21 days) and the anti-inflammatory meloxicam (0.2mg/kg, IM, SID, for five days) were added to the therapeutic protocol to reduce inflammation in the digestive tract and provide greater comfort to the patient.

Fourteen days after the start of treatment and only seven days after the administration of sodium benzoate associated with nystatin, the animal showed visible clinical improvements.

A new cytological analysis was then carried out based on the collected ingluvium content samples to monitor the condition of the bird's microbiota, and the results showed the presence of microorganisms compatible with *M. ornithogaster* (Fig. 01.C). Therefore, we decided to continue treatment with nystatin and sodium benzoate but suspended the use of metoclopramide due to the absence of regurgitation. The animal returned to ingesting food and water, and the supply of baby food was reduced in quantity and frequency (volume of 1.5mL, twice a day). At 21 days after the initial treatment and 14 days after sodium benzoate administration, the animal was actively feeding itself, and the nutrition provided by the baby food was maintained to support the recovery of the animal's weight. A new cytological examination of the microbiota of the ingluvium was performed, and it verified that the number of *M. ornithogaster* was significantly reduced (Fig. 01.D), thus demonstrating the compatibility of the treatment with the general clinical state of the animal.

RESULTS AND DISCUSSION

Macrorhabdiosis was diagnosed based on the animal's history and clinical symptoms as well as a cytological examination of the ingluvium contents, and this case report of the disease in a cockatiel (*Nymphicus hollandicus*) in the state of Rio Grande do Norte. Macrorhabdiosis can affect domestic and wild birds, and it is considered a disease with a worldwide distribution and opportunistic character (OZMEN *et al.*, 2013; PHALEN, 2014). In the study case, the introduction of a new cockatiel into the household was suggested as the probable stressor factor.

In this case, a history of progressive weight loss and a body score of observed on clinical examination suggested a chronic condition. In certain species, such as chicken (*Gallus gallus domesticus*), guinea (*Numida meleagris*), and turkey (*Meleagris gallopavo*), the disease manifests clinically as weight loss, prostration, lack of appetite, and cachexia, and it may progress to death (MARTINS *et al.*, 2006). Regurgitation is a clinical sign of the disease in budgies (OZMEN *et al.*, 2013). The horizontal transmission of macrorhabdiosis occurs primarily through regurgitation feeding (MOORE *et al.*, 2001).

Thus, it is possible to infer that the passage of this agent occurs in the esophagus, ingluvial cavity, and oral cavity, which are possible locations for sample collection. The ingluvium or crop is a dilated section of the esophagus in birds, and it has the function of storing food (HARRISON and LIGHTFOOT, 2006) and presents glands that produce acidic and neutral secretions (RAJABI and NABIPOUR, 2009). Macrorhabdiosis causes ingluvial stasis (PHALEN, 2005, LANZAROT *et al.*, 2013), which leads to the accumulation of substrates and secretions in the organ. These conditions can promote the development of the fungus.

A confirmatory diagnosis was made by direct microscopy of the ingluvial swab smear, which was Gram stained. Moreover, this method was used to evaluate the effectiveness of the treatment of the bird based on an analysis of subsequently collected ingluvium samples. The diagnosis of macrorhabdiosis is based on the patient history, anamnesis, clinical signs (SILVA *et al.*, 2022), proventriculus lavage, ventricle or proventriculus mucosa culture (WERTHER *et al.*, 2000), cloacal swabs (for culture), and polymerase chain reaction (PHALEN, 2014; MADANI, GHORBANI and ARABKHAZAEI, 2014); in addition, mini-FLOTAC has also been proposed as a new and effective method of identifying the agent (BORRELLI *et al.*, 2015).

An ingluvium swab has also been reported as a biological sample for diagnosis of macrorhabdosis, and this technique is more sensitive than cloaca and feces swabs. In the present report, an ingluvium swab helped in the diagnosis and monitoring of the mycotic load during treatment, thus demonstrating its suitability for use in cases of the disease.

After the confirmatory diagnosis of macrorhabdosis, a therapeutic protocol was immediately instituted for the bird, with the goal of promptly restoring its health because the disease can often result in the death of the affected animal (GRESPLAN and RASO, 2014). This protocol was based on supportive therapy in consideration of the clinical signs presented by the bird related to proventriculitis, including ulceration and hemorrhage of the proventriculus mucosa, which caused gastric stasis and, consequently, anorexia due to gastric discomfort resulting from injury (LANZAROT *et al.*, 2013; OZMEN *et al.*, 2013).

Thus, the prokinetic agent metoclopramide was used to increase gastric activity and baby food was provided because it presents easier digestibility and thus can better maintain animal nutrition. Additionally, the treatment protocol included fluid therapy to replace the water volume lost due to reduced water intake and episodes of regurgitation associated with impaired gastrointestinal transit, which caused the ingesta to remain static in the ingluvium (PHALEN, 2005).

The antifungal drugs recommended for the treatment of macrorhabdosis include nystatin (PHALEN, 2014), amphotericin B, and fluconazole (GRESPLAN and RASO, 2014). Initially, treatment with nystatin at 500,000 IU/kg (GRESPLAN and RASO, 2014) was administered; however, after seven days of treatment, the animal did not show clinical improvements and a marked amount of fungi compatible with *M. ornithogaster* was still observed on cytological examination of the ingluvium. Since resistance to nystatin and amphotericin B has been reported for this fungus (PHALEN, 2014; MADANI, GHORBANI and ARABKHAZAEI, 2014), sodium benzoate was included in the therapeutic protocol, and this alternative treatment was administered at dosages of 500mg/L to 1g/L in drinking water for four to eight weeks (MADANI, GHORBANI and ARABKHAZAEI, 2014).

Sodium benzoate is a salt of benzoic acid, and it has a solubility 200 times greater than that of the acid and is used as a food preservative and antifungal agent in foods, beverages, cosmetics, and pharmaceuticals. Sodium benzoate toxicity is rare (WIBBERTMANN *et al.*, 2000), although it is more common during the breeding season when birds under water treatment tend to ingest a greater amount of liquid (HOPPES, 2012). In such cases, birds may experience lethargy, depression, with reduced water consumption (MADANI, GHORBANI and ARABKHAZAEI, 2014). It should be noted that no clinical signs suggestive of poisoning were observed in this report during treatment.

Apple cider vinegar was included in the treatment because macrorhabdosis causes an increase in proventricular pH from 2.7 to values ranging from 7.0 to 7.3 (WERTHER *et al.*, 2000). Since the multiplication of *M. ornithogaster* is favored in an alkaline environment, acidification of the proventriculus through the oral administration of apple cider vinegar or citrus fruit juice makes the environment inhospitable for proliferation (OZMEN *et al.*, 2013). Thus, this acid represents a complementary treatment strategy for this disease and a prophylactic when associated with an adequate diet (GRESPLAN and RASO, 2014) and efficient sanitary management (PHALEN, 2005).

Macrorhabdosis in birds is challenging to treat due to its broad and non-specific symptoms. However, ingluvium swabs can assist in the diagnosis and monitoring of the mycotic load in response to treatment in cases of the disease. The association of nystatin with sodium benzoate promoted the recovery of the affected bird and led to a marked reduction of *M. ornithogaster* in the microbiota of the animal. Thus, the inclusion of this substance contributed to the successful treatment of the studied cockatiel.

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