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Occurrence of *Escherichia coli* non-susceptible to quinolones in fecal and environmental samples from pigs at different ages after fluoroquinolone treatment in piglets or their dams

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submitted by

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Abstract

Despite their indispensability in human medicine fluoroquinolones (FQ) are used in farm animals which inherits the risk of transferring FQ resistant bacteria into the environment and via food chain to human beings. More research is needed in revealing possible transfer mechanisms and factors promoting antibiotic resistance in bacteria. The objectives of this study were to do a follow-up of the presence of quinolone non-susceptible *Escherichia coli* (QNSE) qualitatively and quantitatively in fecal and environmental samples of pigs at four time points and with various FQ backgrounds. 40.9% (95% CI: 37.0-44.9%) of fecal and 14.0% (95% CI: 9.4-19.7%) of environmental samples contained QNSE. Detection rates of QNSE in treated and contact pigs did not differ significantly and were highest in piglets of two- and four-weeks of age. However, the detection rates and counts of QNSE in control pigs were significantly lower compared to treated and contact pigs. 49.6% and 40.0% of isolates in fecal and environmental samples were intermediate or resistant to ciprofloxacin (≥ 3 µg/ml ciprofloxacin), respectively. QNSE were present in the pig's environment and in pigs independent of age or FQ background. New approaches are needed to minimize the emergence and transfer of FQ resistant bacteria from treated pigs to other pigs and the environment.

Keywords
fluoro-/quinolone resistance, pigs, Escherichia coli, fecal samples, environmental samples

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Auftreten von Chinolon-intermediär und -resistenten *Escherichia coli* im Kot von Schweinen unterschiedlichen Alters und in Umgebungsproben nach Fluorchinolonbehandlung der Saugferkel oder deren Muttersau

Zusammenfassung

Trotz ihrer Unentbehrlichkeit in der Humanmedizin und dem Risiko einer Übertragung von Fluorchinolon-resistenten Bakterien in die Umwelt und via Nahrungskette zu den Menschen sind Fluorchinolone bei Nutztieren weiterhin im Einsatz. Weitere Erkenntnisse zu Übertragungsmechanismen und Antibiotikaresistenzen-fördernden Faktoren sind nötig. In dieser Studie wurden Chinolon-unempfindliche *Escherichia coli* (QNSE) qualitativ und quantitativ in Kot- und Umgebungsproben von Schweinen mit unterschiedlicher Fluorchinolon-Vorgeschichte an vier verschiedenen Alterszeitpunkten bestimmt. 40.9 % (95% CI: 37.0-44.9 %) aller Kot- und 14.0 % (95% CI: 9.4-19.7 %) aller Umgebungsproben enthielten QNSE. In zwei- und vierwöchigen Ferkeln waren die Detektionsraten von QNSE am höchsten. In behandelten und Kontaktschweinen unterschied sie sich nicht signifikant. Hingegen waren die Detektionsraten und die Mengen an QNSE in Kontrollschweinen signifikant tiefer verglichen mit behandelten und Kontaktschweinen. 49.6 % und 40.0 % aller *E. coli*-Isolate von Kot- und Umgebungsproben waren intermediär oder resistent gegenüber Ciprofloxacin (≥ 3 µg/ml Ciprofloxacin). Unabhängig des Alters und der Fluorchinolon-Vorgeschichte der Schweine waren QNSE im Kot von und in der Umgebung der Schweine nachweisbar. Neue Bekämpfungskonzepte sind erforderlich um das Auftreten und die Übertragung von Fluorchinolon-resistenten Bakterien von behandelten Schweinen auf andere Schweine und deren Umgebung zu minimieren.

