

## Supplementary data

### File S1. Sampling protocol.

The sampling protocol followed what reported by Hille et al., 2018 [18].

On each farm, the area hosting calves under 60 days of age was divided in three sections: anterior, middle and posterior. Three pooled faecal samples, of at least 10 grams each, were aseptically collected from each of these three sections and immediately put in a sterile container.

The fourth sample consisted of a boot swab sample, which was collected from each calf barn as follows. Boot swabs were commercially available adsorptive polyethylene overboots (Sir Safety System, Assisi, Italy) which were pulled over both feet of the sampling veterinarian. The sampling veterinarian moved across the calf barn, walking along the diagonal of the entire area. On completion of sampling, the boot swabs were carefully removed in order to avoid any losses, inverted to retain of faecal material, and put in a sterile container.

Each sample was individually identified, transported to the laboratory at 4°C and analysed within 12 h from sampling.

**Table S1.** Categorisation of the active principles into the corresponding antimicrobial classes.

<b>Antimicrobial classes and associations</b>	<b>Active Ingredients (AIs)</b>
<b>Amphenicols</b>	
no association	Florfenicol Thiamphenicol
<b>Aminoglycosides</b>	
no association	Gentamicin Kanamycin Paromomycin
with Sulfonamides	Paromomycin (+ Dihydrostreptomycin) Neomycin (+ Streptomycin)
<b>1<sup>st</sup> gen. Cephalosporins</b>	
no association	Cefalexin Cefalexin benzathine Cefalonium Cefapirin Cefapirin benzathine Cefazolin
with Aminoglycosides	Cefalexin
with Rifamycins	Cefacetrile
<b>3<sup>rd</sup> gen. Cephalosporins</b>	
no association	Cefoperazone Ceftiofur
<b>4<sup>th</sup> gen. Cephalosporins</b>	
no association	Cefquinome
<b>Quinolones</b>	
no association	Flumequine
<b>Fluoroquinolones</b>	
no association	Danofloxacin Enrofloxacin Marbofloxacin
<b>Lincosamides</b>	
with Aminoglycosides	Lincomycin
<b>Macrolides</b>	
no association	Gamithromycin Spiramycin Tilmicosin Tylosin Tulathromycin
with Sulfonamides	Erythromycin

<b>Penicillins</b>	
no association	Amoxicillin Ampicillin Procaine benzylpenicillin Cloxacillin Cloxacillin benzathine Penethamate hydriodide Ampicillin (+ Cloxacillin benzathine) Ampicillin (+ Dicloxacillin)
with Clavulanic Acid	Amoxicillin
with Aminoglycosides	Benzylpenicillin Procaine benzylpenicillin Procaine benzylpenicillin (+ Nafcillin) Penethamate hydriodide (+ Benethamine benzylpenicillin)
with Polymyxins	Amoxicillin
<b>Rifamycine</b>	
no association	Rifaximin
<b>Sulfonamides</b>	
no association	Sulfamethoxyipyridazine Sulfadimidine (+ Sulfamerazine + Sulfathiazole)
with Trimethoprim	Sulfadiazine Sulfadimethoxine Sulfadimidine Sulfamonomethoxine
<b>Tetracyclines</b>	
no association	Doxycycline Oxytetracycline
with Penicillins	Chlortetracycline

**Table S2.** Distribution of farms according to the breeding system and the herd size. The stratum-specific ORs are shown.

<b>Breeding system/ herd size</b>	<b>Number of farms</b>	<b>Presence of <i>C. difficile</i></b>	<b>CI95%</b>	<b>OR</b>	<b>P-value</b>
<b>Beef</b>					
50-99	29	5 (17.2%)	0%-35.3%	-	-
100-199	15	2 (13.3%)	0%-35.9%	0.74 (0.13-4.35)	1
≥200	10	3 (30%)	0%-67.3%	2.06 (0.39-10.83)	0.3989
<b>Milk</b>					
50-99	19	4 (21.1%)	0%-45%	-	-
100-199	13	5 (38.5%)	3.7%-73.2%	2.34 (0.49-11.26)	0.4269
≥200	15	1 (6.7%)	0%-23.26%	0.27 (0.02-2.69)	0.3547

**Table S3.** Distribution of antimicrobial classes prescribed on farms and association with *C. difficile*-positive farms. Data on associations of different antimicrobial classes are not shown because they were not significant.

