

Successful treatment of macrorhabdosis in budgerigars (*Melopsittacus undulatus*) using sodium benzoate

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Abstract:

Macrorhabdosis is a debilitating syndrome in budgerigars (*Melopsittacus undulatus*) due to the ascomycetous yeast *Macrorhabdus ornithogaster*. In the present study, occurrences of acute macrorhabdosis resulting in severe mortality in budgerigar fledglings and the effect of different treatment regimens for the control of the disease were investigated. The budgerigar (*Melopsittacus undulatus*) flock consisted of over five hundred breeding adults. The morbidity of chicks reached 90% with more than 50% mortality. The significant clinical and pathological findings included distended abdomen, diarrhoea, ingluvitis, proventriculitis, and mild enteritis. Severe *M. ornithogaster* infection was diagnosed based on cytologic and histologic investigations. Three weeks of nystatin medication in the feed and vinegar administration in the drinking water led to moderate improvement of the flock mortality. After the initial treatment, 500 mg/L sodium benzoate was administered in the drinking water for four weeks. The second treatment regimen was promisingly effective in reducing mortality. However, some sick and retarded birds with *M. ornithogaster* with positive proventricular smears at necropsy were found in the flock. Consequently, a higher dosage of 1 gr/L in drinking water for another four weeks was recommended. After the eight weeks of treatment, no new cases were found in the flock and all dropping samples became negative for the presence of *M. ornithogaster*. Based on these preliminary findings, sodium benzoate can be an efficient and inexpensive alternative to the previous labour intensive and expensive treatment using amphotericin B.

Keywords: budgerigar, macrorhabdosis, *Macrorhabdus ornithogaster*, megabacteriosis, sodium benzoate.

Introduction

Macrorhabdosis or megabacteriosis is the condition caused by the so-called "megabacterium", which is a rod-shaped tomentous Gram-positive, periodic acid-Schiff-positive microorganism found in the isthmus between the proventriculus and ventriculus of birds (Van Herck et al., 1984). It was recently established that the causative agent is an ascomycetous yeast named *Macrorhabdus ornithogaster* (Tomaszewski et al., 2003). Macrorhabdosis was initially reported in budgerigars (*Melopsittacus undulatus*) in 1977, as

a debilitating syndrome (Jones & Carroll, 1977). Since then it was reported in many different avian species either with or without clinical signs or pathologic lesions (Gerlach, 2001). In 2008, macrorhabdosis was first incidentally detected in a budgerigar in Iran (Madani & Nejati, 2008), and pathology and control of the infection was studied thoroughly in a budgerigar flock in Kerman, Iran (Kheirandish & Salehi, 2011). While the incidence of macrorhabdosis, or at least its detection and diagnosis have significantly increased in recent years (Marlier et al., 2006; Phalen et al., 2002; Filippich et al., 1993), information about its biopathologic characteristics and therapeutic measures are scant in the scientific literature.

Control and treatment of *M. ornithogaster* infection are challenging for avian clinicians. Because subclinical infections can occur in many birds without obvious clinical signs, it is almost impossible to keep large aviaries free from the infection (Filippich et al., 2004). On the other hand, it has been reported that many antimicrobial and antifungal drugs like iodine preparations, lufenuron, nystatin, fluconazole, ketoconazole, itraconazole, and terbinafine were not effective in treating the infection (Filippich et al., 1993; Phalen et al., 2002; Phalen, 2005). The most effective treatment has been reported to be amphotericin B, which is given orally or gavage fed at a dose of 100 mg/kg twice a day for a month (Phalen et al., 2002; Phalen, 2005). A water-soluble preparation of amphotericin B when given for 14 days in drinking water was not effective (Phalen et al., 2002). Amphotericin B resistant strains of *M. ornithogaster* were identified in Australia (Filippich et al., 1993). The sensitivity of *M. ornithogaster* to sodium benzoate has been previously shown in limited in vitro (Hanafusa et al., 2007b) and in vivo (Hoppes, 2011) studies. In the present study the clinicopathologic aspects of acute macrorhabdosis (so-called Megabacteriosis) in a budgerigar flock were studied and different therapeutic regimens, including sodium benzoate administration, have been investigated.