Keywords
Fluor-/Chinolonresistenzen, Schweine, Escherichia coli, Kotproben, Umgebungsproben

Introduction

Quinolones, e.g. nalidixic acid, are synthetic antimicrobial agents introduced for the first time in 1963. Chemical modifications enabled fluoroquinolones (FQ) to work against a wide spectrum of bacteria, including *Enterobacteriaceae*, gram positive bacteria and anaerobes, in many different body tissues. The main mechanism of action is to block DNA supercoiling and replication by inhibiting catalyzing topoisomerases which inevitably leads to death of the bacterial cell. The topoisomerase II of *Escherichia coli* (*E. coli*) is built of the subunits GyrA and GyrB. Mutations in the quinolone resistance determining regions (QRDR) of *gyrA* and *gyrB* result in high-level quinolone and fluoroquinolone resistance [1, 2]. According to the classification published by the World Health Organization (WHO), FQ are part of the highest priority critically important antimicrobials (HPCIAs) due to their need in treating patients suffering from zoonotic diseases, e.g. salmonellosis and campylobacteriosis, or protecting neutropenic patients from septicemia [3, 4]. In Switzerland a national strategy against antibiotic resistances was established with Swiss FQ sales figures steadily declining [5, 6]. Restricted prescription and therapeutic guidelines for veterinarians shall promote a responsible and sustainable handling of antibiotics [7, 8]. In Europe, Canada and Japan detection rates of porcine pathogenic FQ resistant *E. coli* isolated from swine lie between 0% and 39% [9, 10]. This is in contrast to China and Brazil which are reporting very high resistance rates (81.0% and 54.4%) in porcine pathogenic and commensal *E. coli* from swine [11, 12]. Number of human beings infected with FQ resistant bacteria has increased since the introduction of FQ into veterinary medicine. An association between the prevalence in swine or poultry herds and the number of diseased patients was proved [13-17]. Slaughter process and kitchen hygiene are two crucial points in transfer of pathogenic bacteria [18, 19]. Nevertheless, animal traffic, liquid manure spread onto croplands and dust of farms are other considerable transmission pathways in livestock and between animals and human beings [20-24]. FQ are mostly excreted unchanged by the body via urine and feces. Although they have a low bioavailability in soil, they form almost undegradable ion-complexes. FQ concentrations have been positively correlated with the abundance of plasmid mediated quinolone resistance genes in soil and wastewater and promote horizontal transfer of antimicrobial resistance genes [1, 25-28].

In pathogenic and commensal *E. coli* isolated from Swiss pigs (sows, weaners and fattening pigs) a low rate of ciprofloxacin resistance (< 2%) was reported [29, 30]. Current research about FQ resistance rates and transfer between FQ treated and not FQ treated pigs is contradictory comparing two German experimental studies. According to the first report in both groups (held in the same room) commensal *E. coli* with MIC ≥ 4 mg/ml enrofloxacin were detected [31]. However, during and 42 days after FQ treatment no ciprofloxacin resistant *E. coli* (MIC ≥ 4 mg/ml enrofloxacin) were observed neither in the treated nor in the untreated contact groups or in the control group (held in a separate room) in a recently published study [32]. Depending on age, fattening pigs showed lower resistance rates compared to pigs of younger ages [33, 34]. To the authors' knowledge, there has not been any individual, quantitative longitudinal study of quinolone non-susceptible *E. coli* (QNSE) in pigs with different FQ background so far. Therefore, a field study was performed to monitor QNSE quantitatively in pigs either treated (intramuscularly, perorally or lactogenically via treated dam), in contact with treated pigs or dams and without any contact to treated or contact pigs (control).

Pigs and their environment were screened at four different time points (two-week old piglets, four-week old piglets, weaners and fattening pigs). Additionally, the isolates' minimal inhibitory concentrations (MICs) of nalidixic acid and ciprofloxacin were tested. The following study questions were included:

Primary outcomes:

- Are there differences in detection rates of QNSE depending on group (G1 to G5 or treated vs. contact vs. control) and the sampled age category?
- Are the counts of QNSE in treated, untreated contact and control weaners and fattening pigs different?
- Do susceptibility rates and minimal inhibitory concentrations (MICs) of nalidixic acid and ciprofloxacin differ between study groups and age categories?

Secondary outcomes:

- Are detection rates and counts of QNSE different between pigs of farms being part of a sow-pool-system (SPS) and pigs of farms not part of a SPS?
- Depending on group category (G1 to G5 or treated vs. contact. vs. control) or the connection to a SPS do susceptibility proportions differ?
- What are the detection rates and counts of QNSE in different materials (dust, slurry, pen wall and floor wipes) of the investigated pigs' environment?
- What are the susceptibility rates and MICs of nalidixic acid and ciprofloxacin in the pigs' environment?

