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Piglets infected with ETEC F4 and F18: effect of *MUC4* and *FUT1* genotypes.

Massacci F.R., Tofani S., Tentellini M., Orsini S., Lovito C., Forte C., Luise D., Bevilacqua C., Marchi L., Bertocchi M., Rogel-Gaillard C., Pezzotti G., Estellé J., Trevisi P., Magistrali C.F.

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Weaning:

- 3rd and 4th week of age
- Switch from highly digestible liquid milk to a less-digestible more-complex solid feed
- Move from maternity building to a post-weaning unit
- Social changes

Lalles *et al*., 2007; Colson *et* al., 2012

INTRODUCTION

Piglets during the weaning are susceptible to diarrhea:

✓ Dysbiosis

✓ Colonization by enteric pathogens

Lalles *et al*., 2007; Gresse *et al.*, 2017

Causes of diarrhoea in post-weaning piglets:

- ✓ *Clostridium perfringens* (Type A, C)
- ✓ *Salmonella*
- ✓ *Escherichia coli*

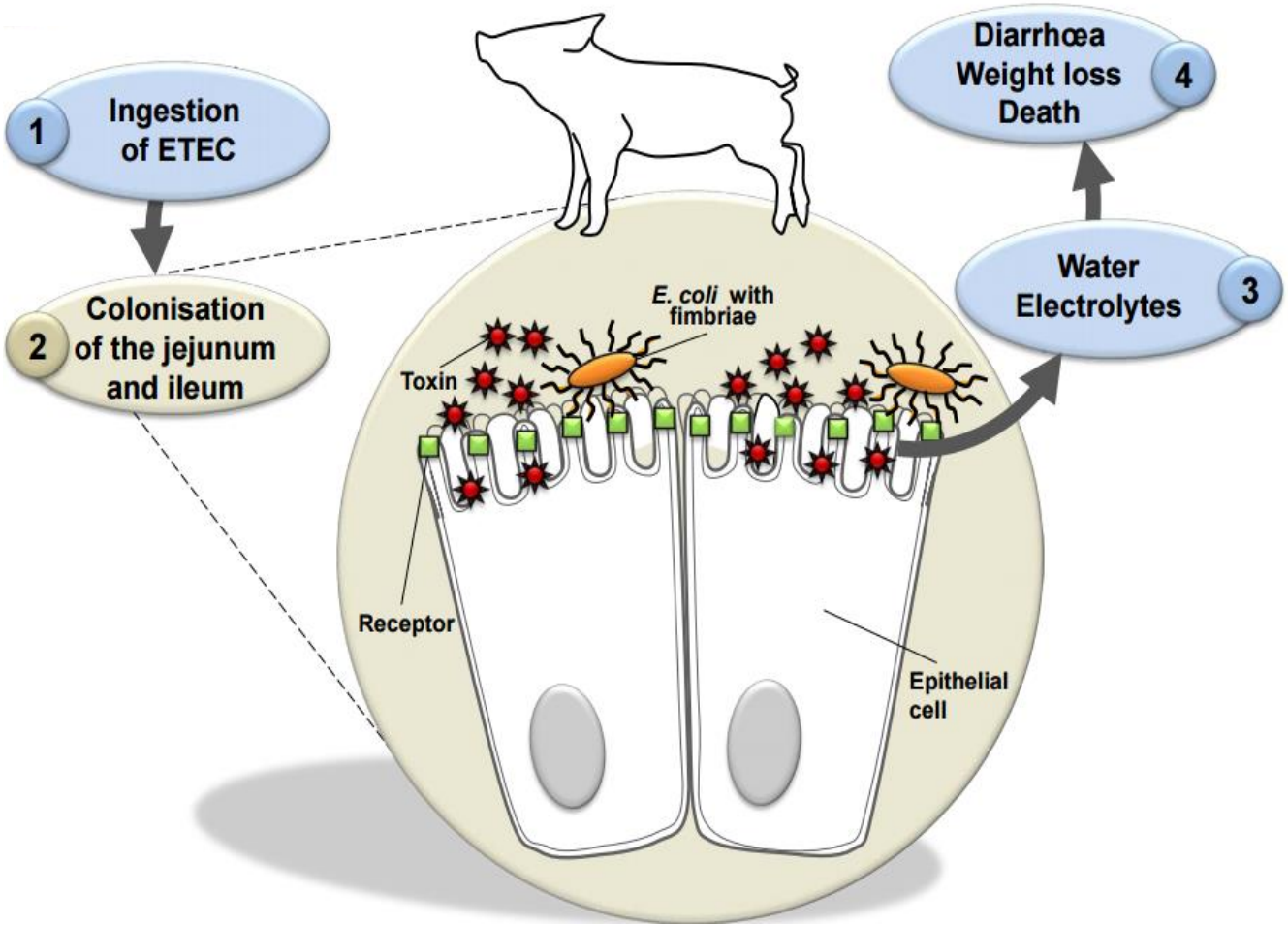
Enterotoxigenic *Escherichia coli* (ETEC)

Post-Weaning Diarrhoea (PWD)

Pathotype	Adhesins	Toxins
ETEC	F4 (K88)	Sta, STb, LT, EAST-1, α-hemolysin
	F18	Sta, STb, LT, EAST-1, Stx2e, α-hemolysin

Luppi *et al.*, 2017

INTRODUCTION



Luppi *et al.*, 2017

Piglets are not equally susceptible to ETEC F4 infection

MUC4

SNP located in intron 7 (g.13:8227C>G) of the *Mucin 4* gene (*MUC4*)

MUC4^{G-} genotypes are considered susceptible phenotypes
MUC4^{CC} genotype is considered resistant phenotype

Jørgensen *et al.*, 2004, Patent number WO2004048606

Piglets are not equally susceptible to ETEC F18 infection

FUT1

SNP located in intron 1 (g.6:54079560T>C) of the *Alpha-fucosyltransferase-1* (*FUT1*)

FUT1^{C-} genotypes are considered susceptible phenotypes
FUT1^{TT} genotype is considered resistant phenotype

Meijerink *et al.*,1997; Wang *et al.*, 2012

ITALY

Reduction in the consumption of antimicrobials was observed in 2016, with a 30% drop in sales (mg/PCU) during the period 2010-2016

Sales of polymyxins fell by 62% in 2016 compared to sales in 2010

- CIA (Critically Important Antimicrobials) list of WHO

PIG PRODUCTION

Antibiotics are mainly administered by the oral route

Concerns have been expressed for the use of oral formulations, since they exert a selective pressure on the gut microbiota

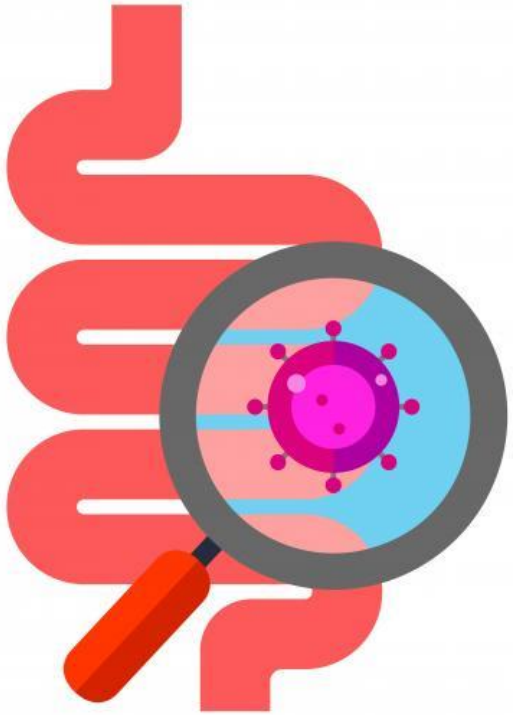
EMA, 2018

AMOXICILLIN

Amoxicillin is the most widely prescribed antibiotic at weaning

- Streptococcosis infection

Burch & Sperling, 2018; Konstantinov *et al.*,2006; Connelly *et al.*, 2018



GUT MICROBIOTA OF PIGLETS

- Weaning
- Host genotypes
- Administration of antibiotics

Mach *et al*.,2015; Konstantinov *et al*.,2006; Messori *et al*.,2013; Blaser *et al*.,2016; Schokker *et al*.,2014; Soler *et al*.,2018

