Study of the fecal microbiota of canaries affected or not by macrorhabdus ornithogaster infection using culture independent approaches

STUDY OF THE FECAL MICROBIOTA OF CANARIES AFFECTED OR NOT BY MACRORHABDUS ORNITHOGASTER INFECTION USING CULTURE INDEPENDENT APPROACHES

P. Robino[1], DVM, PhD, V. Alessandria[2], PhD, I. Ferrocino[2], PhD, L. Cocolin[2], PhD, L. Grosso[1], Graduating student (Vet Med), A. Dogliero[1], DVM, C. Tramuta[1], DBiotech, PhD, L. Galosi[3], Graduating student (Vet Med), G. Rossi[3], DVM, MSc, PhD, ECZM (WPH), P. Nebbia[1], DVM, PhD*

[1] Department of Veterinary Sciences, University of Turin, Grugliasco (TO), Italy
[2] Department of Agriculture, Forestry and Food Science, University of Turin, Grugliasco (TO), Italy
[3] School of Biosciences and Veterinary Medicine, University of Camerino, Matelica (MC), Italy

**Corresponding Author: e-mail address: patrizia.robino@unito.it*

Introduction

Macrorhabdus ornithogaster, also known as "megabacterium" or avian gastric yeast (AGY), is a novel anamorphic ascomycetous yeast that belongs in its own new genus, with a worldwide distribution. *M. ornithogaster* has been found in both psittacine and passerine species, both in captivity and in the wild.

This yeast usually colonises the mucosal surface of the isthmus existing between the proventriculus and ventriculus of a wide diversity of avian species and currently represent a potential threat to bird breeders. Clinical signs include weight loss in spite of a good appetite, regurgitation, diarrhea, depression, maldigestion, ruffled feathers and death (Fig. 1). It is easily visible by Gram stain (Fig. 2) but it is difficult to isolate and to store it in laboratory[1].

Fig. 1: **AGY positive canary** showing ruffled feathers, depression and chronic weight loss.

Fig. 2: **Image of AGY**. Smear prepared from canary fecal sample (1000×, light microscopy, Gram staining).

AIM: to characterize the microbiota diversity of 44 fecal samples of *M. ornithogaster*-infected and non-infected canaries raised in a family owned farm.

Materials and Methods

This study was conducted in canary (*Serinus canaria f. domestica)* breeding center in the North West of Italy (province of Turin). In total 44 animals (fecal samples) were analyzed: 15 originated from non-AGY infected birds (negative at Gram staining) and 29 from AGY infected birds (presence of Gram positive yeasts). Of this last group 16 samples were from asymptomatic animals and 13 from birds with symptoms of AGY-infection. DNA was extracted from fecal samples by using a QIAamp DNA Stool Mini Kit (Qiagen).

The fecal microbiota was investigated, through the application of PCR-DGGE analysis targeting the V3 region of the 16S rRNA genes of bacteria and D1/D2 region of 26S rRNA of yeasts.

Results and Discussion

The PCR-DGGE targeting the D1/D2 region of 26S rRNA showed clearly the presence of *M. ornithogaster* (Fig. 3) in positive at Gram stain animals, confirming a complete correlation between the two tests. The bacterial DGGE profiles were very complex but indicated clearly that the presence of *M. ornithogaster* can affect the bacteria microbiota composition with a possible impact on the animal status (Fig. 4 - 5).

The similarity matrix generated through the bacteria DGGE fingerprints was used to build a PLS-DA, as a function of the canaries status. The results pointed out a different microbial composition in infected -with or without symptoms- and non infected birds (Fig. 6).

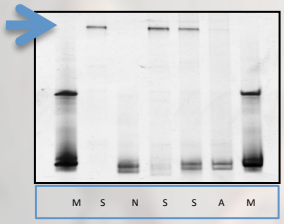

Fig. 3: **DGGE profiles of amplified 26S rRNA** D1/D2 regions obtained from canary stools divided into three groups:
- infected symptomatic (S)
- infected asymptomatic (A)
- non infected (N)

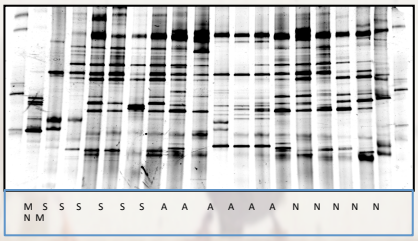

Fig. 4: **DGGE profiles of intestinal bacterial population** in canary stools divided into three groups (S, A, N).

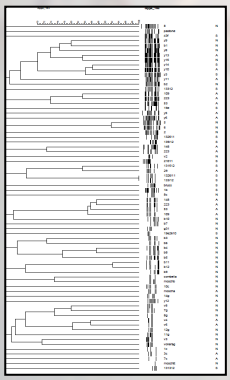

Fig. 5: **Coefficient of similarity of microbiota stool composition.** Dendrogram shows the relatedness of bacterial population in stool samples, divided into three groups (S, A, N), based on DGGE analysis.

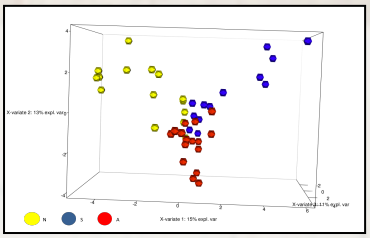

Fig. **6. Microbiota diversity in fecal samples of non infected (N), infected symptomatic (S) and infected without sintoms (A) birds**. The PLS-DA model based on PCR-DGGE shows a clear separation among the three groups of animals.

Future developments: To better understand the relationships between shift in bacterial populations in infected animals we will perform a high-throughput amplicon target sequencing of the fecal samples collected to discover the microbiota diversity as affected by the presence of *M. ornithogaster.*

References

1. Tomaszewski EK, et al. (2003) Phylogenetic analysis identifies the 'megabacterium' of birds as a novel anamorphic ascomycetous yeast, Macrorhabdus ornithogaster gen. nov., sp. nov. Int J Syst Evol Microbiol;53:1201–5. 2. Muyzer G. et al. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695-700. 3. I. Ferrocino et al. (2015) Fecal Microbiota in Healthy Subjects Following Omnivore, Vegetarian and Vegan Diets: Culturable Populations and rRNA DGGE Profiling. Plos One. 1-16. DOI:10.1371/journal.pone.0128669