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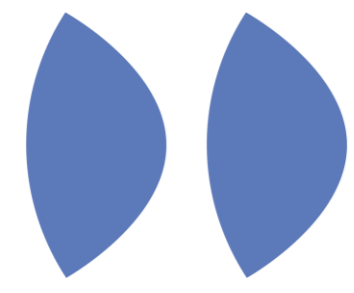
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Thibault Destanque,¹ Tony Rochegue,¹ Joanna Fourquet,² Marisa Haenni,¹ Jean-Yves Madec,¹ Claire Hoede,² Agnese Lupo¹

ANSES - Université de Lyon, Unité Antibiorésistance et Virulence Bactériennes, Lyon, France

Université Fédérale de Toulouse, INRAE, MIAT, 31326, Castanet-Tolosan, France



Contact: agnese.lupo@anses.fr

Background

Amoxicillin is largely used in human and veterinary medicine.

Its impact on the microbiota composition and selection of antibiotic resistance genes (ARGs) has been investigated in humans, but similar studies in animals are lacking.

The objective of this study was to analyze the impact of amoxicillin therapy on the intestinal microbiota of calves and its resistome.

Material and methods

Feces were collected from three calves receiving intramuscular amoxicillin before (T0), at the end (T1), one week after (T2) the treatment and from three healthy calves, not receiving amoxicillin (Table 1).

After DNA extraction, shotgun sequencing was performed using NovaSeq (Illumina). MetagWGS pipeline v.2.3 was used for the analysis. ARGs (n=44) were quantified by qPCR with hydrolytic probes.

Results

At T0, Actinobacteria, Bacteroidetes and Firmicutes were the most abundant phyla in both treated and untreated pre-weaned calves. At T1, Bacteroidetes and Proteobacteria raised in treated calves (Figure 1A) along with genera Bacteroides, Odoribacter, and Escherichia, hosting potentially pathogenic species (Figure 1B).

Species alpha-diversity stagnated in treated calves whereas it increased in non-treated ones (Figure 2).

ARGs (*bla*_{TEM}, *tet*(A), *str*A and *str*B raised in treated calves at T1 and remained more abundant at T2 than T0 (Figure 3).

Calif	Farm	Breed	Sex	Age (days)	Number of amoxicillin injections	T0	T1	T2	Health_status
I2	A	Charolais/Montbeliard	F	8	4	2018-10-07	2018-10-14	2018-10-21	Omphalitis
I6	NA	Charolais/Montbeliard	M	6	4	2019-06-01	2019-06-12	2019-06-17	Omphalitis
I15	F	Prim' Holstein	F	26	4	2019-08-05	2019-08-12	2019-08-19	Omphalitis
NC3	G	Montbeliard	M	7	NA	2020-03-06	2020-03-13	2020-03-20	Healthy
NC4	A	Montbeliard	F	8	NA	2020-03-06	2020-03-13	2020-03-20	Healthy
NC5	A	Charolais/Montbeliard	M	7	NA	2020-03-06	2020-03-13	2020-03-20	Healthy