AIM of the study

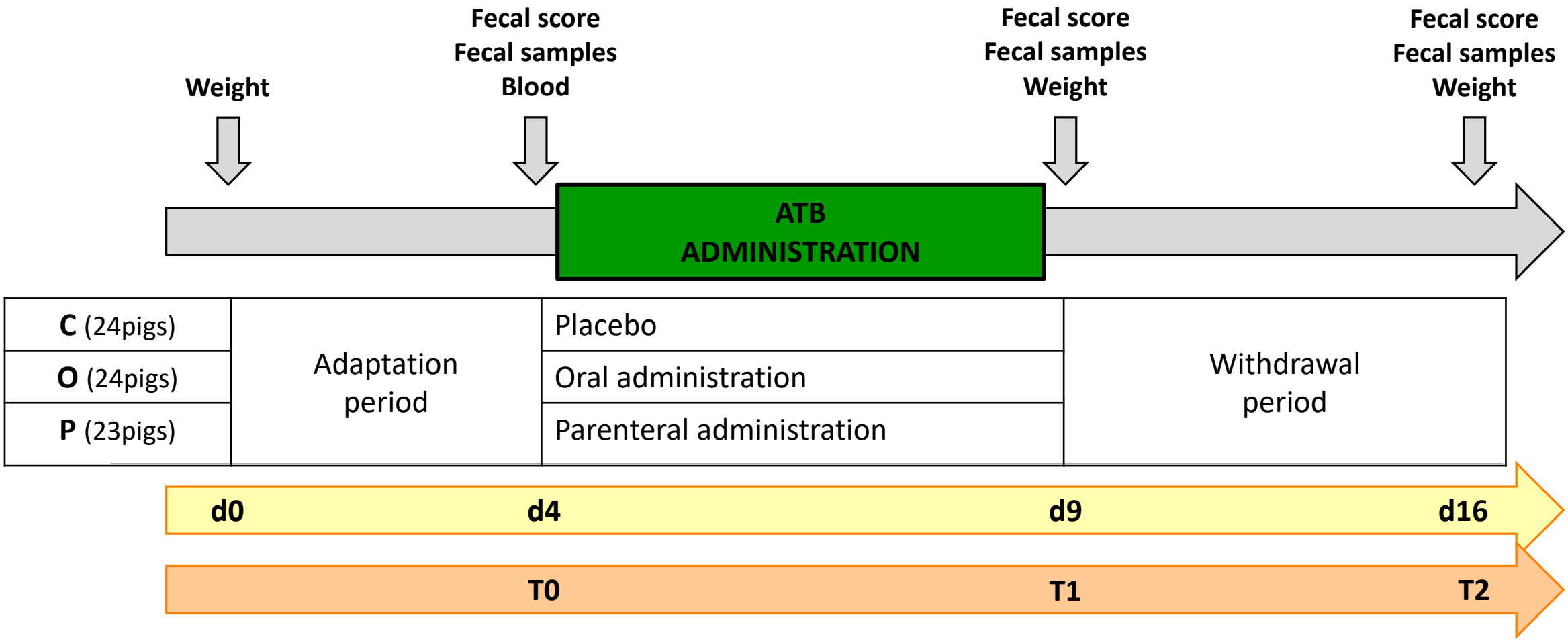
To understand the role of three main factors during a natural infection by ETEC F4 and ETEC F18:

1. Host genotypes: *MUC4* and *FUT1*

2. Oral *vs.* parenteral route of amoxicillin administration

3. Gut microbiota composition

MATERIALS & METHODS



Balanced for sex, weight, litters of origin, *MUC4* and *FUT1* genotypes
MORTALITY: 1 piglet removed from the study

MATERIALS & METHODS

Fecal scores:

- 0= normal stools
- 1= loose stools
- 2= watery diarrhoea

Fecal samples:

- Microbiological culture and antimicrobial susceptibility test
- PCR ETEC
- 16S rRNA gene (V3-V4 regions)

Blood:

- *MUC4* and *FUT1* genotyping

Bioinformatical and biostatistical analysis:

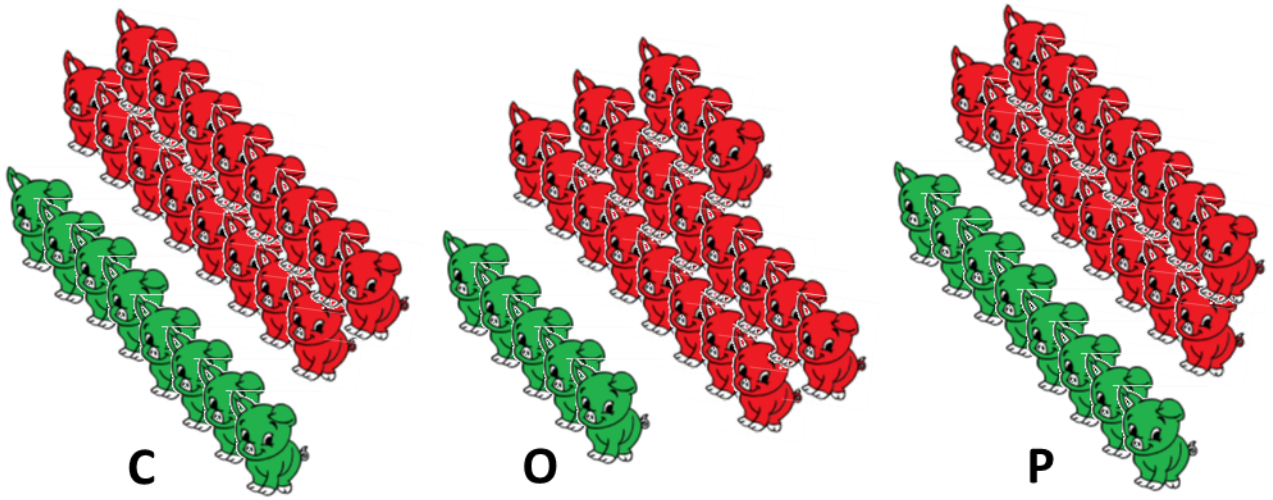
- QIIME
- R software

RESULTS

Course of natural infection

ETEC F4

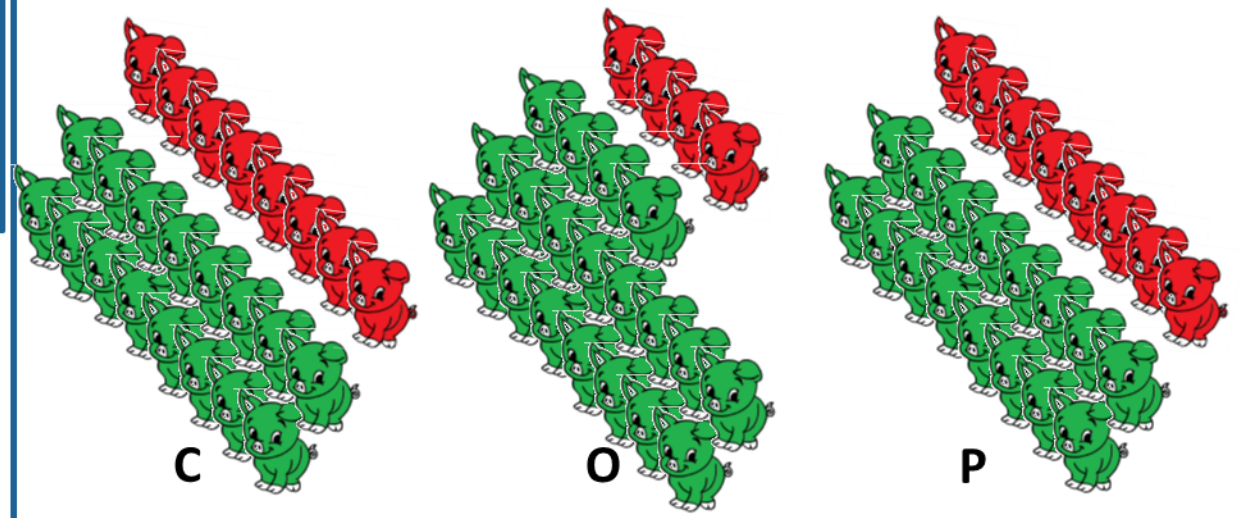
Fisher tests; $p>0.05$



C	O	P
16 (66%)	19 (79%)	15 (65%)
8 (34%)	5 (21%)	8 (35%)

ETEC F18

Fisher tests; $p>0.05$



C	O	P
8 (34%)	4 (17%)	8 (35%)
16 (66%)	20 (83%)	15 (65%)

