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Assessment of the efficacy of amphotericin B for reduction of Macrorhabdus ornithogaster shedding in budgerigars

ALEKSANDRA LEDWOŃ, PIOTR SZELESZCZUK*, MICHAŁ CZOPOWICZ**

Division of Pathology Exotic, Laboratory, Wildlife Animals and Fish, Department of Pathology and Veterinary Diagnostics;
*Division of Avian Diseases, Department of Pathology and Veterinary Diagnostics,
**Autonomous Laboratory of Veterinary Epidemiology and Economics, Faculty of Veterinary Medicine,
Warsaw University of Life Sciences, 159 Nowoursynowska Str., 02-776 Warsaw, Poland

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Ledwoń A., Szeleszczuk P., Czopowicz M.

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Summary

M. ornithogaster infections are relatively common in budgerigars and other birds from the order Psittaciformes, as well as in species from the order Passeriformes, such as canaries. The treatment of these infections is usually based on the use of amphotericin B (amB). The aim of this study was to reduce M. ornithogaster infection in a colony comprising 25 budgerigars, in which no clinical signs were observed (with the exception of one parakeet) and the amount of fungi in faeces was low. The colony was subjected to a 30-day treatment with amB at a dose of 100 mg/kg, administered into the crop by means of a "ball-tipped" gavage needle every 12 h. Additionally, biochemical analyses of blood were performed in order to assess the adverse effects of the drug. The results did not show any significant changes in the biochemical parameters of blood, or in the general health condition of the budgerigars. The therapy did not lead to the complete elimination of the pathogen, although a significant decrease in the degree of infestation was noted. In the case of the one parakeet with clinical symptoms, the termination of treatment resulted in intensification of M. ornithogaster invasion.

Keywords: amphotericin B, budgerigar, Macrorhabdus ornithogaster, megabacteria

Macrorhabdus ornithogaster is an anamorphic ascomycetous yeast that is the only known member of its genus (15). In mucosal scrapings and in faeces, the organism is a stiff, straight rod, 20 to 80 µm long and 2 to 3 µm wide, with rounded ends. It is Gram positive, but only the cytoplasm stains with the Gram stain (1). *M. ornithogaster* colonizes the isthmus of the proventriculus and gizzard of birds and has not been identified elsewhere in the body or in the environment (14). *M. ornithogaster* infection is most prevalent in captive-bred budgerigars (*Melopsitticus undulatus*), parrotlets (*Forpus spp.*), and canaries (*Serinus canaria*), and can be found in these birds throughout the world (2, 3, 7). Studies conducted by Piasecki et al. (13) revealed the presence of these fungi in feces of captive birds, as well as free-ranging pigeons and passerines in Poland. *M. ornithogaster* was detected in 28.7% of exotic birds and 26.1% of wild birds (13). *M. ornithogaster* has been associated with a chronic wasting condition in the budgerigar, but can affect many other psittacine and non-psittacine birds (2, 3, 5). This pathogen can be associated with a lymphoplasmacytic gastritis in poultry (9) and chronic fatal wasting disease in young ostriches (8). In medicine, invasive fungal infections are treated with amphotericin B (amB). Its efficacy, however, is limited, with response rates from 10% to 80%. Moreover, amB is toxic, especially for the kidneys.

Amphotericin B deoxycholate, a polyene antimicrobial agent, has been in use since the 1950s, mainly to treat fungal infections. Its common toxicities are well-described in the literature and include infusion-related reactions with fevers and chills, renal dysfunction, cytopenias and nausea/vomiting (16). The aim of the study was to assess the efficacy of amphotericin B for reduction of *Macrorhabdus ornithogaster* shedding in a budgerigar colony and the influence of the drug on birds' condition and blood biochemical parameters.

Material and methods

Ethics statement. The authors obtained a positive opinion from the Local Ethics Committee prior to using budgerigars in the experiment.