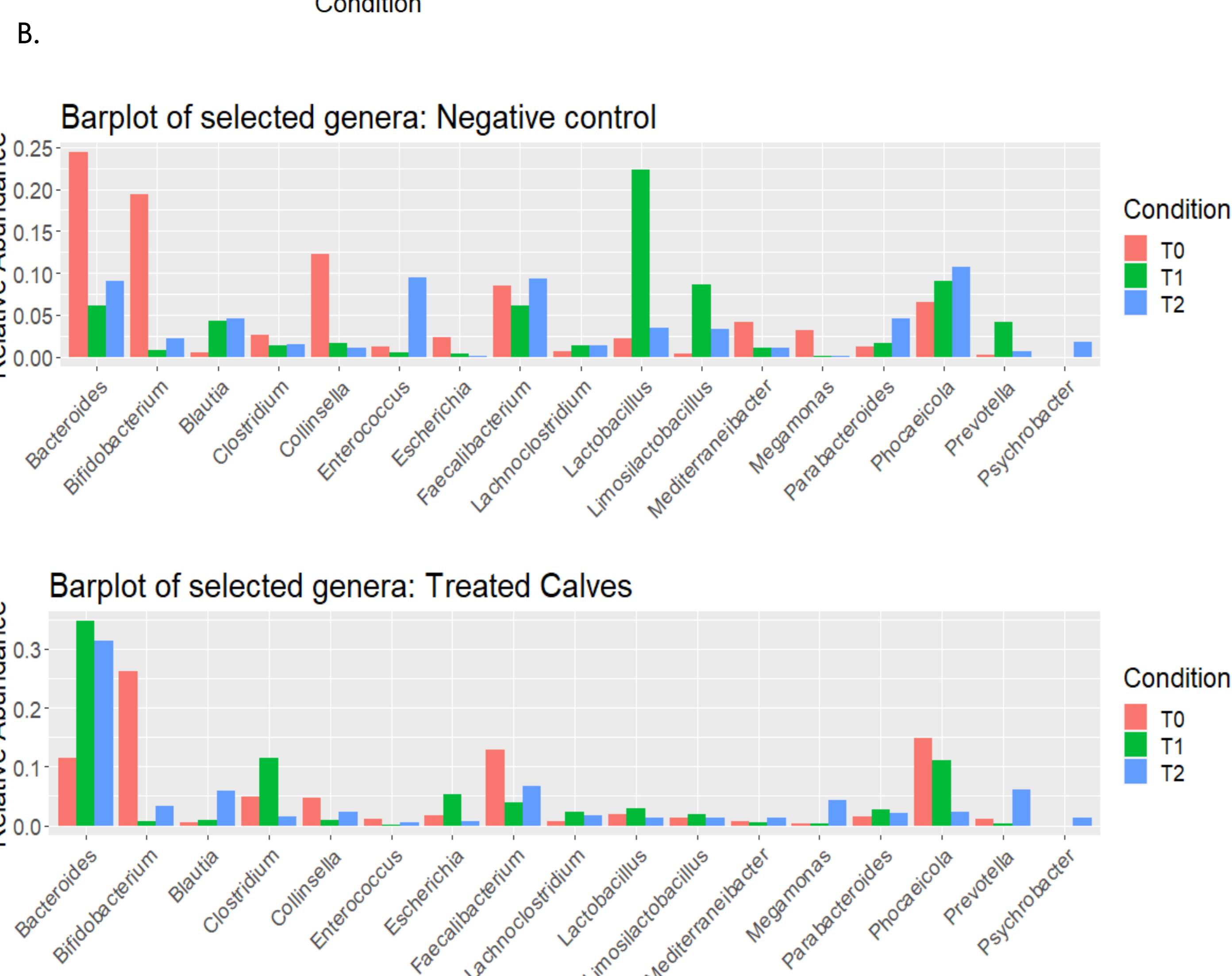