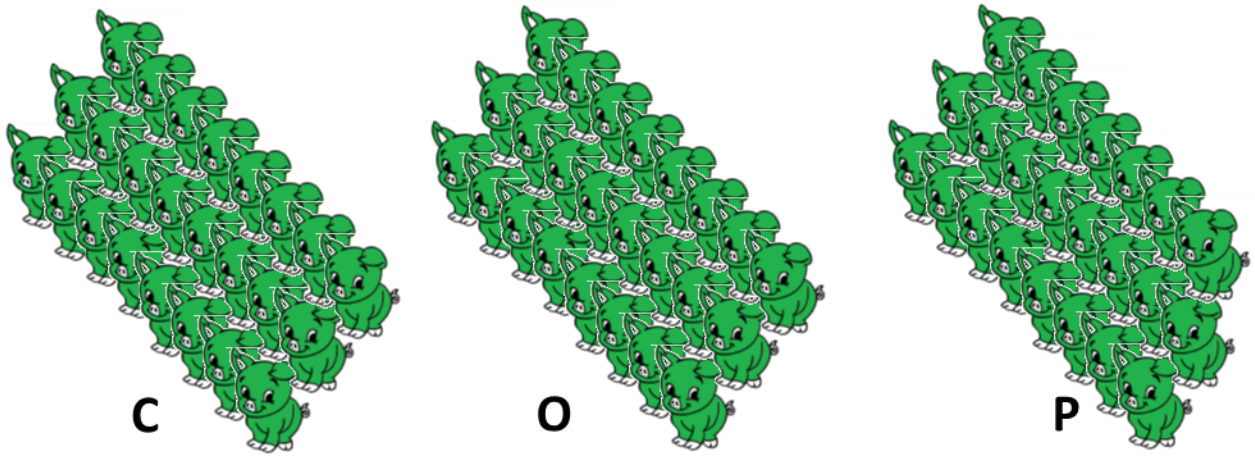
T0: end of adaptation period

RESULTS

Course of natural infection

ETEC F4

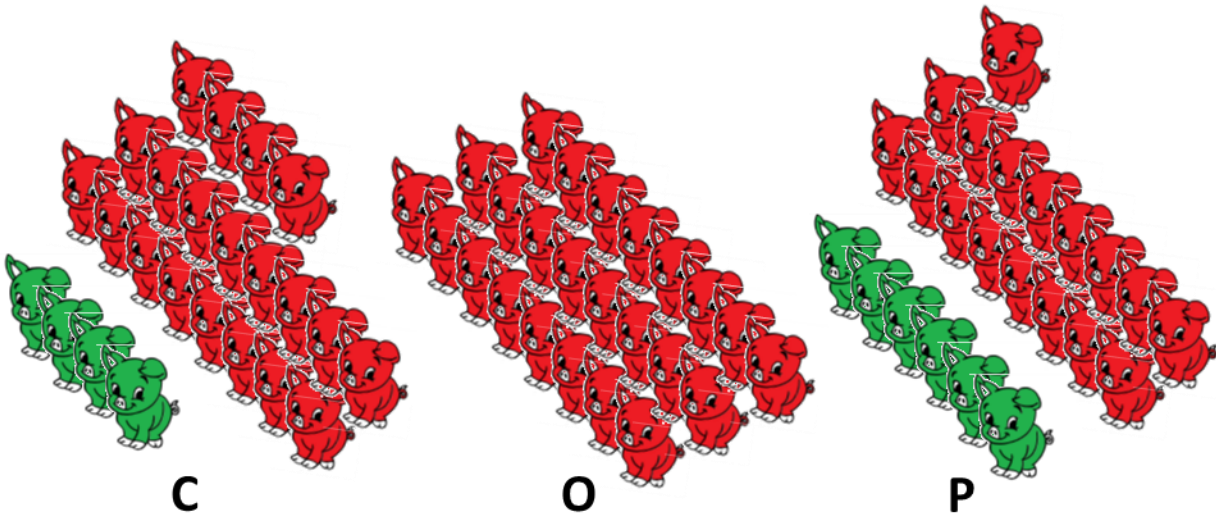
Fisher tests; $p>0.05$



C	O	P
0	0	0
24 (100%)	24 (100%)	23 (100%)

ETEC F18

Fisher tests; $p=0.01$



C	O	P
20 (83%)	24 (100%)	17 (74%)
4 (17%)	0	6 (26%)

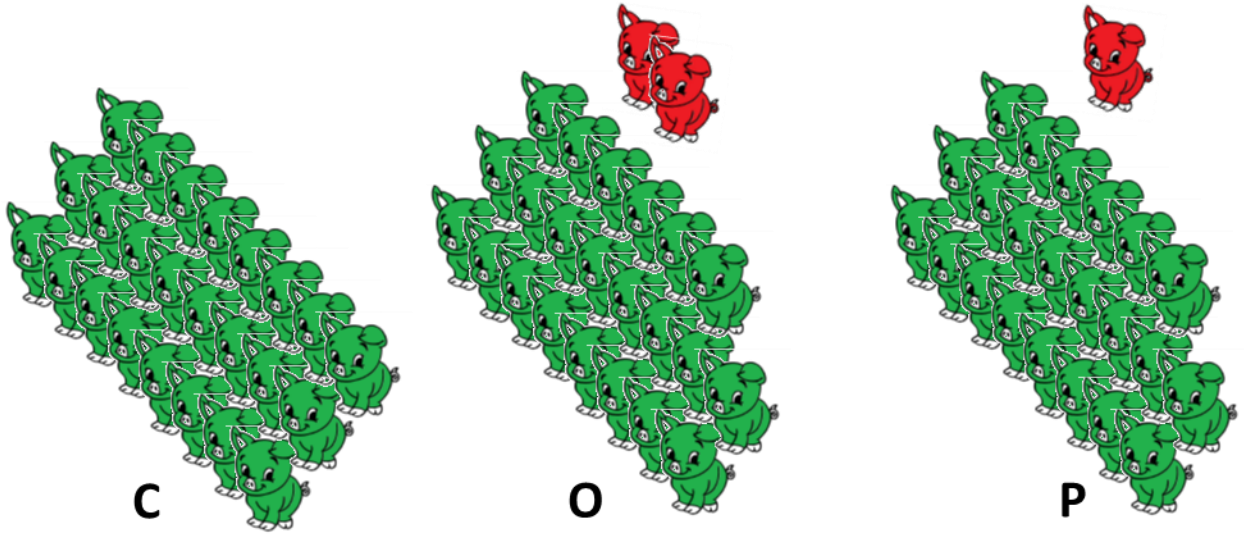
T1: end of amoxicillin administration

RESULTS

Course of natural infection

ETEC F4

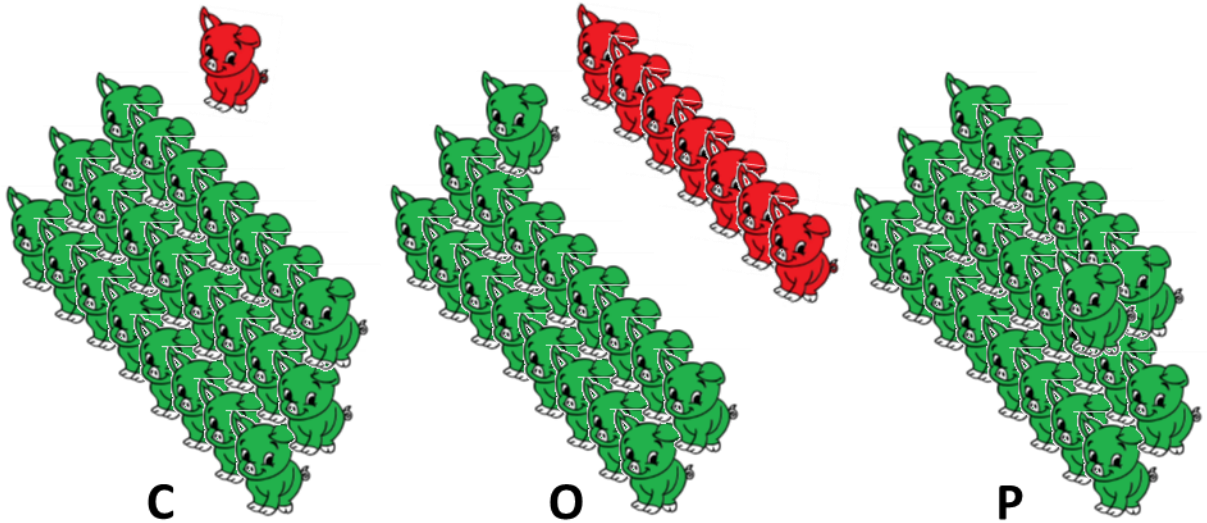
Fisher tests; $p>0.05$



C	O	P
0	2 (8%)	1 (4%)
24 (100%)	22 (92%)	22 (96%)

ETEC F18

Fisher tests; $p=0.004$



C	O	P
1 (4%)	7 (29%)	0
23 (96%)	17 (71%)	23 (100%)

T2: end of the withdrawal period

Antimicrobial susceptibility

The ETEC F4 and ETEC F18 isolates were classified as **multi-resistant to antibiotics**

Beta-lactams
Phenicols
Quinolones
Sulphonamides
Tetracycline
→ ETEC F4 and F18: Resistant

Streptomycin
→ ETEC F4: Susceptible
ETEC F18: Resistant

Cephalosporins
Gentamicin
Kanamycin
→ ETEC F4 and F18: Susceptible

Is there an association between host genotypes and the ETEC infection?

RESULTS

MUC4 vs. ETEC F4

T0

	R	S	Tot
-	12	9	21
+	7	43	50
Tot	19	52	

Fisher tests; *p*=0.003

T1

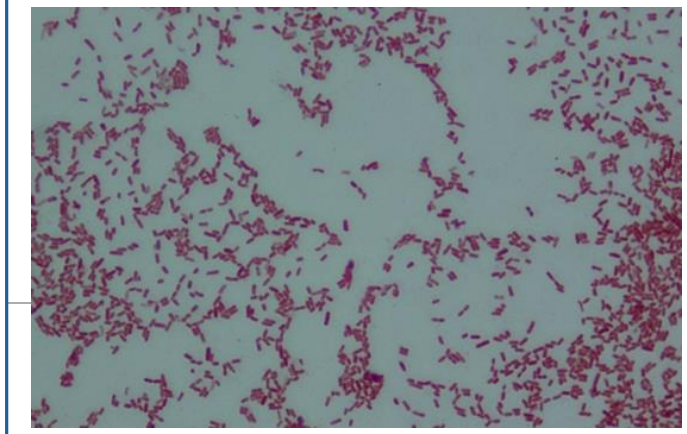
	R	S	Tot
-	19	52	71
+	0	0	0
Tot	19	52	

Fisher tests; *p*>0.05

T2

	R	S	Tot
-	17	51	68
+	2	1	3
Tot	19	52	

Fisher tests; *p*>0.05



Is there an association between host genotypes and the ETEC infection?