Twenty-five budgerigars, aged about 6 months, came from an experimental colony, which was in a period of quarantine. Birds were fed a commercial seed mix, Prestige Premium (Versele Laga, Belgium), supplemented with a vitamin mixture, Ornitovit Papużki (Dolfos, Poland), and cuttle fish bone (Vadigran, Belgium). The birds were fed *ad libitum* and had unlimited access to fresh water. The study lasted 6 weeks. Before treatment, all budgerigars except one, showed a low intensity of *Macrorhabdus ornitogaster* shedding. Parakeets were randomly allocated into an experimental group (n = 25) and a control group (n = 8). The birds were placed in cages, 2 birds in each. In order to eliminate the pathogen from the experimental group, the parakeets were treated with amphotericin B (Ampho-moronal® Suspension 100 mg/ml, Dermapharm AG). The product was diluted fivefold in water and administered via the esophagal tube at a dose of 100 mg/kg (0.2 ml/40 g b.w.) by a "ball-tipped" gavage every 12 hours for 30 days (3, 5, 12). Parakeets from both groups were weighed every week (7 times in total). Blood was collected from all budgerigars 3 times: just before treatment (week 0), at the end of treatment (week 4) and two weeks after the completion of treatment (week 6). It was collected from the jugular vein with tuberculin syringes containing lithium heparin, centrifuged and sent to the Idexx Laboratories (Germany) for biochemical tests: AST, bile acids, total protein, albumin, cholinesterase, uric acid, CK, LDH, inorganic phosphate, calcium, potassium and α-amylase. Samples were obtained and examined individually. Faeces were taken from transport boxes in which the birds were left individually for 15-30 minutes. Wet mount from fresh faeces and one to two drops of 0.9% NaCl were examined microscopically (magnification of 100 and 400 times). The samples were collected during treatment (days 0, 7, 14, 21, 28) and after treatment (days 5 and 12). The degree of infection was evaluated on an ordinal scale from "0" to "5", where „0" – negative; „1" – 1-4 cells of *M. ornithogaster* in the microscopic slide; „2" – 5-20 cells in the microscopic slide; „3" – 1-2 macrorhabdus in the field of view (400 ×); „4" – 3-5 macrorhabdus in the field of view (400 ×) and „5" – more than 6 cells of *M. ornithogaster* in the field of view (400 ×).

Statistical analysis. The normality of the distributions of body weight and biochemical parameters was evaluated by the Shapiro-Wilk test. A repeated measures ANOVA with Tukey's post-hoc test was used to search for significant differences in the aforementioned factors between the experimental and control groups at each time point of the study.

The Friedman test and Daniel's post-hoc test were used to compare the degree of infection with *M. ornithogaster* in the experimental group in the successive time points of the study (14).

Numerical variables were reported as arithmetic mean and standard deviation (SD), whereas categorical variables were presented as median and interquartile range (IQR).

The level of significance (α) was 0.05 in all analyses. Statistical analyses were performed with Statistica 10 software (Statsoft Inc.).

Results and discussion

No side effects, such as regurgitation, diarrhea or polyuria, were observed during treatment. There was no significant difference in the average body weight between the two groups during the entire study (time/group interaction p = 0.0632) (Fig. 1).

All biochemical parameters remained stable and were comparable between the two groups during the entire study (Tab. 1). The levels of LDH and creatine kinase (CK) in both groups were decreased in relation to the reference values, but no information about pathologies causing a decreased activity of these enzymes in birds was found in the literature.

Tab. 1. Biochemical parameters of the budgerigars' blood before treatment (week 0), at the end of treatment (week 4) and 2 weeks after treatment (week 6)

Parameter	Week 0.		Week 4.		Week 6.		Reference range	p-value***
	E (n = 25)	C (n = 8)	E (n = 25)	C (n = 8)	E (n = 25)	C (n = 8)		
AST (GOT) [U/l]	228.4 (52.2)	233 (24.0)	252.7 (45.4)	324.0 (144.2)	231.0 (25.8)	309.5 (140.7)	150-350*	0.2791
Bile acids [µmol/l]	29.1 (14.4)	51.1 (5.7)	33.6 (8.0)	50.1 (25.6)	31.1 (5.6)	54.1 (24.3)	15-70**	0.9720
Total protein [g/l]	20.0 (2.0)	21.5 (0.7)	22.0 (1.7)	20.5 (2.1)	21.3 (1.2)	21.0 (2.8)	2.5-4.5**	0.3501
Albumin [g/l]	5.5 (0.5)	7.5 (0.7)	6.3 (0.0)	5.5 (0.7)	6.0 (0.0)	6.5 (0.7)	7.9-13.5**	0.3629
Cholinesterase [kU/l]	2.85 (0.52)	3.65 (0.64)	3.07 (0.37)	3.25 (0.07)	3.26 (0.48)	3.45 (0.07)	> 2.5**	0.1038
Uric acid [µmol/l]	335.5 (87.2)	421.5 (72.8)	334.6 (57.6)	380.0 (124.5)	308.8 (51.4)	433.0 (22.6)	268-833*	0.6052
CPK [U/l]	29.2 (8.5)	60.0 (24.0)	44.4 (12.6)	46.5 (27.6)	86.3 (15.2)	239.5 (23.3)	90-300*	< 0.0001#
LDH [U/l]	50.4 (12.9)	48.5 (4.9)	64.9 (10.2)	65.0 (1.4)	77.5 (8.1)	81.0 (8.5)	150-450*	0.8266
Inorganic phosphate [mmol/l]	1.21 (0.10)	1.30 (0.14)	1.17 (0.20)	1.25 (0.21)	0.91 (0.12)	1.40 (0.51)	0.9-1.6*	0.0647
Calcium [mmol/l]	1.73 (0.20)	1.80 (0.28)	1.88 (0.08)	1.85 (0.07)	1.81 (0.04)	1.85 (0.07)	1.6-2.7*	0.7430
Potassium [mmol/l]	2.16 (0.15)	3.05 (0.07)	2.64 (0.24)	2.80 (0.00)	2.65 (0.15)	3.15 (0.07)	2.2-3.9*	0.0098#
a-amylase [U/l]	435.7 (117.2)	361.0 (45.3)	374.7 (55.6)	342.0 (8.5)	483.8 (201.1)	348.5 (43.1)	200-500**	0.6357