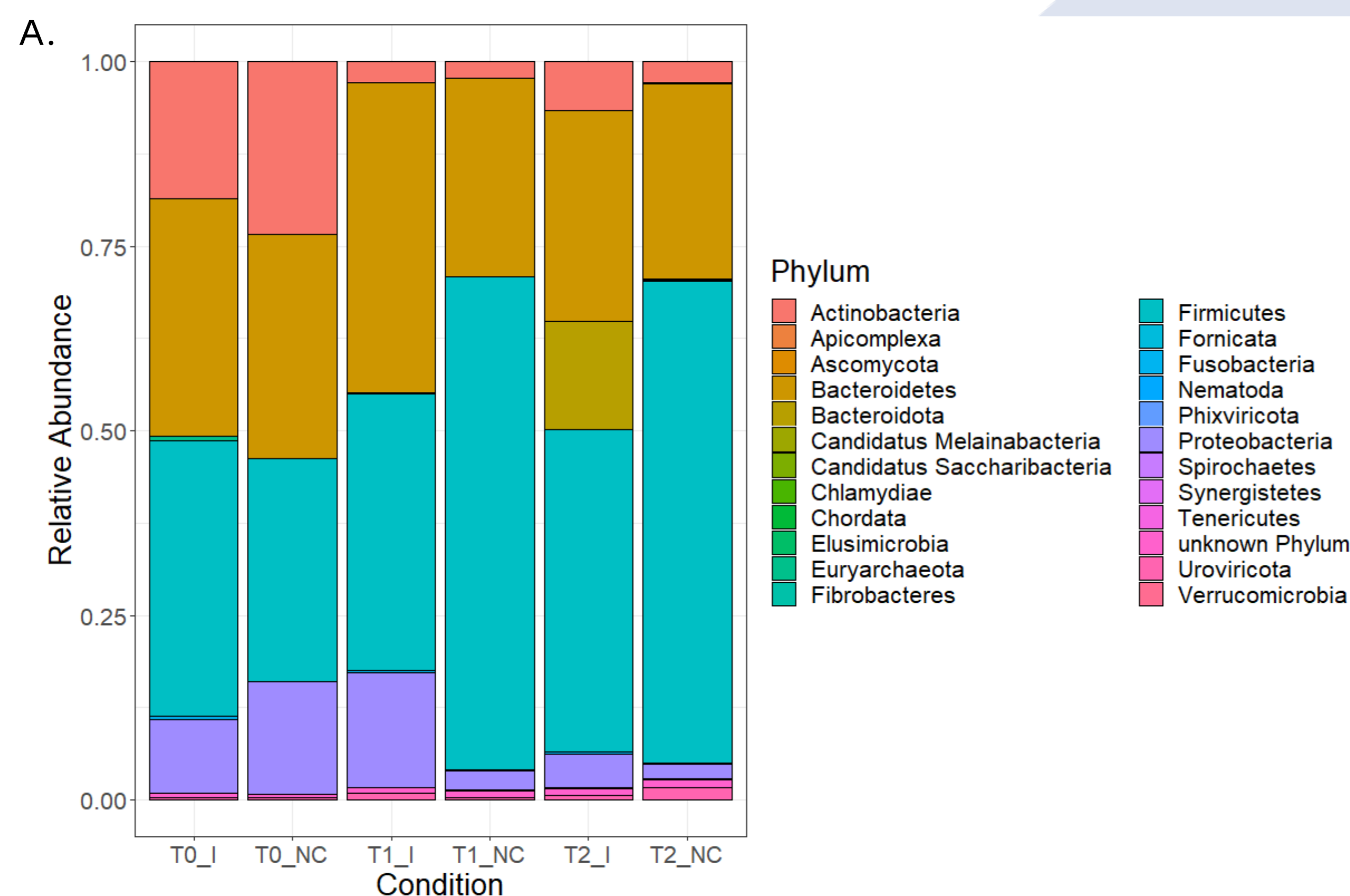