RESULTS

FUT1 vs. ETEC F18

T0

	R	S	Tot
-	12	38	50
+	1	20	71
Tot	13	58	

Fisher tests; $p>0.05$

T1

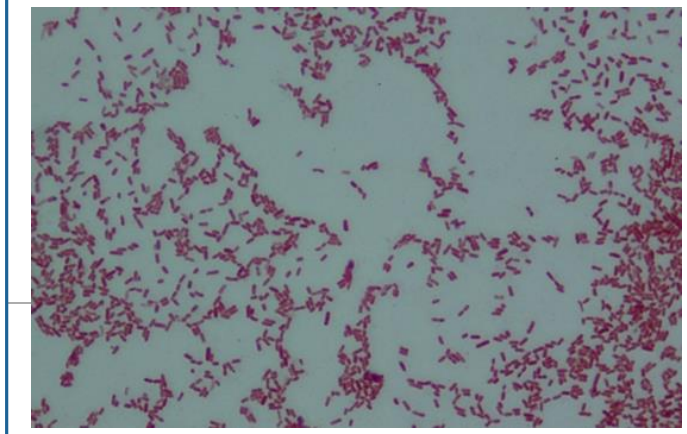
	R	S	Tot
-	5	5	10
+	8	53	61
Tot	13	58	

Fisher tests; $p=0.001$

T2

	R	S	Tot
-	13	50	63
+	0	8	8
Tot	13	58	

Fisher tests; $p>0.05$



Is there an association between host genotypes and diarrhoea?

RESULTS

MUC4 vs. diarrhoea

T0

	R	S	Tot
-	10	44	54
+	9	8	17
Tot	19	52	

Fisher tests; *p*=0.01

T1

	R	S	Tot
-	12	34	46
+	7	18	25
Tot	19	52	

Fisher tests; *p*>0.05

T2

	R	S	Tot
-	16	45	61
+	3	7	10
Tot	19	52	

Fisher tests; *p*>0.05

***FUT1* vs. diarrhoea** Fisher tests; *p*>0.05

Is there an association between the amoxicillin administration and the ETEC infection?

RESULTS

Amoxicillin *vs.* ETEC F4

T1

	C	O	P	Tot
-	24	24	23	71
+	0	0	0	0
Tot	24	24	23	

Fisher tests; $p>0.05$

T2

	C	O	P	Tot
-	24	22	22	68
+	0	2	1	3
Tot	24	24	23	

Fisher tests; $p>0.05$

Is there an association between the amoxicillin administration and the ETEC infection?

RESULTS

Amoxicillin *vs.* ETEC F18

T1

	C	O	P	Tot
-	4	0	6	10
+	20	24	17	61
Tot	24	24	23	

Fisher tests; *p*=0.017

T2

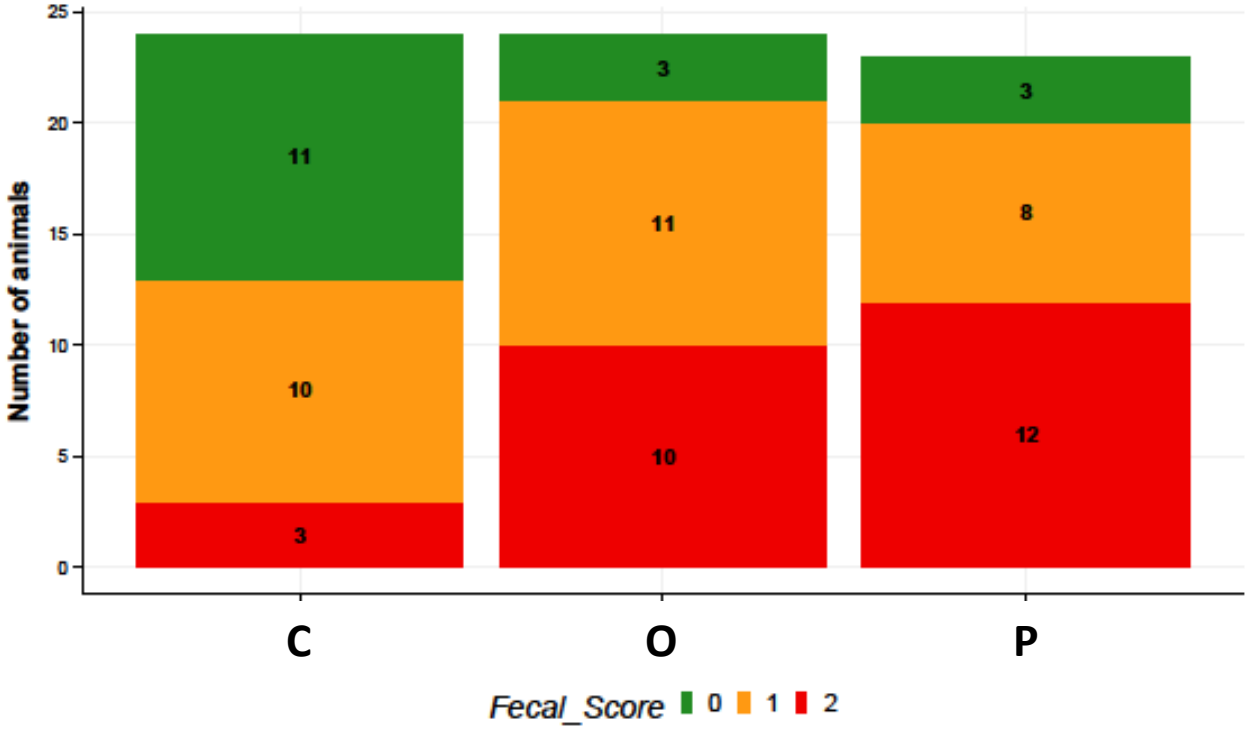
	C	O	P	Tot
-	23	17	23	63
+	1	7	0	8
Tot	24	24	23	

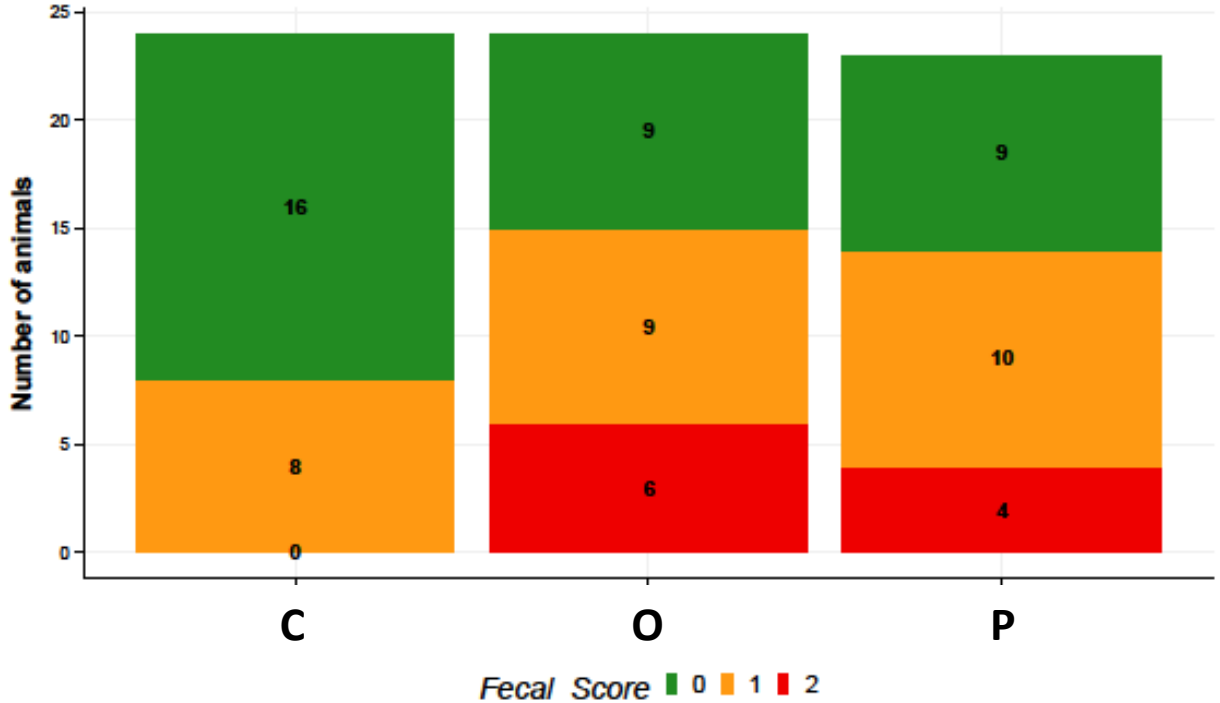
Fisher tests; *p*=0.004

RESULTS

Is there an association between the amoxicillin administration and diarrhea?