Explanations: E – experimental group; C – control group; * IDEXX Laboratories; ** by Harrison (8); *** Repeated measures ANOVA (time/group interaction); # A post-hoc test revealed a parallel increase in both groups (insignificant difference between groups)

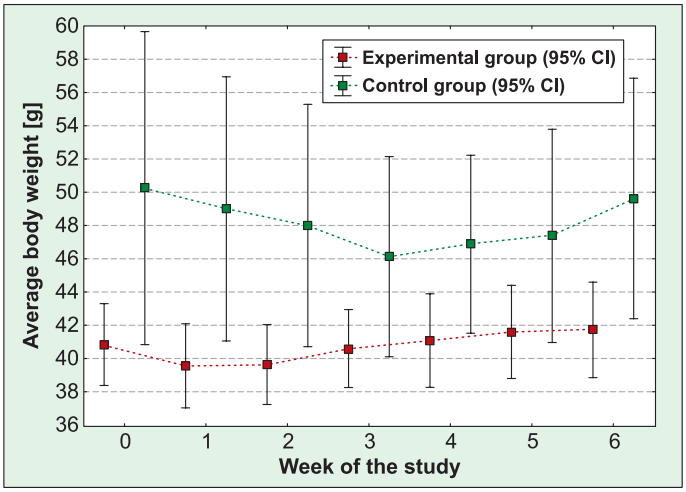

Fig. 1. Body weight of the budgerigars before treatment (week 0), during treatment (weeks 1-4) and after treatment (weeks 5-6) (CI 95% – 95% confidence interval for the average body weight)

The degree of *M. ornithogaster* shedding decreased significantly during the study (Friedman $p < 0.0001$). The significant reduction took place during the first week of treatment (Daniel's $p = 0.0004$) (Fig. 2).

The thirty-day treatment with a relatively high dose of amphotericin B did not cause any adverse effects for the general health condition of the budgerigars, and no significant changes in biochemical parameters of blood were noted, despite previous reports on the nephrotoxic effect of this drug (6, 16). However, the one-month treatment did not fully eliminate the shedding of *M. ornithogaster*. It merely caused a significant reduction in the number of yeast cells, which was noted from the second week of drug administration onwards. Additionally, in the parakeet that had showed the highest degree of fungal infection before treatment, a relapse of *M. ornithogaster* invasion was observed after the termination of treatment. The authors' observations based on their clinical experience indicate that there is a substantial inter-subject variability in terms of the response

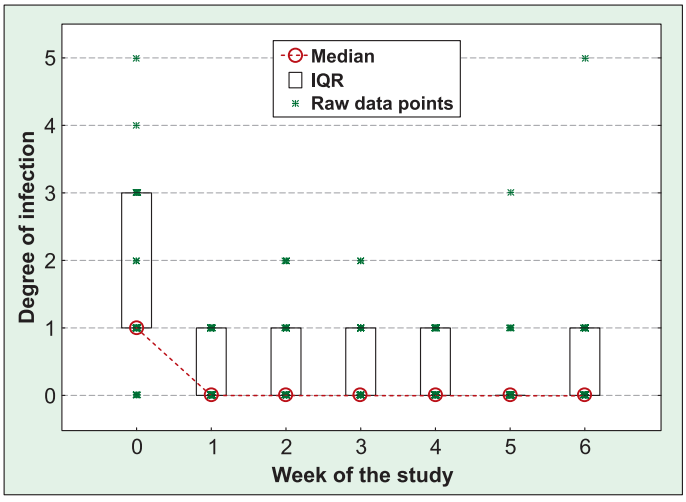

Fig. 2. Degree of infection with *M. ornithogaster* before treatment (week 0), during treatment (weeks 1-4) and after treatment (weeks 5-6)