Materials and Methods

Birds. A breeding budgerigar (*Melopsittacus undulates*) flock with more than five hundred adult birds was referred to the Pet Bird Clinic of the Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran. The flock had a six months history of high mortality and poor growth in fledglings. Previous treatment with ketoconazole and blue vitriol were not effective. The morbidity of four-day-old to one-month-old hatchlings and fledglings had reached 90% with more than 50% mortality at the referral time. Based on the history presented by the owner, more than 3000 chicks had died or were culled during the last six months.

Preliminary diagnostic work-up: Thorough clinical examination of five submitted live fledglings was performed. Faecal wet smears and faecal Gram stained smears were investigated. All five birds were euthanized and a thorough necropsy was performed. In addition two dead chicks were also referred for necropsy. Heart blood, liver, and crop mucosal samples were cultured on blood and MacConkey agar. The distal intestines of chicks were cultured on selenite broth to investigate *Salmonella* infection. Fresh wet smears from the crop, proventriculo-ventricular junction, and distal intestine, and also Giemsa stained contact smears from livers, spleens, and lungs of all chicks were microscopically investigated. Thin sections from the liver, kidney, lung, gonad, adrenal, proventriculus, gizzard, spleen, duodenum, jejunum, crop, thyroid and parathyroid of some birds were studied for histopathologic lesions using haematoxylin and eosin staining.

First treatment regimen and control measures: In the first step immediately after initial presumptive diagnosis based on clinical and necropsy findings, treating the flock with nystatin (JaBer Ebne Hayyan, Tehran, Iran; 500 mg/kg feed) in addition to the use of vinegar (10 ml/L of drinking water) for five to six weeks was recommended.

Second treatment regimen: According to the outcome of first regimen, which was evaluated three weeks after its initiation, the second treatment plan using sodium benzoate (Merck, Darmstadt, Germany; 1 gr/L drinking water) was started.

Diagnostic follow-up: Droppings were collected from each cage for 24 hours and were evaluated weekly during the treatment for the presence of *M. ornithogaster*. Based on our instruction, the owner brought two to three birds back to the clinic on three different occasions over the next six months for follow-up. Besides faecal smears, samples were taken from the crop of referred live birds to detect *M. ornithogaster* using crop lavage. Complete post-mortem examination including gross pathology, microbial culture, cytologic investigation of gastrointestinal

contents, liver, spleen and lung tissue of five additional moribund or dead birds were performed on three occasions during next six months.

Results

In the clinical examination, the fledglings had distended abdomens and droppings attached to the vent region. Another sign was accumulation of dried droppings around the toes of fledglings (Fig. 1a). While growth retardation and occasionally cachexia were evident in some birds, no feather dystrophy or follicular degeneration indicating French molt or budgerigar fledgling disease (BFD) could be observed in referred cases during the whole study.

The wet smear investigation of droppings showed the presence of large numbers of large rod-shaped bacilli resembling *M. ornithogaster* in all specimens.

Necropsy findings indicated proventriculitis and proventricular dilatation. Typical diphtheric ingluvititis was obvious in fledglings' cadavers (Fig. 1b). The crop mucosa showed pseudomembranous necrosis. The proventricular walls of all necropsied chicks seemed to be enormously thickened and tiny haemorrhages were evident at the mucosal surface while a thick mucoid layer covered the mucosa of the proventriculus and some parts of the intestines (Fig. 2). Some chicks had dark brown to black intestinal contents indicating antemortem haemorrhagic diathesis in the intestinal tract, which could be a sign of severe debilitation and/or anorexia in small birds. All wet smears prepared from the proventriculo-ventricular junction had too many large rods resembling *M. ornithogaster*. The Gram stained smears from the proventricular mucosa had too many Gram-positive rods of *M. ornithogaster* and also some Gram-negative bacilli. No bacteria could be isolated from the heart blood and the liver samples of the chicks. Gram-negative coliform bacilli other than *Escherichia coli* and also coagulase-negative Staphylococci were isolated from the crop content of some chicks. All intestinal samples were also negative for *Salmonella*.

