

Supplementary data

**Intestinal exposure to ceftiofur and cefquinome after intramuscular treatment and the impact of ceftiofur on the pig faecal microbiome and resistome**

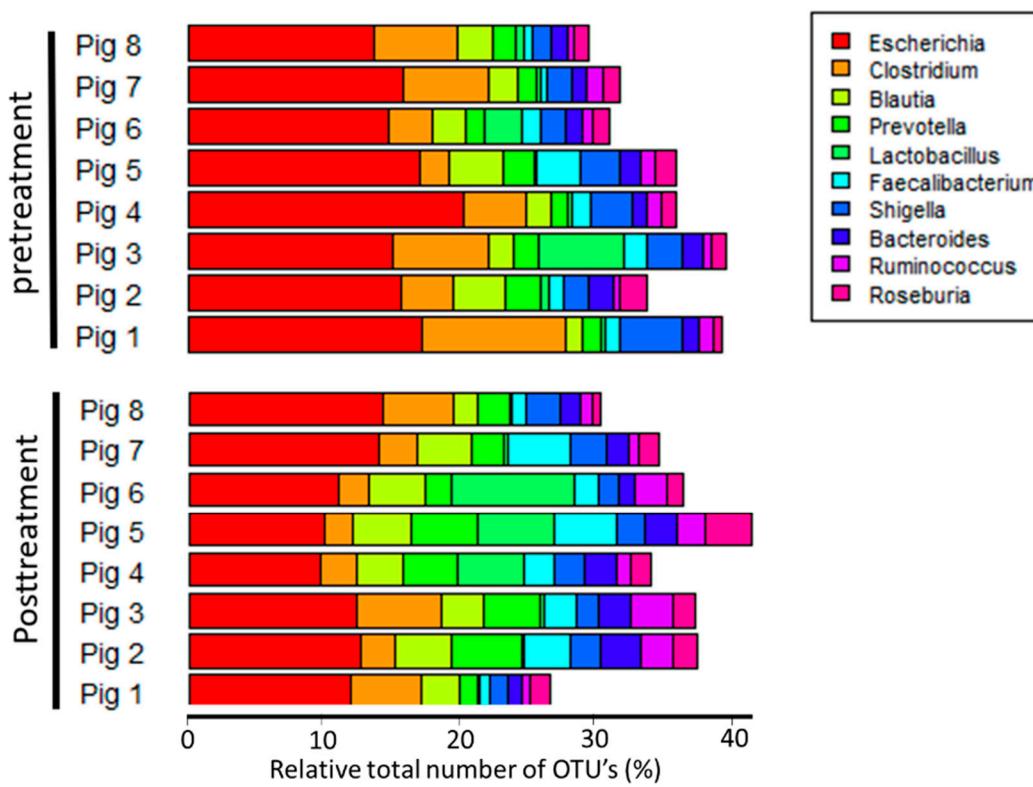
Sofie Rutjens<sup>1</sup>, Nick Vereecke<sup>2</sup>, Ward De Spiegelaere<sup>3</sup>, Siska Croubels<sup>1</sup>, and Mathias Devreese<sup>1,\*</sup>

<sup>1</sup> Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium; Sofie.Rutjens@UGent.be, Siska.Croubels@UGent.be

<sup>2</sup> PathoSense BV, 2500 Lier, Belgium & Department of Translational Physiology, Infectiology and Public Health, Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium; Nick.Vereecke@pathosense.com

<sup>3</sup> Department of Morphology, Imaging, Orthopedics, Rehabilitation and Nutrition, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium; Ward.DeSpiegelaere@UGent.be

\* Correspondence: Mathias.Devreese@UGent.be; Tel.: 0032 9 264 73 47



**Figure S1.** Genus level horizontal bar plot of the relative total number of OTU's of the top 10 genera. Faecal microbiomes for each animal (n=8) were analysed pre-treatment and 9 hours after the last dose of the daily intramuscular administration of 3 mg.kg<sup>-1</sup> body weight of ceftiofur for 3 consecutive days. Collection times and animal numbers are displayed on the side.

**Table S1.** Mean ( $\pm$  standard deviation, n=8) resistance gene counts corrected to the relative (pre-treatment to post-treatment, i.e. 9 hours after the last dose of the daily intramuscular administration of 3 mg.kg<sup>-1</sup> body weight of ceftiofur for 3 consecutive days) base counts of the top 20 genera. *Tet* = tetracycline, *AME* = aminoglycoside-modifying enzyme, *Van* = vancomycine, *CfxA* = beta-lactamase.