Material and methods

Study population

Sampling was performed on 24 Swiss pig farms between May 2017 and May 2018: three small to medium-sized farrow-to-finish farms (75 - 150 sows), two small farrowing farms (22 and 14 farrowing pens connected to a sow-pool-system with 1'200 sows), four small farrow and rearing farms (two farms with twelve and 30 sows, two farms with 22 and 23 farrowing pens connected to a sow-pool-system with 1'200 sows) one medium-sized rearing and finishing farm (400 weaner and 480 fattening pigs) and 14 small to medium-sized fattening farms (40 - 1'500 fattening pigs). Thirteen farms were part of a sow-pool-system (SPS): two farrowing farms, two farrow and rearing farms, one rearing and finishing farm and eight fattening farms. Inclusion criteria for study farms was a regular FQ usage restricted to either piglets or sows (in lactating sows: one farrow-to-finish farm, one farrow and rearing farm, two farrowing farms; in piglets: two farrow and rearing farms). Additionally, farms with no use of FQ in any age category for at least three to 34 months (two farrow-to-finish farms, one being part of a SPS, one farrow and rearing farm, one rearing and fattening farm and all 14 fattening farms) were included in order to compare the dissemination of quinolone non-susceptible *E. coli*. The main indications for FQ treatment in sows and piglets were either postpartum dysgalactia syndrome (PPDS) in sows or septic arthritis or diarrhea in piglets. Treatments were carried out by the farmers following their private veterinarian's medical prescriptions including single and multiple FQ treatments (according to the drugs' summary of products characteristics (SPC) parenteral in sows: Baytril®5% (enrofloxacin), 2.5 mg/kg SID; Marbocyl®10% (marbofloxacin), 2 mg/kg SID, parenteral or oral in piglets: Marbocyl®2% (marbofloxacin), 2 mg/kg SID; Baytril®0.5% (enrofloxacin), 1.7 mg/kg SID). Two piglets received a second FQ treatment after weaning (Baytril®0.5% (enrofloxacin), 1.7 mg/kg SID) but the remaining sows and their progeny did not receive any additional FQ treatment during study conduction apart from the initial FQ treatment. Other reported antimicrobials used during the study were sulfadoxin-trimethoprim (parenteral in sows), amoxicillin, benzylpenicillin and in combination with streptomycin (parenteral in piglets), colistin, sulfadimidine-sulfathiazole-trimethoprim, chlortetracycline and chlortetracycline-sulfadimidin-tylosin (oral during weaning or fattening). Sampled pigs were divided into five groups. In two groups (G1 and G3, summarized as Trt) either the dams or the piglets were treated with fluoroquinolones (G1: sows FQ treated, piglets not FQ treated; G3: sows not FQ treated, piglets FQ treated). On every farm sampling of contact pigs was performed forming groups G2 and G4 (contact sows and piglets not FQ treated, summarized as Ctat). Contact pigs are summarised as pigs not treated with FQs but held in the same group with FQ treated pigs, i.e. having direct contact, or pigs not treated with FQs but held in the same room or farm, i.e. having indirect contact. Control pigs from farms without FQ use for more than three months belonged to the fifth group (G5 = Ctrl: sows and piglets from FQ free farms). Information on group formation and farm structure are summarized in Table 1. Study designers did not have any influence on the distribution of group animals from the farrowing to the fattening units. Thus, one fattening unit received pigs belonging to the third group (G3) but no pigs belonging to the fourth group (G4).

group		code	FQ treatment of sows	FQ treatment of piglets	farrow-to-finish	farrowing	farrow and rearing	rearing and finishing	fattening
G1	Trt	S+P-	+	-	1	2 (SPS)	1 (SPS)	1 (SPS)	7 (7 SPS)
G2	Ctat	S-P-	-	-					
G3	Trt	S-P+	-	+			2 (1 SPS)		6 (5 SPS)
G4	Ctat	S-P-	-	-					5 (5 SPS)
G5	Ctrl	FQ free	-	-	2		1		3 (1 SPS)
TOTAL					**3**	**2 (SPS)**	**4 (2 SPS)**	**1 (SPS)**	**14 (8 SPS)***

Table 1 Group formation and distribution of farm structures: S = sows, P = piglets, + = FQ use, - = no FQ use, SPS = part of a sow-pool-system, * = 14 farms received weaners from different groups (G1-G5)