Histologic investigation revealed diffused renal tubular necrosis with some round circular to amorphous eosinophilic spheroid materials in some tubuli indicating antemortem dehydration. Nonspecific small necrotic foci in the liver of some chicks with typical anisokaryosis and bile duct hyperplasia, and severe pancreatic zymogen depletion were occasionally found in some tissue samples. No viral inclusion bodies could be found in the studied specimens. No microscopic lesions could be found in thyroid, parathyroid, and spleen tissues. The most significant lesions were found in the proventriculus and the crop. Too many rod-shaped *M. ornithogaster* gathered together and formed a broom stick appearance that invaded the mucosa of the proventriculus and the crop (Fig. 3 and 4). Submucosal cellular infiltration was minimal but degeneration, necrosis and sloughing of the epithelial cells were present in some sections. Acute macrorhabdosis was confirmed as the definitive diagnosis based on the clinical, necropsy and histological findings.

Follow-up necropsies and microscopic investigation of the wet smears prepared from either proventricular mucosa of die-offs or faecal samples after three weeks of initial control measures, including the administration of nystatin in the feed and vinegar in the drinking water, showed only a slight improvement while the mortality of the remaining chicks continued. So it was decided to change the treatment regimen to sodium benzoate administration via drinking water. The treatment with 1 gr/L of sodium benzoate resulted in depression and lethargy in some affected birds and obvious decreased water consumption in the flock. Consequently the dose of medication decreased to the level of 500 mg/L of drinking water and it continued until eight weeks. Interestingly, the droppings examined during the treatment were negative for the presence of *M. ornithogaster* from day 20 after medication and mortality of chicks almost ceased. In addition, the occasional necropsy examination of some birds after eight weeks of the treatment showed typically reduced lesions and a subjectively decreased number of proventricular yeasts. Even after this

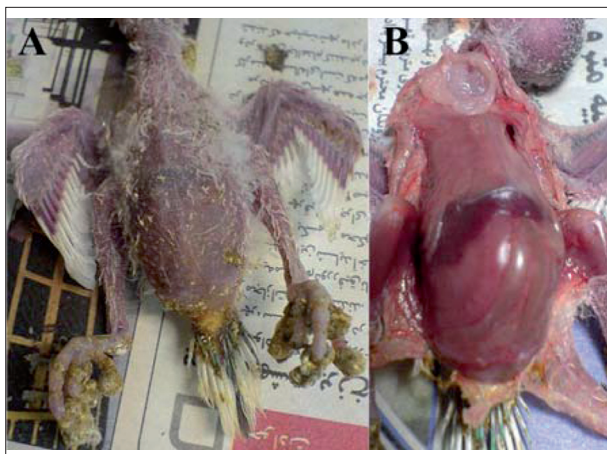


Fig. 1. A) Heavy plantar contamination of the feet in a two-week-old budgerigar affected by severe macrorhabdosis. Diarrhoea and khaki coloured dropping can be a clinical sign of macrorhabdosis in budgerigars. B) Distended abdomen and diphtheric ingluvitis in budgerigar hatchling. Typical thickening of crop and mucosal diphtheric lesion can be the result of severe macrorhabdosis.

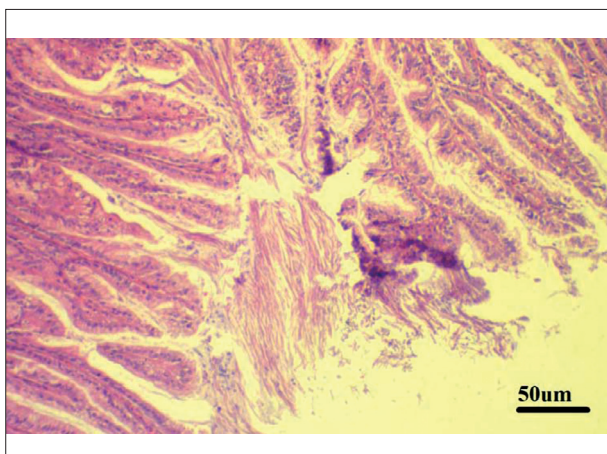


Fig. 3. Photomicrograph of the proventricular mucosa affected by acute macrorhabdosis. Aggregation of a massive number of rod to filamentous shaped *M. ornithogaster* resembling a broom stick and their invasion of the submucosa are evident. H&E staining. Bar = 50µm.

eye catching result in the control and the treatment, there were still birds with droppings attached to the vent region. While faecal samples were negative in those birds, a few *M. ornithogaster* could still be found in the wet smears obtained from the proventricular mucosa during post-mortem examination.