Amoxicillin *vs.* diarrhea

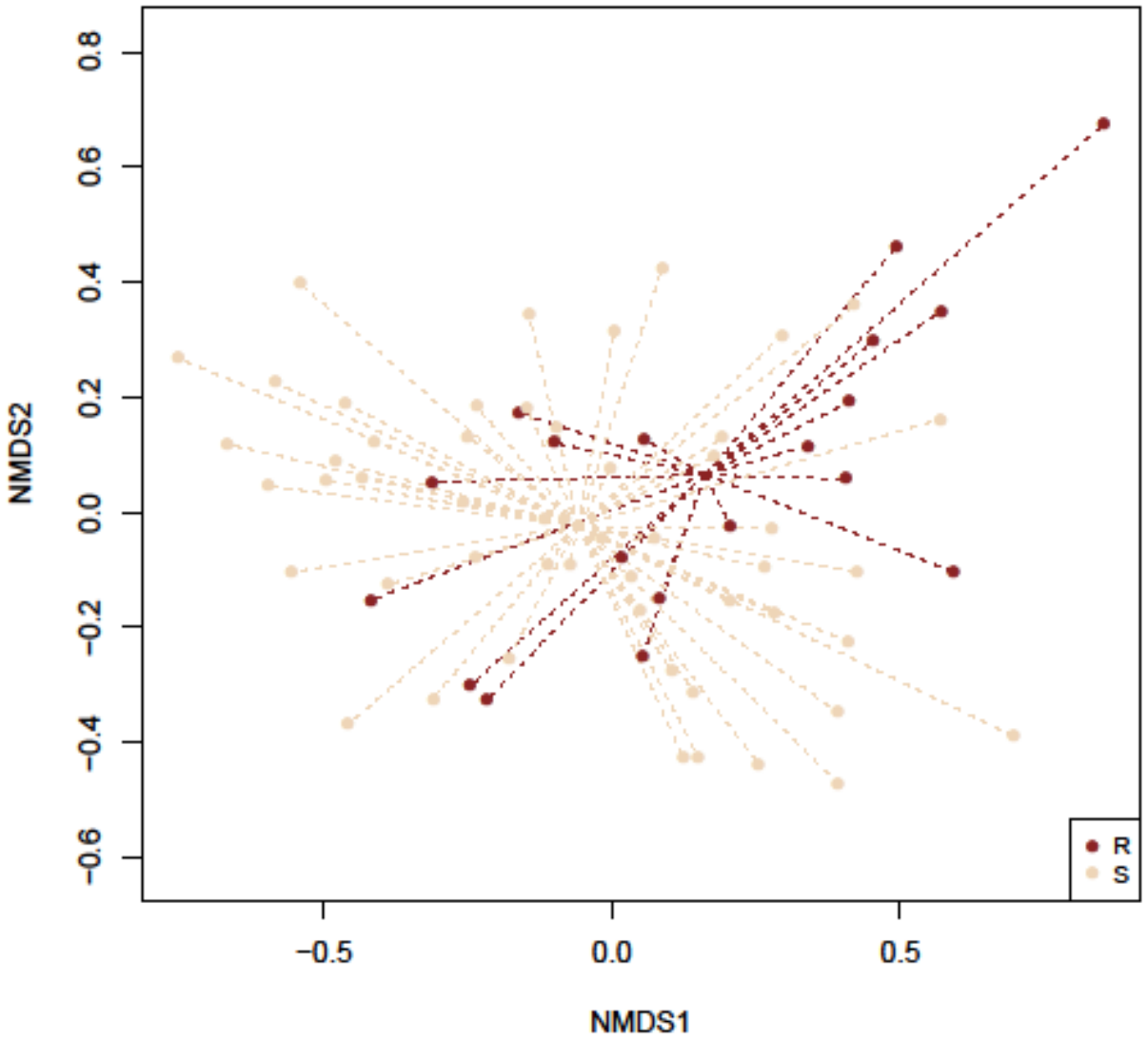




T1 (Fisher test; p=0.009)

T2 (Fisher test; p=0.02)

Gut microbiota



Envfit test, *p*=0.018

T0

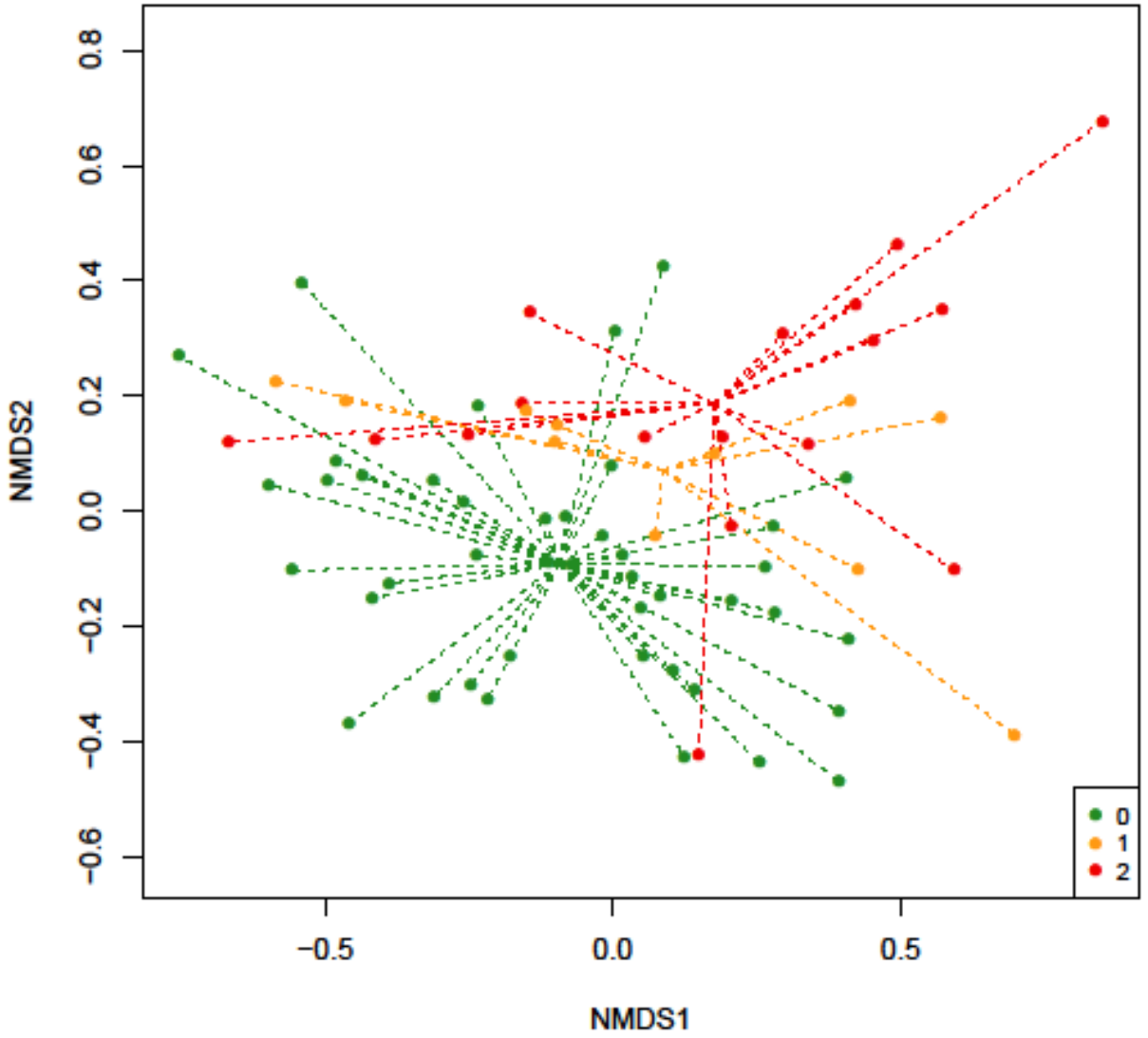
MUC4 (Adonis test, $p = 0.004$)

68 DA OTUs

Oscillospira genera and the *Actinobacillus porcinus* more abundant in the resistant *MUC4* genotype

