GUT MICROBIAL ECOLOGY DURING *CAMPYLOBACTER* INFECTION IN CHICKENS: A DESCRIPTION OF COMMUNITY CHANGES VIA METATAXONOMIC CHARACTERIZATION

Orsini M[1], Patuzzi I[1], Cibin V[1], Petrin S[1], Tiengo A[1], Barco L[1], Ricci A[1], Losasso C[1]

[1]*Department of General and Experimental Microbiology, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy;*

INTRODUCTION

Campylobacter jejuni is the most common cause of foodborne illness in Europe. Among other matrices, undercooked chicken meat has been identified as main source of this pathogen.
Many studies have shown that modification of the intestinal microbiota may be effective in competing with *Campylobacter* spp. for intestinal colonization and methods based on competitive exclusion have been extensively explored. The importance of characterizing the intestinal microbiota of chickens is widely documented, but most of the studies published so far are mainly based on cultural methods or on simplified approaches based on molecular biology.
In this work, we used **16S rRNA gene sequencing** to characterize the intestinal microbial community in the first weeks of life of broilers infected with *Campylobacter*. Evidences obtained through this study can be used to identify options to reduce the incidence of infection at primary production level based on the targeted influence of the intestinal microbiota, i.e. on the selection of a bacterial population typical of animals with a low risk of infection. Moreover, this approach can be used to classify farms in terms of risk for the presence and spread of *Campylobacter*.

MATERIALS AND METHODS

For this study, we selected **four broiler farms**, half of them being positive for *Campylobacter*during the sampling period. Five samples were collected from each farm at different time points (**7th, 14th, 18th, 21th** and **28th day** of life), for a total of **110 caecal samples**, each with one biological replicate. For the two positive farms, ten samples (five positive and five negative for *Campylobacter* spp.) were collected for the time points when infection was first detected.
V3-V4 16S rRNA gene regions were amplified and sequenced using HiSeq2500 Illumina platform. Reads were filtered and cleaned using in-house methods and pre-processed using QIIME pipeline to obtain the final OTU table. OTUs proportional abundance, number and taxonomic classification were investigated with the aim of describing microbial communities diversity in terms of alpha and beta indices. A differential abundance analysis was performed to check for differences in temporal evolution of *Campylobacter* both between negative and positive farms and between the 2 positive farms. Population dynamics was investigated through the reconstruction of inter-*genera* interaction networks that were inferred starting from data on the microbial community composition.

RESULTS & CONCLUSIONS

FARM	D7	D14	D18	D21	D28
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	7	16
4	0	5	16	16	16

Table 1 Number of animals per farm and time point in which *Campylobacter* spp. was isolated

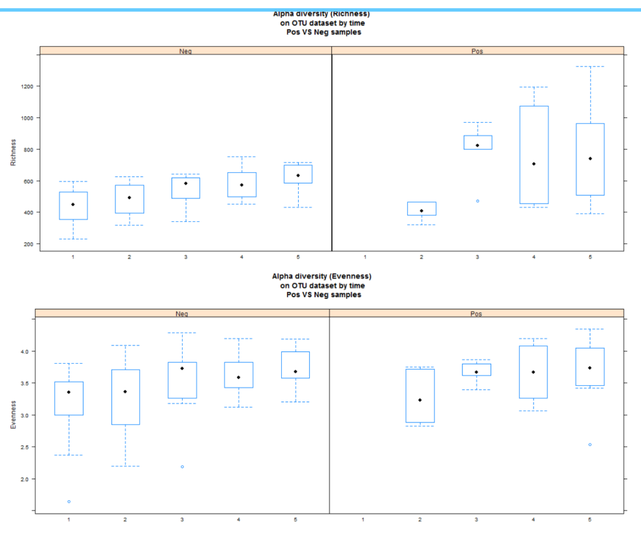

Figure 1 Comparison of richness and evenness alpha diversity indices at OTU level between negative and positive samples.