Fecal sampling

Farrowing farms were contacted after the sows' expected delivery date. When FQ treatment was reported, farms were visited approximately two weeks after farrowing. Sampling was performed in two different steps in the farrowing units: In farms with FQ use in sows three piglets from every sow (treated or untreated) were randomly picked and a pooled fecal sample of approximately 1g to 5g was taken rectally or during defecation. This procedure was used to ensure sufficient sample material for the following laboratory procedures. In farms with FQ use in piglets we were able to collect single fecal samples of the same amount described above. Contact and treated piglets were picked from the same litter if piglets were suffering from septic arthritis. Since farmers performed metaphylaxis (treating all piglets) in litters suffering from diarrhea, a different litter was selected for control sampling. Gloves were changed after each sample to avoid cross-contamination. Untreated and treated sows or piglets were always held in the same room or farm and direct contact between litters and sows was possible through pen barring. A numbered ear tag of contrasting color in the left ear and the four-digit number of the Swiss animal movement data base in the right ear ensured group and individual identification for the following samplings. Because of a low prevalence of ciprofloxacin resistance in *E. coli* from Swiss pigs and a study recommending pooled samples only if prevalence of resistance is > 2% we preferred to collect single fecal samples in the following samplings to record the pigs' individual courses [29, 30, 35]. Sampling was timed by the dates when pigs were moved to another facility to assess quinolone susceptibility status in every animal before and after movement: in the farrowing unit piglets were resampled at four weeks of age shortly before moving to the rearing unit. Subsequently, feces were collected at the end of rearing around ten weeks (weaners) and at least two weeks after moving to the fattening unit (fattening pigs at an age around twelve weeks). Because there had been no use of FQ for more than three months in the farrowing unit we forewent collecting piglet feces from group 5 (Figure 1).

Due to subsequent processing collected native feces were individually kept in a stool tube and stored at - 20°C on the same day.

All sampling procedures were approved by cantonal veterinary authorities (licence number: LU 03/14).

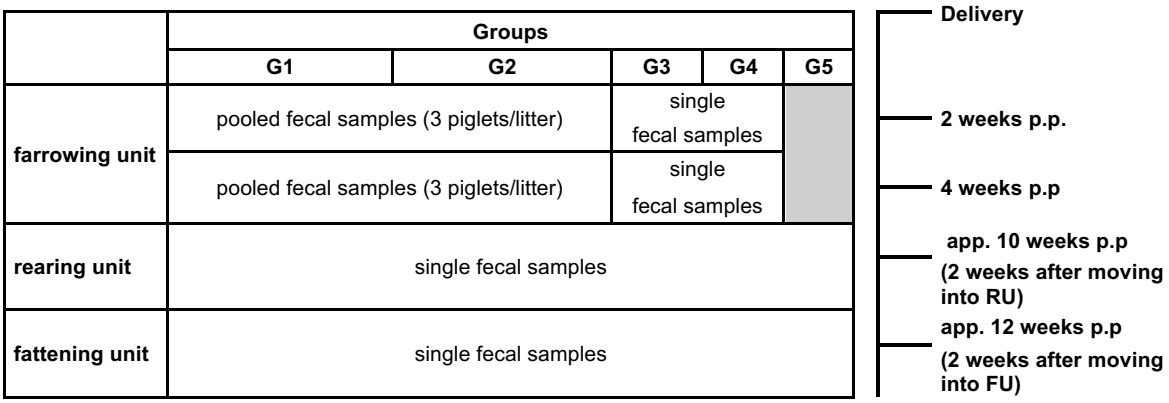

Figure 1 Fecal sampling: p.p. = postpartum, app. = approximately, RU = rearing unit, FU = fattening unit

Environmental sampling

On every farm an approximate surface size of 0.06 sqm of unclean floors and walls from pens with sampled pigs were wiped with a gauze sponge moistened with sterile 0.85% saline solution. This was performed during sampling of each age category (two times in farrowing units, once in rearing and twice in fattening units). Additionally, a dust sample was collected in the same procedure from different horizontal surfaces (e.g. window sills, feed or water pipelines and lids of piglet nests) near the pens. From the slurry pit samples at different depths and with different consistency were mixed in a bucket. A homogenous sample of approximately 300 to 500 ml was taken and stored at - 20°C. After animal movement and the farmers' individual cleaning procedures wipe and slurry samples were retaken. During the second visit in the farrowing barns (sampling of four-week old piglets) and in the fattening units with either continuous flow system or limited accessibility to slurry pits we forewent collecting a second slurry sample.