RESULTS

Gut microbiota



Envfit test, *p*=0.0004

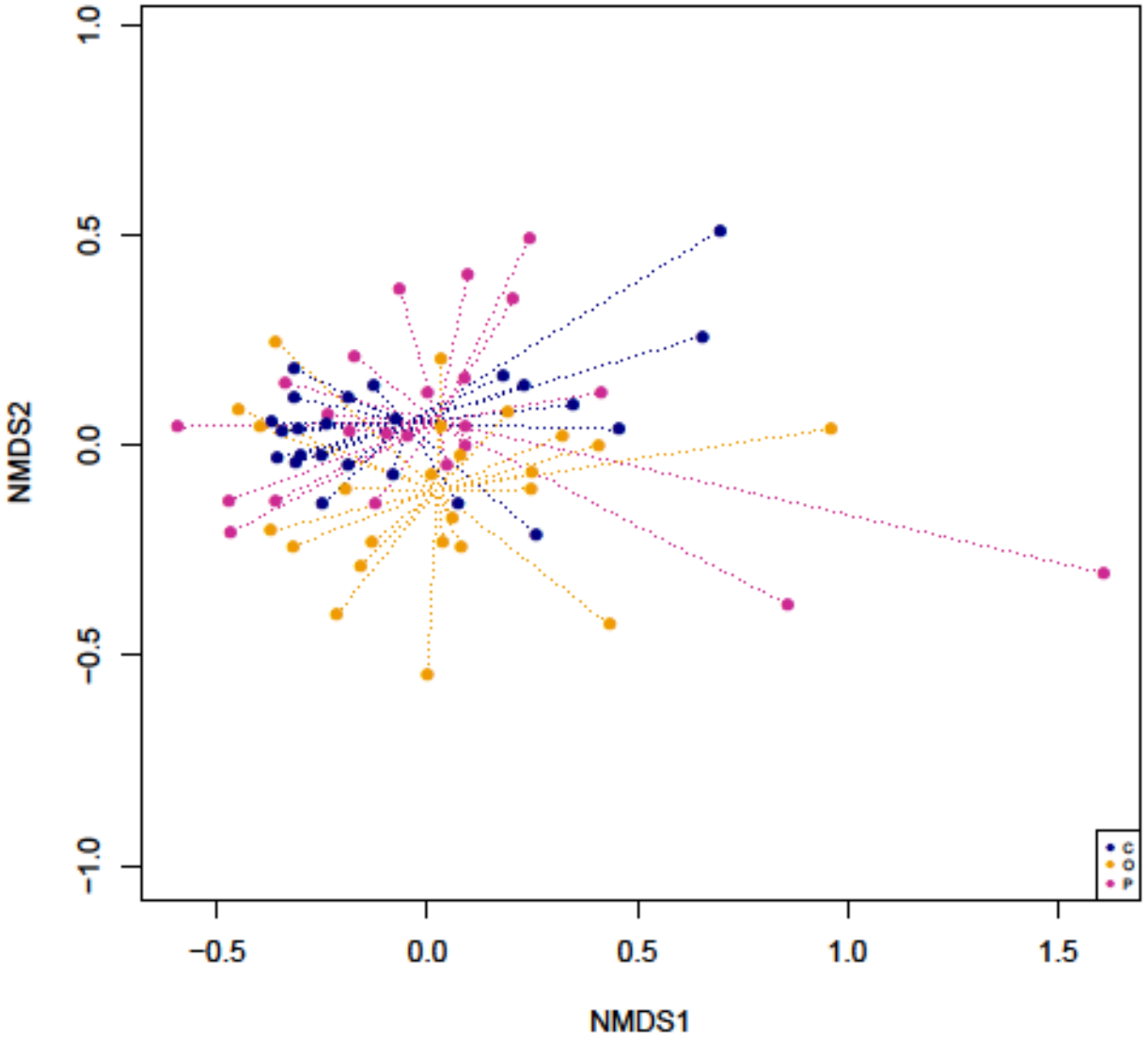
T0

Fecal score (Adonis test, $p = 0.001$)

153 DA OTUs

Ruminococcaceae and *Christensenellaceae* families more abundant in non-diarrhoeic animals

Gut microbiota



Envfit test, *p*=0.02

T1

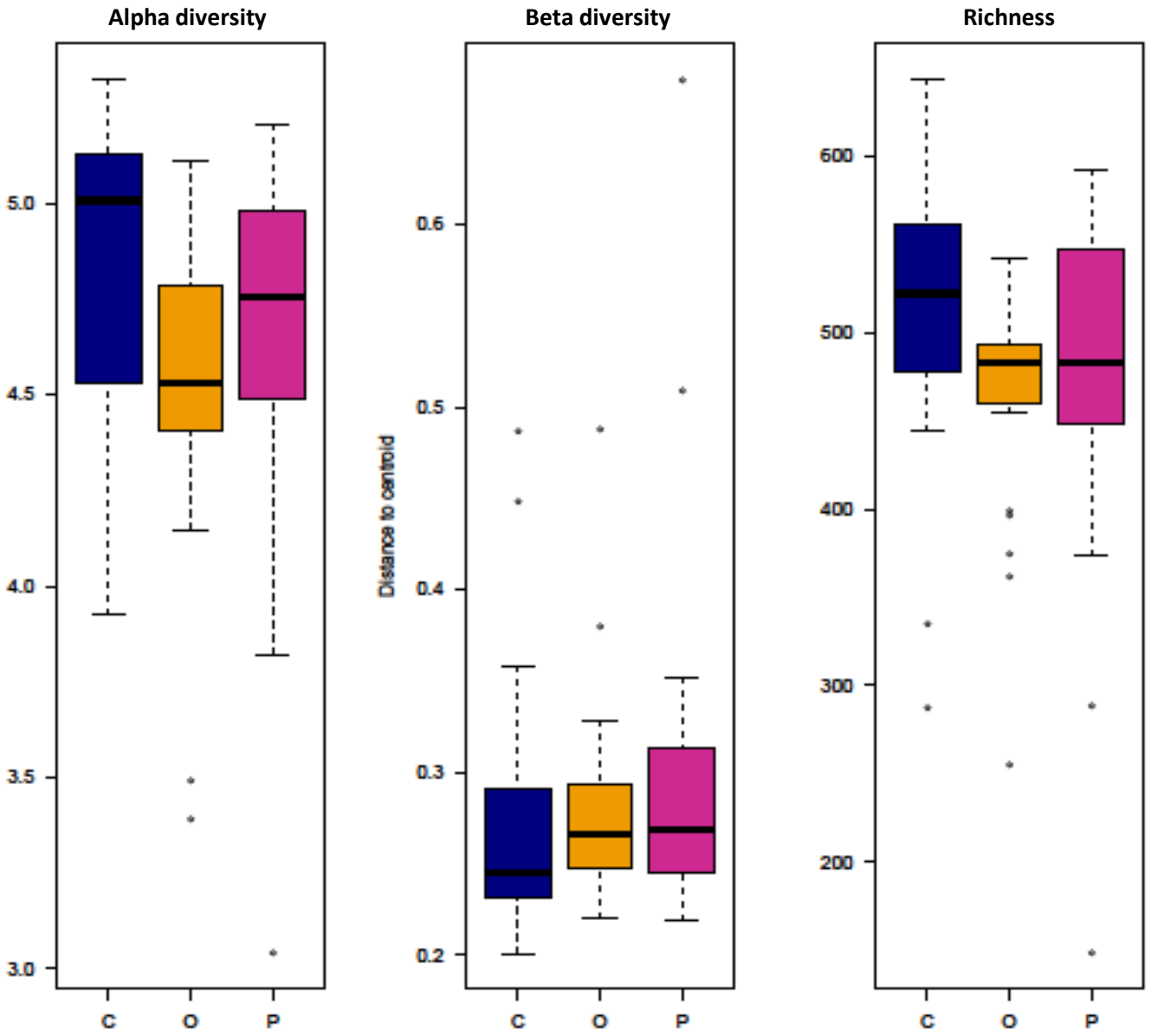
Amoxicillin administration
(Adonis test, $p = 0.0009$)