Additionally, weak birds that were still sick despite the treatment were isolated from the flock and placed in separate cages. Apparently healthy pairs were placed back together again and the nests



Fig. 2. Proventricular lesion of acute macrorhabdosis in a budgerigar fledgling. Increased thickness of proventricular wall, enormous mucoid content, mucosal hyperaemia and a focal haemorrhage in the isthmus between the proventriculus and the gizzard can be observed.

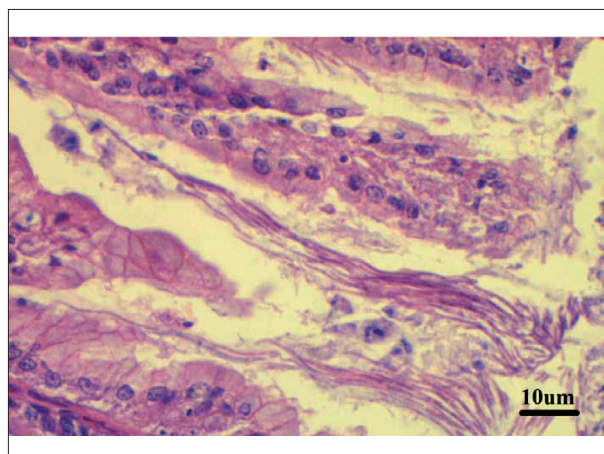


Fig. 4. Colonization and invasion of *M. ornithogaster* within proventricular mucosa. Higher magnification of photomicrograph depicted in Fig 3. Haematoxylin and Eosin. Bar = 10µm.

were returned into the cages. Furthermore, the dosage of sodium benzoate was returned to 1 gr/L of drinking water for another four weeks and until the chicks hatched. Interestingly, necropsy examination and analysis of the droppings of the birds after four weeks of treatment with the higher dosage showed highly desirable results and it seemed to be very efficient in the control of *M. ornithogaster* in the flock, as the last two weak birds which were euthanized and examined, were negative for the presence of *M. ornithogaster* in their proventriculus, the privilege site of

subclinical infection.

Discussion and Conclusion

In this study, clinical signs and pathologic findings of acute macrorhabdosis in budgerigars causing severe loss were thoroughly investigated. The efficiency of the administration of sodium benzoate in drinking water in the affected budgerigar flock, which previously had been unsuccessfully treated with a variety of different drugs, were further clinically evaluated for the first time in Iran. Although this study was not conducted under strictly controlled experimental infection conditions, the results seem to put forward new scopes of studies on the effects of sodium benzoate on *M. ornithogaster* infection in budgerigars.

Macrorhabdosis was reported as the leading cause of illness and death in exhibition budgerigars in England (Baker, 1992; Baker, 1996). It was shown that almost one third of healthy budgerigars could carry *M. ornithogaster* in their stomach without any obvious clinical signs (Baker, 1997), but abundant faecal shedding can be an indication of clinical disease (Filippich et al., 2004). Due to its fastidious nature, isolation is not an appropriate procedure for diagnosis and as a consequence in vitro drug sensitivity studies are rare for *M. ornithogaster* (Hanafusa et al., 2007a; Hanafusa et al., 2007b). *M. ornithogaster* could be stained by different conventional staining methods, such as Giemsa, Gram, and PAS, but a fresh wet smear is the most sensitive and of course easiest method for its detection (Gerlach, 2001; Filippich et al., 2004). As a result of misdiagnosis of other materials and debris, particularly plant fibres, due to an inexperienced microscopist, other more specific methods, such as Calcofluor-white M2R staining (Moore et al., 2001) and PCR (Tomaszewski et al., 2003) have been used in some studies, but neither of them have become a routine diagnostic method.