Antimicrobial class prescribed on farm	N° of farms	<i>C. difficile</i> positive farms (%)	CI 95%	P-value
<b>Amphenicols</b>				0.670
Yes	13	2 (15.4%)	0%-35%	
No	88	18 (20.5%)	12%-28.9%	
<b>Aminoglycosides</b>				0.261
Yes	30	8 (26.7%)	10.9%-42.5%	
No	71	12 (16.9%)	8.2%-25.6%	
<b>1<sup>ST</sup> gen. Cephalosporins</b>				0.626
Yes	35	6 (17.1%)	4.7%-29.6%	
No	66	14 (21.2%)	11.4%-31.1%	
<b>3<sup>RD</sup> gen. Cephalosporins</b>				0.727
Yes	37	8 (21.6%)	8.4%-34.9%	
No	64	12 (18.8%)	9.2%-28.3%	
<b>4<sup>TH</sup> gen. Cephalosporins</b>				0.887
Yes	29	6 (20.7%)	6%-35.4%	
No	72	14 (19.4%)	10.3%-28.6%	
<b>Quinolones</b>				
Yes	1	0 (0%)		
No	100	20 (20%)	12.2%-27.8%	
<b>Fluoroquinolones</b>				0.699
Yes	67	14 (20.9%)	11.2%-30.6%	
No	34	6 (17.7%)	4.8%-30.5%	
<b>Lincosamides (with aminoglycosides)</b>				0.940
Yes	31	6 (19.3%)	5.5%-33.3%	
No	70	14 (20%)	10.6%-29.4%	
<b>Macrolides</b>				0.396
Yes	42	10 (23.8%)	10.9%-36.7%	
No	59	10 (16.9%)	7.4%-26.5%	
<b>Penicillins</b>				0.027*
Yes	68	18 (26.5%)	16%-37%	
No	33	2 (6.1%)	0%-14.2%	
<b>Rifamycins</b>				0.419
Yes	28	7 (25%)	9%-41%	
No	73	13 (17.8%)	9%-26.6%	
<b>Sulfonamides</b>				
Yes	5	0 (0%)		
No	96	20 (20.8%)	12.7%-29%	
<b>Tetracyclines</b>				0.341
Yes	41	10 (24.4%)	11.3%-37.5%	
No	60	10 (16.7%)	7.2%-26.1%	

**Table S4.** Descriptive statistics relating to the average number of treated animals for each antibiotic class. Data on associations are not shown.

<b>Antimicrobial class/prescribed on farm</b>	<b>Number of treated animals/100 animals (±SD)</b>	<b>P-value</b>
<b>Amphenicols</b>		0.6764
<i>C. difficile</i> positive farms	0.016 (±0.67)	
<i>C. difficile</i> negative farms	0.0123 (±0.0386)	
<b>Aminoglycosides</b>		0.116
<i>C. difficile</i> positive farms	0.0714 (±0.1083)	
<i>C. difficile</i> negative farms	0.027 (±0.0594)	
<b>1<sup>ST</sup> gen. Cephalosporins</b>		0.3908
<i>C. difficile</i> positive farms	0.0169 (±0.0824)	
<i>C. difficile</i> negative farms	0.0457 (±0.0823)	
<b>3<sup>RD</sup> gen. Cephalosporins</b>		0.9567
<i>C. difficile</i> positive farms	0.0372 (±0.0986)	
<i>C. difficile</i> negative farms	0.0417 (±0.0908)	
<b>4<sup>TH</sup> gen. Cephalosporins</b>		0.8812
<i>C. difficile</i> positive farms	0.0475 (±0.1253)	
<i>C. difficile</i> negative farms	0.0424 (±0.1505)	
<b>Quinolones</b>		0.6193
<i>C. difficile</i> positive farms	0	
<i>C. difficile</i> negative farms	0.0002 (±0.0014)	
<b>Fluoroquinolones</b>		0.2015
<i>C. difficile</i> positive farms	0.0806 (±0.0855)	
<i>C. difficile</i> negative farms	0.054 (±0.0873)	
<b>Lincosamides (with aminoglycosides)</b>		0.8839
<i>C. difficile</i> positive farms	0.0273 (±0.0619)	
<i>C. difficile</i> negative farms	0.0176 (±0.0412)	
<b>Macrolides</b>		0.8415
<i>C. difficile</i> positive farms	0.038 (±0.0646)	
<i>C. difficile</i> negative farms	0.0552 (±0.0957)	
<b>Penicillins<sup>1</sup></b>		<b>0.0215*</b>
<i>C. difficile</i> positive farms	0.1076 (±0.1439)	
<i>C. difficile</i> negative farms	0.0642 (±0.1069)	
<b>Rifamycins</b>		0.2846
<i>C. difficile</i> positive farms	0.0123 (±0.0194)	
<i>C. difficile</i> negative farms	0.0094 (±0.0203)	
<b>Sulfonamides</b>		0.257
<i>C. difficile</i> positive farms	0	
<i>C. difficile</i> negative farms	0.0045 (±0.0241)	
<b>Tetracyclines</b>		0.4235
<i>C. difficile</i> positive farms	0.0001 (±0.0006)	
<i>C. difficile</i> negative farms	0.0001 (±0.0006)	