187 DA OTUs

RESULTS

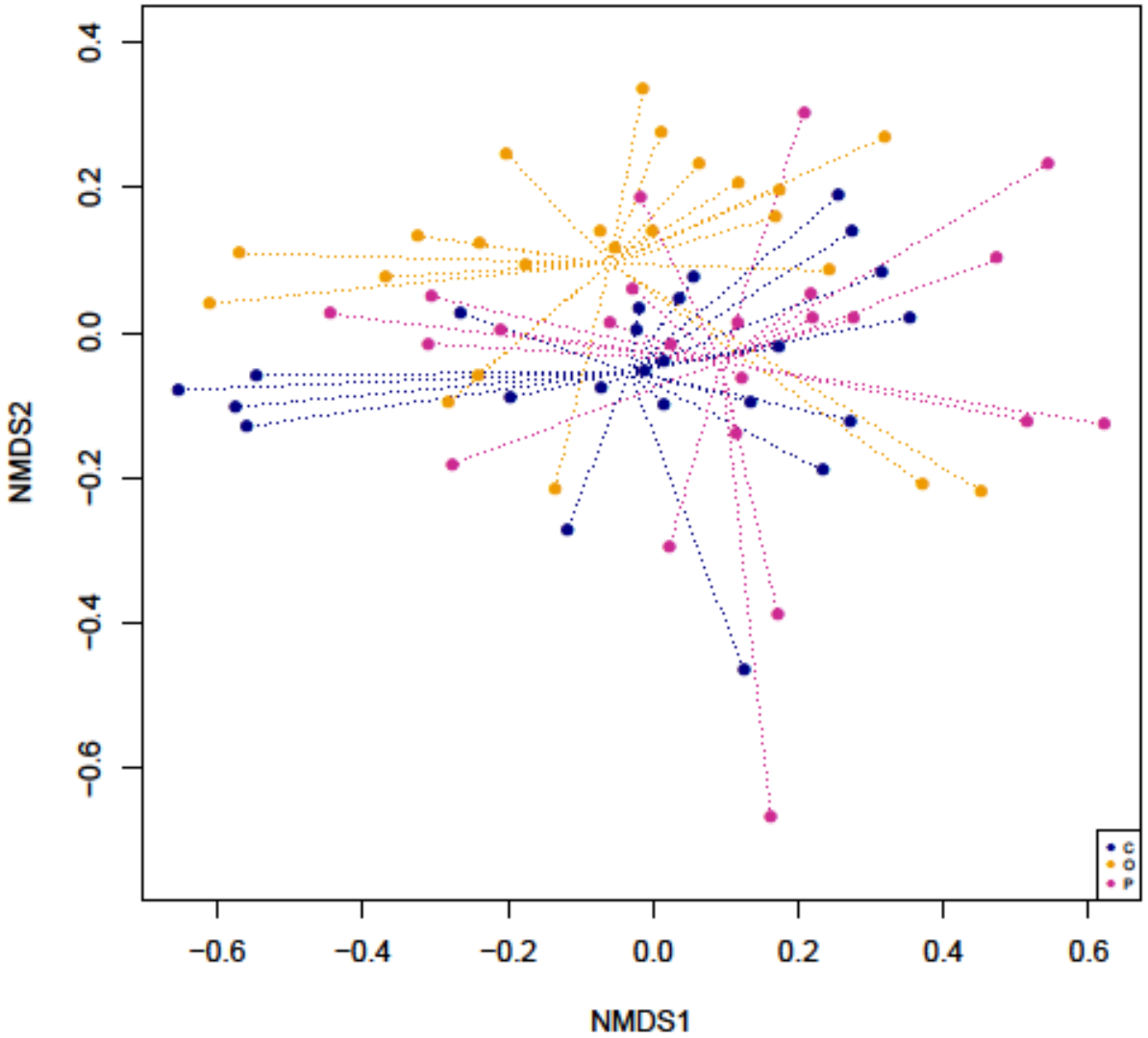
Gut microbiota

T1



***p*=0.03** *p*>0.05 *p*>0.05

Gut microbiota



Envfit test, *p*=0.03

T2

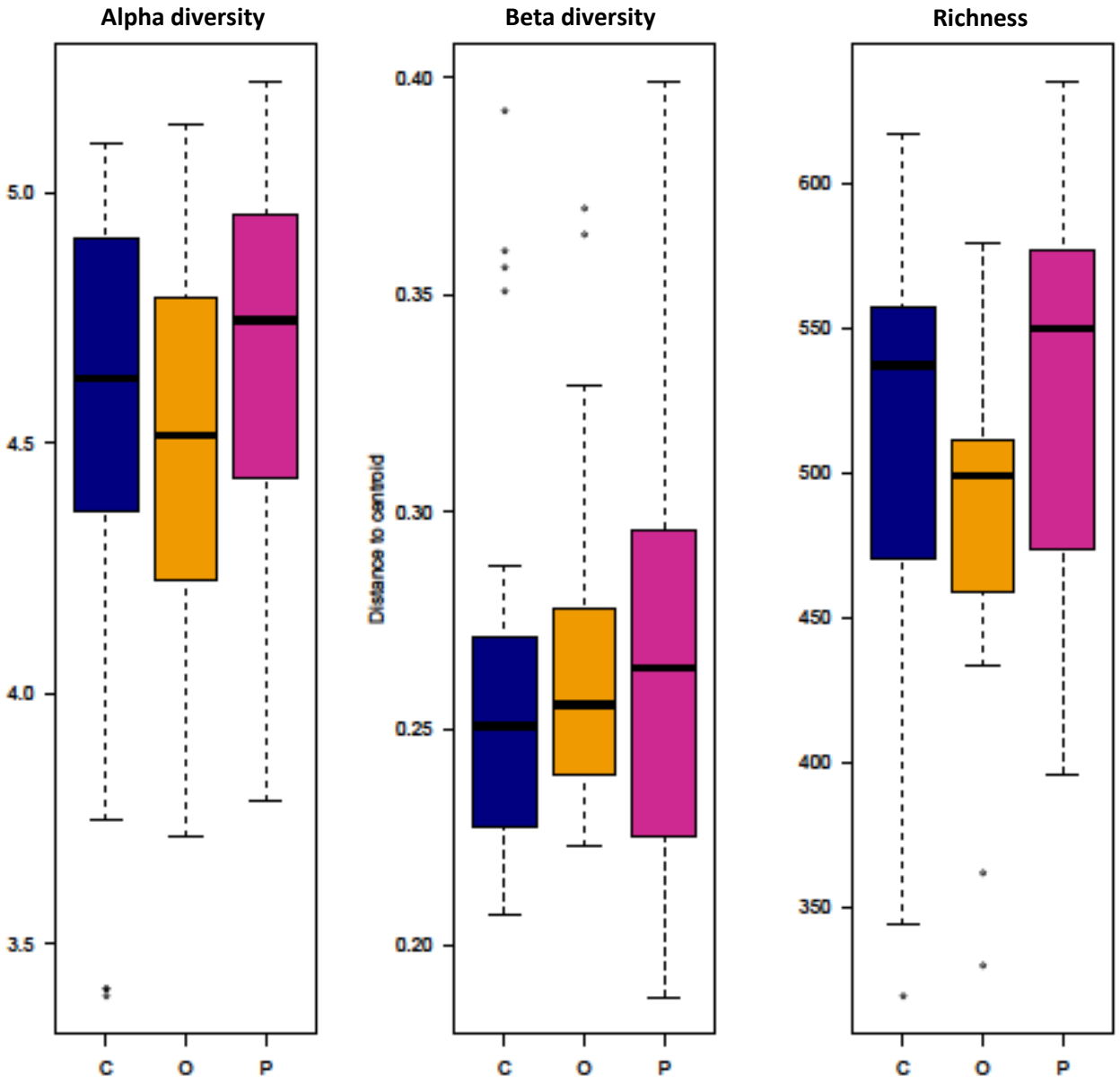
Amoxicillin administration
(Adonis test, $p = 0.0001$)

124 DA OTUs

RESULTS

Gut microbiota

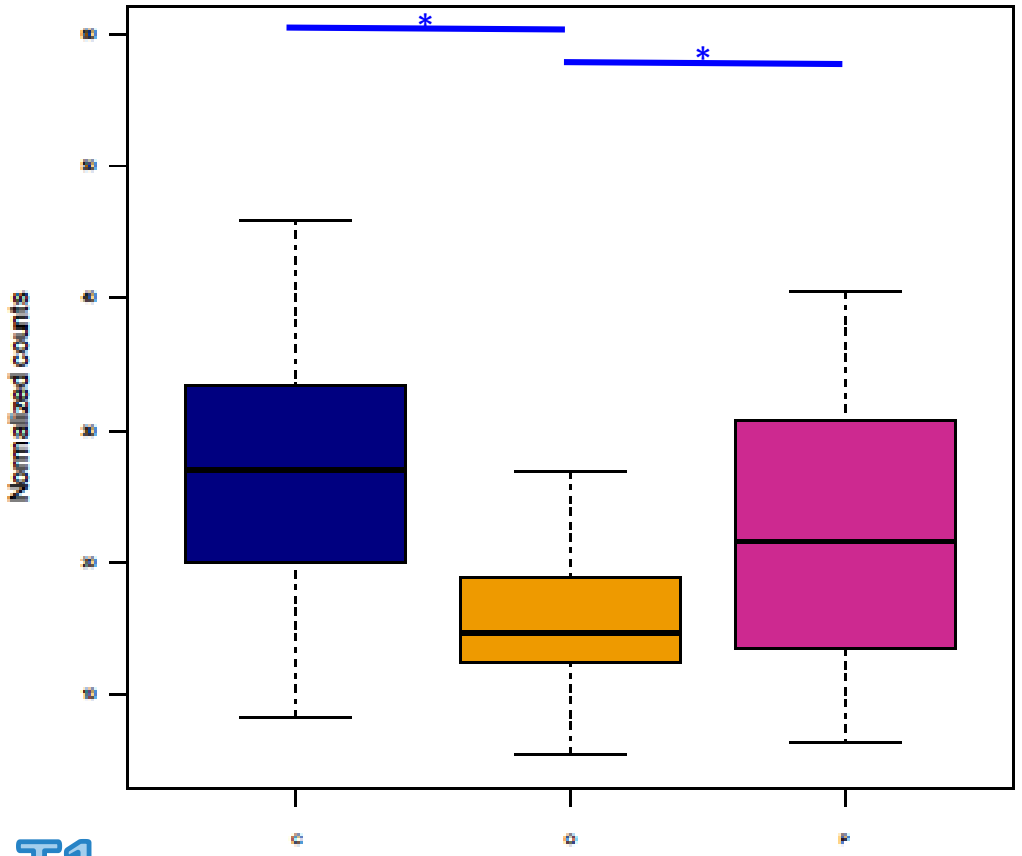
T2



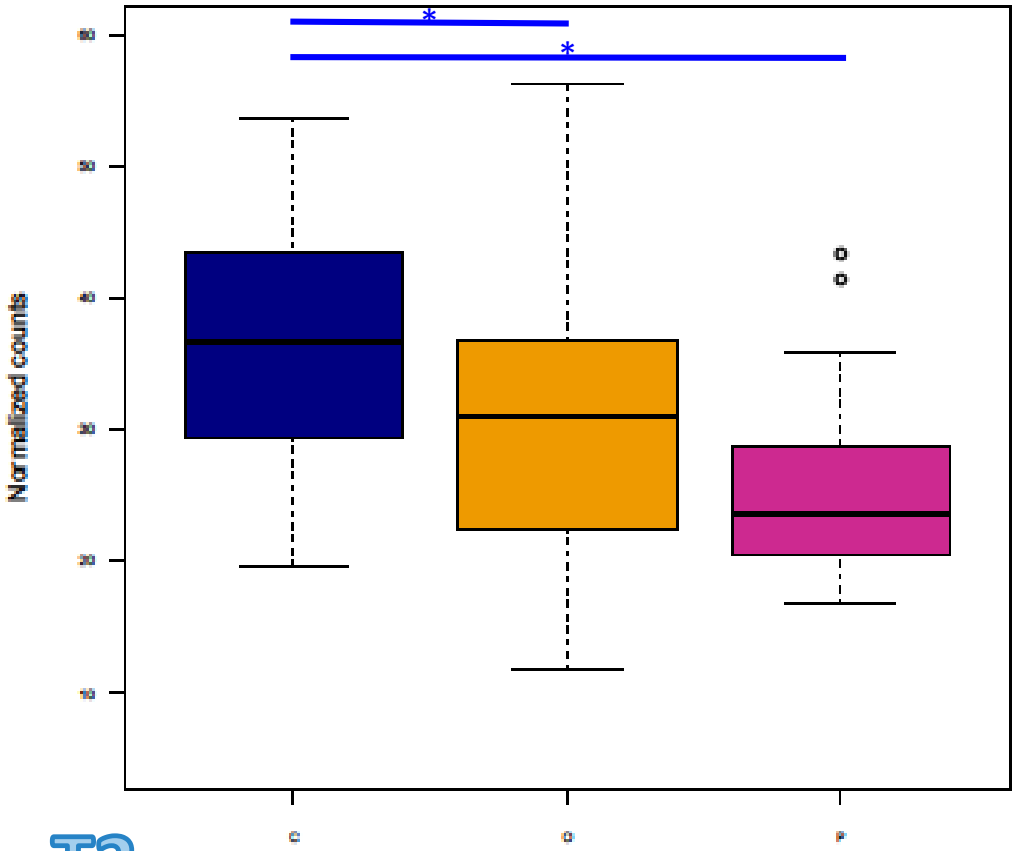
p>0.05 *p*>0.05 *p*>0.05

The case of the *Lactobacillus* genus

Gut microbiota



T1



T2

DISCUSSION

Common situation occurring in commercial pig herds during the weaning period

- ✓ animals are naturally infected by ETEC strains and simultaneously treated with antibiotics