Laboratory methods

Samples were thawed at 7°C overnight and tested semi-quantitatively for the presence of non-susceptible *E. coli* to quinolone. Approximately 1 g or 1 ml of sample was diluted 1:10 with 0.85% saline solution and homogenized in a Stomacher® (Seward Stomacher® 400 Laboratory Blender BA 7021, West Sussex, UK). The homogenate was streaken in different dilutions by the pour plate method on selective Rapid-*E. coli* 2 agar plates (Biorad®, Munich, Germany) supplemented with 8 mg/L nalidixic acid, 10 mg/L vancomycin and 5 mg/L amphotericin B. After overnight incubation at 37°C presumptive positive *E. coli* colonies (β-D-glucuronidase and β-D-galactosidase positive, presented as purple and round colonies) were counted. Plates with a massive *E. coli* growth making counting impossible were given an approximate number of 100'000 counts per plate. In assessing the rate of resistance in an animal stock results from a study only slightly differed between testing a single or multiple isolates per fecal sample [36]. Hence, from each sample one *E. coli* colony was randomly picked and stocked. Isolates underwent disk diffusion (DD) susceptibility testing including

antibiotics (Becton Dickinson and company, Sparks, MD USA) nalidixic acid (NA30) and ciprofloxacin (CIP5). In isolates intermediate and resistant to nalidixic acid, the minimal inhibitory concentration (MIC in µg/ml) to nalidixic acid and ciprofloxacin was assessed using ETEST® strips (BioMérieux, Marcy l'Etoile, France). Performance and interpretation of susceptibility testing followed the guidelines of the Clinical and Laboratory Standards Institute [37]. Due to a lack of animal specific breakpoints, MICs were interpreted according to human pathogen specific breakpoints published by Clinical and Laboratory Standards Institute [37] and by epidemiological cut-offs (ECOFFs *Escherichia coli*; $ECOFF_{Nalidixic\ acid}$: 8 mg/L, $ECOFF_{ciprofloxacin}$: 0.064 mg/L) published by The European Committee on Antimicrobial Susceptibility Testing [38]. MIC values of nalidixic acid and ciprofloxacin were defined as intermediate resistant if lying between > 16 µg/ml and < 32 µg/ml and > 1 µg/ml and < 4 µg/ml, respectively.

Data analysis and statistical evaluation

Descriptive statistics and confidence intervals (95% CI)

Descriptive and inferential statistics were performed in IBM® SPSS® Statistics for Macintosh Version 25.0 and the software program R Version 3.5.1 [39]. QNSE counts were expressed as log CFU/g or ml except zero QNSE counts (expressed as 0 CFU/g or ml). The number of samples with detection of QNSE divided by the total number of tested samples is described as detection rate (%). FQ susceptibility proportions (%) of isolated *E. coli* are described. Binomial and multinomial confidence intervals (95% CI) were obtained using Jeffreys approach [40] and MultinomCI() from the package by DescTools [41]. Non overlapping confidence intervals were considered to be significantly different.

Mixed effects models

A hurdle Poisson mixed effects model was used to assess if the counts of quinolone non-susceptible *E. coli* (QNSE) in weaners and fattening pigs (using the original size scales, for further information view annex) differed between the groups Trt (treated pigs; G1 and G3), Ctat (contact pigs; G2 and G4) and Ctrl (control pigs; G5, FQ free) with the package GLMMadaptive [42]. Because we did not have any influence on the distribution of weaners into the rearing units, the rearing units were integrated as a random effect. The detection of at least one QNSE is described as the outcome, i.e. as a “count”. Samples with zero QNSE counts are described as “zero count” and were tested in the zero-count part. The groups Trt, Ctat and Ctrl are described as the predictor variables, i.e. as fixed effects of the models. The hurdle models comprise two parts: the zero-count part is considered to be binomial (logistic regression) and the positive-count part is treated as a Poisson distribution. Additionally, models with a zero-inflated negative binomial distribution were also tested. Model fit was assessed by likelihood ratio tests. Additionally the factor “connection to a sow-pool-system” was included as fixed effect. QNSE count results from the mixed effects models (original size scales) were transformed in log CFU/g.