Treatment and control of the infection, especially in large collections, could be challenging for avian clinicians (Gerlach, 2001; Phalen, 2005). Although in some instances widely available antifungal drugs, such as nystatin, have

been used for treatment both per os (Scullion & Scullion, 2004) and in drinking water (Kheirandish & Salehi, 2011), in the present case the administration of nystatin in the feed for three weeks was not successful in reducing morbidity and mortality. It should be mentioned that nystatin is not water-soluble and water medication of this drug for large aviaries is questionable. It was demonstrated that lowering gastrointestinal pH with drinking water acidification using different organic acids could have some therapeutic effects on *M. ornithogaster* infection (Gerlach, 2001; Filippich, 2004; Phalen, 2005), but in our experience it was not a fully efficient method for the treatment of heavy infection. Despite the partial efficiency of amphotericin B in drinking water in some experiments (Christensen et al., 1997; Filippich & Hendrikz, 1998), it is now believed that the most efficient method for the treatment is gavage feeding of amphotericin B (100 mg/kg) twice a day for 30 days (Filippich et al., 2004; Phalen, 2005). There is no need to elaborate on the difficulty and demanding nature of performing such a treatment in large bird colonies. In agreement with the results of the present study, successful treatment of the infection in a large budgerigar aviary using sodium benzoate, which is a less expensive and water-soluble alternative to amphotericin B, has been once reported previously (Hoppes, 2011). Benzoic acid is a natural ingredient in many foodstuffs and plant extracts (Anonymous, 2000; SCCP, 2005). Undissociated benzoic acid is responsible for antimicrobial activity, but as it is only slightly soluble in water, its salt, sodium benzoate with 200 times more water solubility, is often used as a preservative and antifungal agent in food, beverages, cosmetics, and pharmaceuticals (Anonymous, 2000). Fungal growth was inhibited in a pH dependent manner by concentrations of sodium benzoate ranging from 100-60000 mg/L (Anonymous, 2000). The toxicity of sodium benzoate is rare and its LD50 for rats was reported to be more than 1700 mg/kg (Anonymous, 2000; SCCP, 2005). Sodium benzoate may cause slight skin irritation in healthy human subjects, but in patients with urticaria or asthma, exacerbation of

symptoms was observed (Anonymous, 2000). In spite of its safety for use in animals and humans, lethargy, depression and reduced water consumption were observed in the flock during the initiation of the treatment with 1 gr/L of sodium benzoate. Unfortunately, the daily water consumption of birds was not accurately measured during the treatment process. However, according to the breeder's claim, except for the higher dose introduction of sodium benzoate (1 gr/L) in the drinking water, the treatment did not significantly reduce the water consumption of the birds and did not cause abnormal mortality. Reducing the dosage to 500 mg/L alleviated the initial adverse effects. Afterwards, returning the dosage to its former state was well tolerated by the birds during the final four weeks of the treatment process, indicating possible adaptation. It was also indicated in another report, that breeding pairs and chicks were more susceptible to the adverse effects of sodium benzoate, probably due to more water consumption (Hoppe, 2011).

Viral inclusion bodies or other specific macroscopic and/or microscopic lesions like feather dystrophy or French molt indicating common viral diseases of budgerigars like polyomaviruses and circoviruses were not observed in this budgerigar flock. As long as there was no attempt in the detection of such viruses using virological or molecular methods, their possible association with the presented situation should be kept in mind.

According to this study, the use of sodium benzoate in the treatment and control of *M. ornithogaster* infection in budgerigars that are not producing eggs and rearing their chicks, will achieve acceptable results as compared to the use of other antifungals, such as nystatin. In addition, in the follow-up, there were no occurrences of abnormal mortality and other adverse effects on reproduction of the birds treated with this substance. Even after the subsequent investigation following over two cycles of reproduction, the birds showed almost complete recovery and they were returned to their maximum performance after the treatment with sodium benzoate. Mortality of

chicks after the treatment regimen was reduced to its acceptable range of less than 0.1% in a week.

Studying the effect of sodium benzoate on *M. ornithogaster* infection in experimentally infected budgerigars is highly recommended to assess its impact more accurately.

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