LIMIT OF THE STUDY:

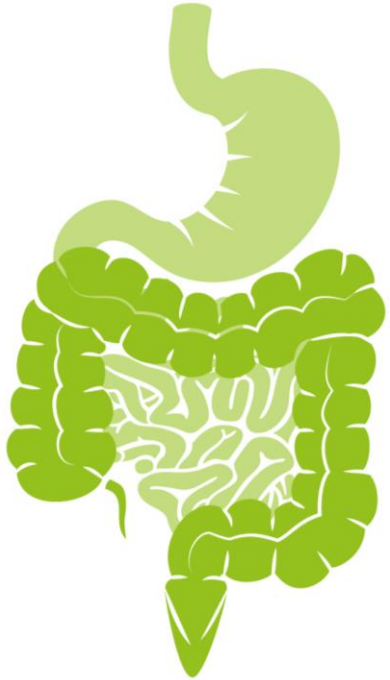
Infectious load was not homogeneous in the animals

DISCUSSION

Susceptible *MUC4* genotypes associated with ETEC F4

Susceptible *FUT1* genotypes associated with ETEC F18

Confirming precedent studies and the role of these genes in the host susceptibility to the infection



Intestinal microbiota is mainly influenced by the *MUC4* genotypes

Casini *et al*., 2016; Jørgensen *et al*., 2004; Luise *et al*., 2019; Meijerink *et al*., 1997; Poulsen *et al*., 2018; Zhang *et al*., 2017

DISCUSSION

Different composition of the faecal microbiota in diarrheic animals compared to non-diarrheic animals

confirming the role of dysbiosis in the development of diarrhoea

Bacteroides, Parabacteroides, Fusobacterium genera and *Pasteurellaceae* family

Ruminococcaceae and *Christensenellaceae* families

Luise *et al.*, 2019; Messori *et al.*, 2012; Bian *et al.,* 2016; Massacci *et al.*, Submitted; Soler *et al.*, 2018

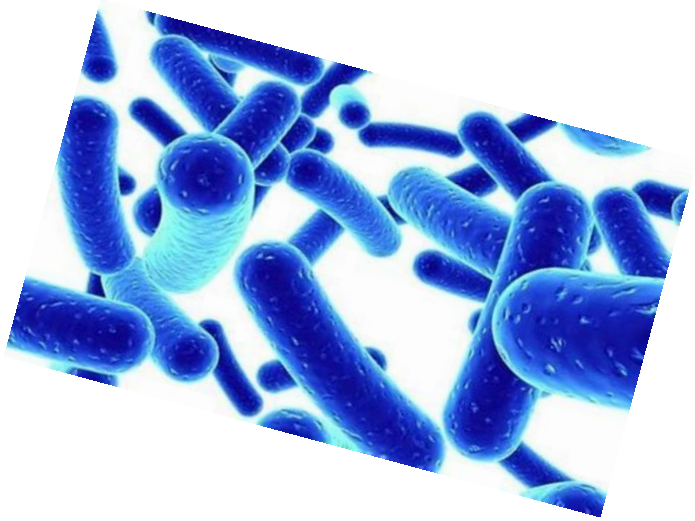
DISCUSSION

Pigs administered with amoxicillin were at higher risk for diarrhoea and ETEC F18 when compared to non-treated piglets

Pigs administered with amoxicillin showed a disrupted gut microbiota when compared to non-treated piglets

Burch & Sperling, 2018; Konstantinov *et al.*,2006; Connelly *et al.*, 2018

The case of the *Lactobacillus* genus

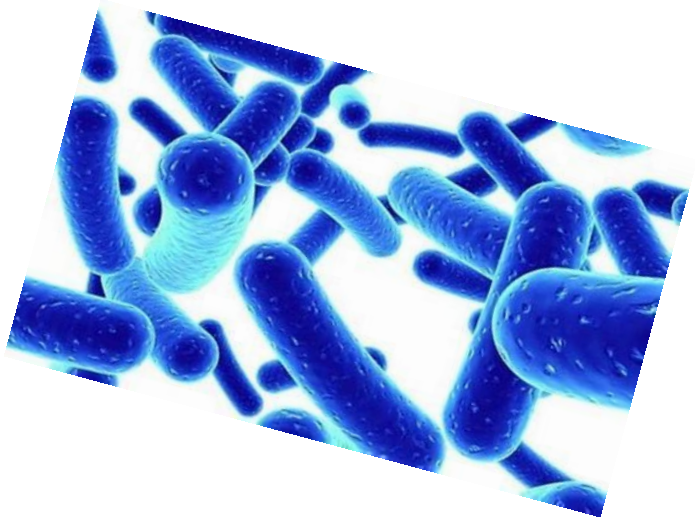


In the group that received amoxicillin orally, we described a decreased abundance of the commensal *Lactobacillus*

Clinical activity of amoxicillin

Burch & Sperling, 2018; Konstantinov *et al.*,2006; Connelly *et al.*, 2018

The case of the *Lactobacillus* genus



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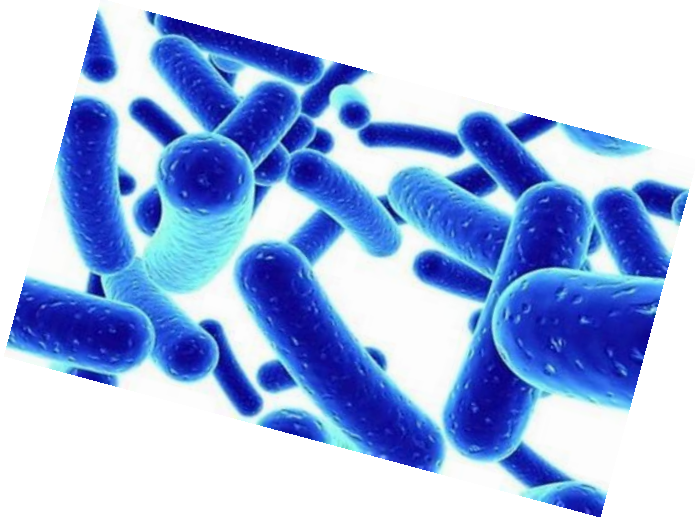
Clinical activity of amoxicillin

Decrease at weaning increases the risk of enteritis:

- Bacteria belonging to this genus play a major role in disease prevention

Burch & Sperling, 2018; Konstantinov *et al.*,2006; Connelly *et al.*, 2018

The case of the *Lactobacillus* genus



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Clinical activity of amoxicillin

Decrease at weaning increases the risk of enteritis:

- Bacteria belonging to this genus play a major role in disease prevention

Lower effect of parenteral than oral administration

Burch & Sperling, 2018; Konstantinov *et al.*,2006; Connelly *et al.*, 2018

CONCLUSION

MUC4 and *FUT1* were confirmed as genetic markers for the susceptibility to ETEC infections

ETEC F4 and ETEC F18 multi-drug resistant

Amoxicillin treatment affect the gut microbiota

Casini *et al*., 2016; Jørgensen *et a*l., 2004; Luise *et al*., 2019; Meijerink *et al*., 1997; Poulsen *et al*., 2018; Zhang *et al*., 2017 Burch & Sperling, 2018; Konstantinov *et al*.,2006; Connelly *et al*., 2018

CONCLUSION

Amoxicillin treatment may produce adverse outcomes on pig health in course of multi-resistant ETEC infection

- stronger effect when the antibiotic is orally administered than parenterally

Amoxicillin may help the ETEC colonization

- Antibiotics therapy causes alterations of the intestinal microbial composition, enabling *C. difficile* or *Salmonella* colonization

Mullish *et al*., 2018; Crowther *et a*l., 2015; Divek *et al*., 2018

CONCLUSION

Alternative control measures should be included in farm management practices to preserve a balanced and stable gut microbiota in weaners

Selection of resistant genotypes

New antibiotics

Vaccination

Probiotics

Eubiosis

Journal of Animal Breeding and Genetics

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"Host genotype and amoxicillin administration affect the incidence of diarrhoea and faecal microbiota of weaned piglets during a natural multi-resistant ETEC infection."

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DELL'UMBRIA E DELLE MARCHE "TOGO ROSATI"

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Claudio Forte
Carmela Lovito
Serenella Orsini
Michele Tentellini

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