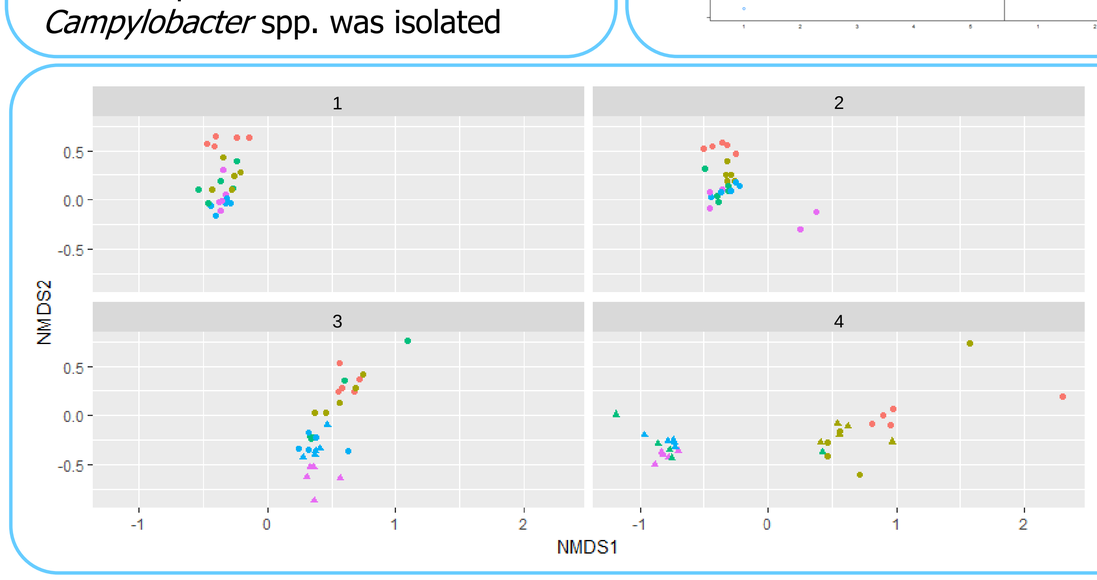

Figure 2 NMDS plot of the beta matrix calculated with Bray-Curtis distance on the abundances obtained from the OTU table, divided by farm. The shape is associated with the state of the sample (Pos / Neg), the color with the sampling instant (t ∈ [1,5])

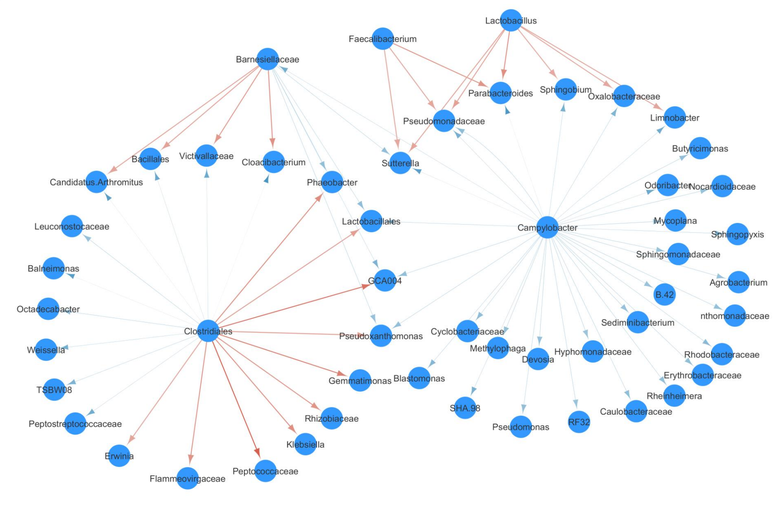

Figure 3 *Campylobacter* interaction network. Red arrows represent positive interactions, while blue arrows represent negative ones.

- A difference in the colonization rate and timing was observed in the four flocks that became positive for *C. jejuni* over the five investigated time points
- An appreciable difference was detected in alpha diversity between negative and positive samples

- A key role of *Faecalibacterium* and *Lactobacillus* genera was found. Interestingly, these two taxa were not directly contrasted by any other community component but instead, they were found to exert positive action over six different taxa (i.e. *Limnobacter*, *Parabacteroides*, *Pseudomonadaceae*, *Sutterella*, *Sphingobium* and *Oxalobacteraceae*) that in turn were exposed to the negative action triggered by *Campylobacter* itself
- Microbial interaction networks could be used as a tool to delineate new strategies against *Campylobacter* infections in poultry as well as to classify poultry farms in terms of presence of *Campylobacter*