Results

Demographic data of fecal samples (groups and age categories)

In this study we included 218 pigs of which eleven pigs could not be followed up until the fattening unit (three pigs died, one pig was euthanized, seven pigs were undetectable). The greater part (n = 117, 53.7%) was born at farms which were connected to a sow-pool-system (SPS). Overall 621 fecal samples (116 fecal samples from two-week old piglets, 104 from four-week old piglets, 206 from pigs during rearing and 195 from fattening pigs) were tested and used for further analysis. In 40.9% (254/621) of fecal samples quinolone non-susceptible *E. coli* (QNSE) were detected (Table 2). Two-week old piglets showed the highest

		Groups					
		G1	G2	G3	G4	G5	TOTAL
FARU	2w p.p.	94.4% (17/18), p CI = 76.8 - 99.4% x_0 = 1 ø = 7.0 m = 6.6	100.0% (18/18), p CI = 87.1 - 100.0% x_0 = 0 ø = 6.7 m = 5.8	92.5% (37/40) CI = 81.3 - 97.9% x_0 = 3 ø = 7.1 m = 5.6	92.5% (37/40) CI = 81.3 - 97.9% x_0 = 3 ø = 5.8 m = 4.6		94.0% (109/116) CI = 88.5 - 97.3% x_0 = 7 ø = 6.9 m = 5.7
	4w p.p.	94.1% (16/17), p CI = 75.6 - 99.4% x_0 = 1 ø = 6.4 m = 5.1	91.7% (11/12), p CI = 67.1 - 99.1% x_0 = 1 ø = 6.4 m = 6.3	72.5% (29/40) CI = 57.4 - 84.5% x_0 = 11 ø = 5.4 m = 3.8	91.4% (32/35) CI = 78.8 - 97.6% x_0 = 3 ø = 5.1 m = 3.8		84.6% (88/104) CI = 76.7 - 90.6% x_0 = 16 ø = 5.9 m = 4.3
RU		13.3% (6/45) CI = 5.7 - 25.5% x_0 = 39 ø = 2.3 m = 0.0	15.4% (6/39) CI = 6.6 - 29.0% x_0 = 33 ø = 3.1 m = 0.0	10.8% (4/37) CI = 3.7 - 23.7% x_0 = 33 ø = 1.0 m = 0.0	2.5% (1/40) CI = 0.2 - 11.1% x_0 = 39 ø = 3.4 m = 0.0	11.1% (5/45) CI = 4.3 - 22.7% x_0 = 40 ø = 1.7 m = 0.0	10.7% (22/206) CI = 7.0 - 15.5% x_0 = 184 ø = 2.9 m = 0.0
FU		23.8% (10/42) CI = 12.9 - 38.2% x_0 = 32 ø = 2.9 m = 0.0	14.3% (6/42) CI = 6.1 - 27.1% x_0 = 36 ø = 2.3 m = 0.0	29.6% (8/27) CI = 15.1 - 48.3% x_0 = 19 ø = 2.2 m = 0.0	25.0% (9/39) CI = 12.0 - 38.0% x_0 = 30 ø = 2.1 m = 0.0	4.4% (2/45) CI = 0.9 - 13.6% x_0 = 43 ø = 1.9 m = 0.0	15.6% (35/195) CI = 13.0 - 23.8% x_0 = 160 ø = 2.5 m = 0.0
TOTAL		40.2% (49/122) CI = 31.7 - 49.1% x_0 = 73 ø = 6.3 m = 0.0	36.9% (41/111) CI = 28.3 - 46.2% x_0 = 70 ø = 6.1 m = 0.0	54.2% (78/144) CI = 46.0 - 62.2% x_0 = 66 ø = 6.6 m = 100.0	51.3% (79/154) CI = 43.4 - 59.2% x_0 = 75 ø = 5.3 m = 100.0	7.8% (7/90) CI = 3.5 - 14.7% x_0 = 83 ø = 1.8 m = 0.0	40.9% (254/621) CI = 37.0 - 44.9% x_0 = 367 ø = 6.2 m = 0.0