Genus	<i>Tet</i> resistance genes		<i>AME</i> resistance genes		<i>VAN</i> resistance genes		Macrolide resistance genes		Lincosamide resistance genes		<i>CfxA</i> resistance genes	
	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3
<i>Anaerobutyricum</i>	2.1 ± 2.1	2.2 ± 2.5	0.1 ± 0.4	0.1 ± 0.2	0	0	0.4 ± 0.7	0.1 ± 0.2	0.6 ± 1.2	0	0	0
<i>Bacillus</i>	0	0	0.3 ± 0.5	0.2 ± 0.5	0	0	0	0.3 ± 0.6	0.8 ± 2.1	0	0	0
<i>Bacteroides</i>	8.3 ± 6.6	7.8 ± 6.6	0	0	0	0	9.5 ± 7.3	5.9 ± 2.8	2.0 ± 4.7	0.2 ± 0.4	4.7 ± 2.5	7.6 ± 6.4
<i>Blautia</i>	0.5 ± 1.1	1.2 ± 1.1	0.3 ± 0.5	0.2 ± 0.4	0	0	0	0.1 ± 0.2	0.4 ± 0.5	0.2 ± 0.5	0	0
<i>Clostridium</i>	6.8 ± 3.5	9.8 ± 7.9	0.6 ± 0.7	0*	0	0	0.9 ± 1.0	2.4 ± 3.5	0.6 ± 1.4	2.8 ± 5.0	0	0
<i>Escherichia</i>	0	0	0.1 ± 0.4	0.7 ± 1.0	0	0	0.1 ± 0.4	0.1 ± 0.4	0.6 ± 0.5	0.7 ± 0.8	0	0
<i>Faecalibacterium</i>	7.8 ± 6.0	6.8 ± 3.9	0	0.1 ± 0.2	0	0	0.1 ± 0.4	0	0.6 ± 0.7	0.6 ± 0.5	0	0
<i>Lachnoclostridium</i>	1.9 ± 2.0	1.4 ± 1.0	0.1 ± 0.4	0.1 ± 0.3	0	0	0.3 ± 0.5	0.1 ± 0.3	0.1 ± 0.4	0.2 ± 0.4	0	0
<i>Lactobacillus</i>	3.3 ± 4.8	9.7 ± 19.3	0	0	0	0	2.1 ± 4.8	0.3 ± 0.6	5.0 ± 3.2	7.9 ± 19.6	0	0
<i>Leishmania</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Methanobrevibacter</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oscillibacter</i>	1.4 ± 1.8	3.2 ± 3.8	0.1 ± 0.4	0	0	0	0	0	0.4 ± 0.5	0	0	0
<i>Prevotella</i>	0	0.1 ± 0.2	0	0	0	0	0	0	0	0	2.8 ± 2.0	0.7 ± 0.7*
<i>Romboutsia</i>	0.1 ± 0.4	0	0.1 ± 0.4	0	0	0	0.3 ± 0.5	0	0	0	0	0
<i>Roseburia</i>	2.5 ± 2.2	3.2 ± 2.0	0.3 ± 0.7	0.2 ± 0.3	0	0	0.8 ± 0.7	0.9 ± 1.0	0.1 ± 0.4	0.6 ± 0.9	0	0
<i>Ruminococcus</i>	0.5 ± 0.8	0.4 ± 0.8	0.3 ± 0.5	0	0	0	0	0.3 ± 0.6	0.2 ± 0.5	0.2 ± 0.4	0	0
<i>Salmonella</i>	0	0	0.5 ± 0.5	0.1 ± 0.3	0	0	0	0	0	0	0	0
<i>Shigella</i>	0	0	0	0	0	0	0	0	0.6 ± 1.4	0	0	0
<i>Streptococcus</i>	7.9 ± 5.7	13.3 ± 7.0	0.3 ± 0.5	0.1 ± 0.2	1.6 ± 1.6	1.2 ± 1.4	0.3 ± 0.7	0.5 ± 0.5	2.5 ± 3.3	1.5 ± 1.3	0	0
<i>Treponema</i>	0.1 ± 0.4	0	0	0	0	0	0	0	0.8 ± 1.2	0.6 ± 1.6	0	0

Significance codes: '\*'  $p$ -value <0.05