of budgerigars to treatment. In some birds, a two-week therapy permanently eradicated the shedding of the fungi, whereas in other parakeets the recurrence of megabacteria in faeces was noted even after a month-long treatment. Similar observations were reported by Filippich and Perry (4, 10), who achieved a full recovery in 28 out of 30 parakeets, but they did not show the efficacy of amphotericin B administration with drinking water. Different results were obtained by Gestier (http://www.vetafarm.com.au/pages/Megabacteria-in--Australian-Budgerigars.html), who treated budgerigars with a water-soluble complex of amphotericin B and cyclodextrin administered to birds with drinking water. Despite an irregular uptake of water by parakeets, negative results of the presence of *M. ornithogaster* in birds' faeces were noted already after 5 days of therapy. It should be pointed out, however, that in that study Gram-staining was used as a method of evaluation, which is not the standard analytical method for determining this type of infections and substantially limits the volume of samples investigated. It seems that there are two main factors determining the susceptibility of macrorhabdosis to treatment: individual traits of the infected bird and the resistance of the fungus to therapy (2, 11), but the authors have not carried out research in this area.

References

1. *Baker J. R.*: Megabacteriosis in exhibition budgerigars. Vet. Rec. 1992, 131, 12-14.
2. *Filippich L. J., Hendrikz J. K.*: Prevalence of megabacteria in budgerigar colonies. Aust. Vet. J. 1998, 76, 92-95.
3. *Filippich L. J., Parker M. G.*: Megabacteriosis and proventricular/ventricular disease in psittacines and passerines. Proc. Assoc. Avian Vet. 1994, p. 287-293.
4. *Filippich L. J., Perry R. A.*: Drug trials against megabacteria in budgerigars (Melopsittacus undulatus). Aust. Vet. Pract. 1993, 23, 184-189.
5. *Gerlach H.*: Megabacteriosis. Semin. Avian Exotic Pet. Med. 2001, 10, 12-19.
6. *Hamill R. J.*: Amphotericin B formulations: a comparative review of efficacy and toxicity. Drugs 2013, 73, 919-934.
7. *Hannafusa Y., Bradley A., Tomaszewski E. E., Libal M. C., Phalen D. N.*: Growth and metabolic characterization of Macrorhabdus ornithogaster. J. Vet. Diagn. Invest. 2007, 19, 256-265.
8. *Huchzermeyer F. W., Henton M. M., Keffen R. H.*: High mortality associated with megabacteriosis of proventriculus and gizzard in ostrich chicks. Vet. Rec. 1993, 133, 143-144.
9. *Mutlu O. F., Seckin S., Ravelhofer K., Hildebrand R. A., Grimm F.*: Proventriculitis in domestic fowl (Gallus gallus var. dom. L., 1758) caused by megabacteria. Tierärztl. Prax. 1997, 25, 460-462.
10. *Perry R. A.*: Megabacterium Associated Disease (M.A.D.). Avian Diagnostics, Proc. Post Graduate Committee in Veterinary Science 1993, p. 3-39.
11. *Phalen D.*: Diagnosis and management of Macrorhabdus ornithogaster (formerly megabacteria). Vet. Clin. Exot. Anim. 2005, 8, 299-306.
12. *Phalen D.*: Implications of macrorhabdus in clinical disorders, [in:] Harrison G., Lightfoot T. L. (eds): Clinical Avian Medicine. Spix Publishing, Inc. Palm Beach, Florida 2006, p. 705-709.
13. *Piasecki T., Prochowska S., Celmer Ż., Sochacka A., Bednarski M.*: Occurrence of Macrorhabdus ornithogaster in exotic and wild birds in Poland. Med. Weter. 2012, 68, 245-249.
14. *Sheldon M. R., Fillyaw M. J., Thompson W. D.*: The use and interpretation of the Friedman test in the analysis of ordinal-scale data in repeated measures designs. Physiother. Res. Int. 1996, 1, 221-228.
15. *Tomaszewski E. K., Logan K. S., Snowden K. F., Kurtzman C. P., Phalen D. N.*: Phylogenetic analysis identifies the 'megabacterium' of birds as a novel anamorphic ascomycetous yeast, Macrorhabdus ornithogaster gen. nov., sp. nov. Int. J. Systematic Evolution Microbiol. 2003, 53, 1201-1205.
16. *Wade R. L., Chaudhari P., Natoli J. L., Taylor R. J., Nathanson B. H., Horn D. L.*: Nephrotoxicity and other adverse events among inpatients receiving liposomal amphotericin B or amphotericin B lipid complex. Diagn. Microbiol. Infect. Dis. 2013, 76, 361-367.

Corresponding author: Aleksandra Ledwoń PhD DMV, Nowoursynowska 159, 02-776 Warsaw, Poland; e-mail: aleksandra_ledwon@sggw.pl