Figure 1. A. Relative abundance of phyla in feces of two groups of pre-weaned calves one treated with intramuscular amoxicillin (I) and the other one untreated, serving as control (NC). The feces were analyzed before amoxicillin treatment (T0), at the end of the treatment (T1) and one week after withdrawal (T2). B. Relative abundance at the genus level.

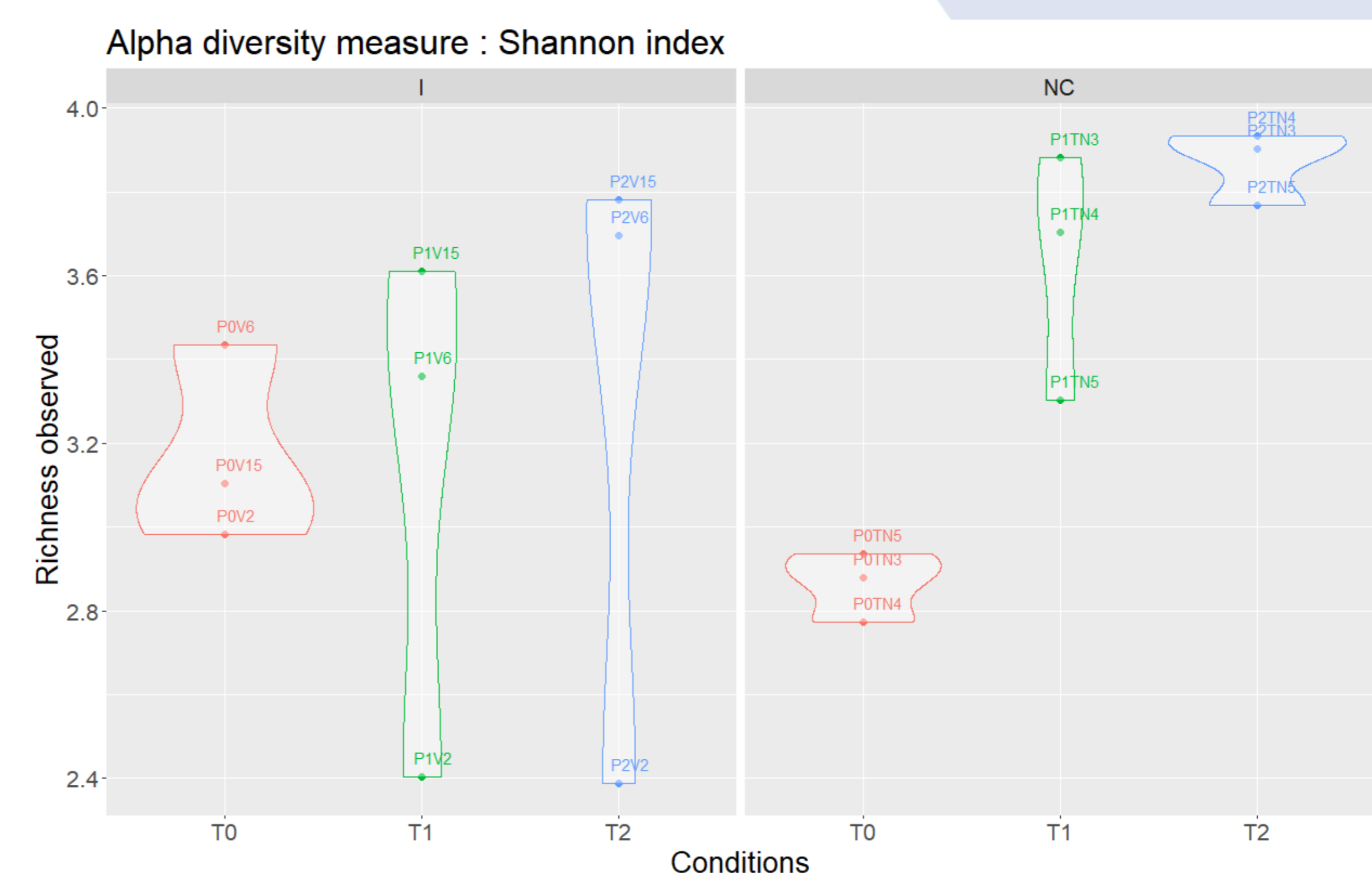


Figure 2. Species' alpha diversity calculated according to Shannon index. I: injected; NC: negative control.

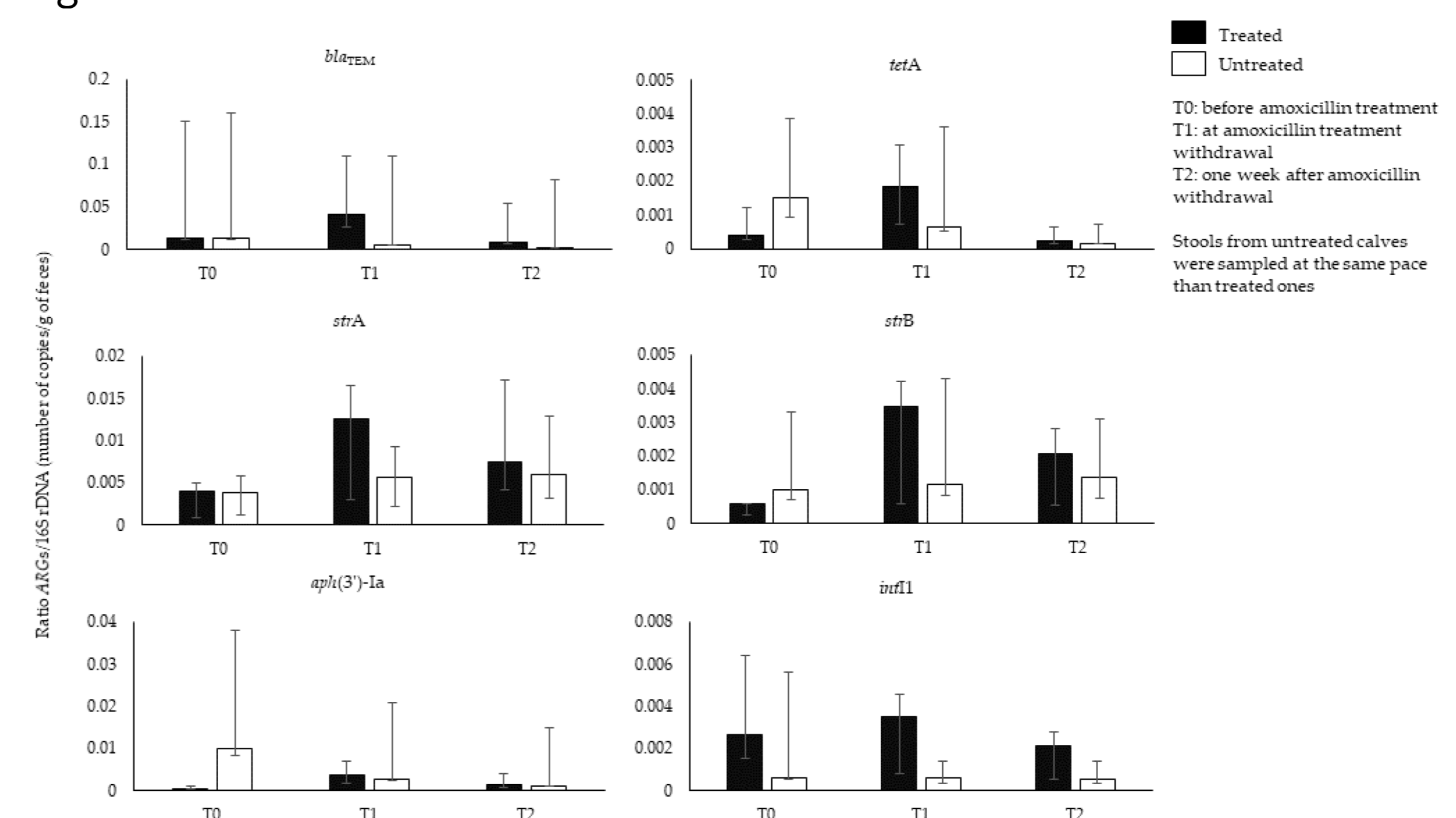


Figure 3. Representation of antibiotic resistance genes/16S rDNA (number of copies/g of feces) by qPCR in treated and untreated calves, before amoxicillin treatment (T0), at amoxicillin withdrawal (T1), one week after amoxicillin withdrawal (T2). Stools from untreated calves were sampled at the same pace than treated ones.

Conclusion

Amoxicillin affects the composition of pre-weaned calves' microbiota and selects ARGs.

The stagnation of alpha diversity in pre-weaned, treated calves suggests a delay in microbiota maturation with unknown consequences for the host health.

Standardization of procedures/data analysis are necessary for precisely comparing the impact of different antibiotic therapies, which could help improving antibiotics usage preserving microbiota health and mitigating the resistance crisis.