Table 2 Detection rates and corresponding 95% confidence intervals (CI), x_0 = number of samples with zero quinolone non-susceptible *E. coli* detected, mean (= ø) and median (= m) log colony forming unit per gram feces (log CFU/g) of quinolone non-susceptible *E.coli* in fecal samples from pigs of different age and group, median = 0.0 were expressed in colony forming unit per gram feces (CFU/g), FARU = farrowing unit, 2/4w p.p. = two and four weeks postpartum, RU = rearing unit, FU = fattening unit, p = pooled samples

detection rate (94.0%, 109/116) followed by four-week old piglets (84.6%, 88/104), fattening pigs (15.6%, 35/195) and weaners (10.7%, 22/206). Confidence intervals differed between piglets (two- and four-week old, 95% CI = 76.7 - 97.3%) and weaners (95% CI = 7.0 - 15.5%) and piglets and fattening pigs (95% CI = 13.0-23.8%), respectively. Detection rate of QNSE was lower in samples of weaners (10.7%, 95% CI = 7.0 - 15.5%) compared to samples of fattening pigs (15.6%, 95% CI = 13.0 - 23.8%) but 95% CI did not differ significantly. QNSE were found to be existent in weaners and fattening pigs from farms without FQ use (G5 = 7.8%, 7/90). Over all ages, the detection rate of QNSE was significantly lowest in G5 (7.8%, 95% CI = 3.5 - 14.7%) compared to G1 to G4 (36.9-54.2%, 95% CI = 28.3 - 62.2%). In the farrowing unit contact animals (G2 and G4) showed similar or higher detection rates compared to treated piglets and piglets from treated sows (G3 and G1) but with overlapping 95% CI. In the rearing unit detection rates decreased in all groups (G1-G4) but reincreased in three groups (G1, G3, G4) two to ten times during the fattening unit. In group 5 no increase of the detection rate was observed between the rearing and the fattening unit. However, there were no significant differences concerning the 95% CI between groups 1 to 5 comparing

age. Detection rates in weaners (SPS+: 11.8%, 95% CI: 6.7 - 18.9%, SPS-: 9.4%, 95% CI: 4.7 - 16.5%) and fattening pigs (SPS+: 15.7%, 95% CI: 9.6 - 23.7%, SPS-: 20.4%, 95% CI: 13.2 - 29.5%) did not differ depending on the connection to a SPS. From piglets to fattening pigs there is a decrease in mean and median log counts of quinolone non-susceptible *E. coli* colony forming units per gram feces (log CFU/g feces). Highest means were detected in two-week old piglets from G3 (ø = 7.1 log CFU/g feces) and G1 (ø = 7.0 log CFU/g feces). In the rearing and fattening unit, the lowest means were observed in G3 (ø = 1.0 log CFU/g feces) and G5 (ø = 1.9 log CFU/g feces). According to the route of antibiotic application during suckling period (intramuscularly vs. perorally) detection rates did not differ significantly (overlapping 95% CIs) in the farrowing (two-week old piglets: 93.8% (i.m., 95% CI = 74.3 - 99.4%) and 91.7% (p.o., 95% CI = 75.8 - 98.3%), four-week old piglets: 87.5% (i.m., 95% CI = 65.5 - 97.4%) and 62.5% (p.o., 95% CI = 42.6 - 79.6%)), rearing (weaner: 13.3% (i.m., 95% CI = 2.8 - 36.4%) and 9.1% (p.o., 95% CI = 1.9 - 26.1%)) and fattening unit (fattening pigs: 12.5% (i.m., 95% CI = 1.3 - 45.4%) and 36.8% (p.o., 95% CI = 18.2 - 59.2%)).

Quantitative and qualitative detection of QNSE – hurdle models

The factor “connection to a sow-pool-system” was excluded after the hurdle models were not converging including this factor. The lowest means of colony forming units per gram feces in weaners and fattening pigs were observed in group Ctrl (control group; G5, weaner: ø = 1.7 log CFU/g feces; fattening pig: ø = 1.9 log CFU/g feces) compared to group Trt (treated pigs; G1 and G3, weaner: ø = 2.1 log CFU/g feces; fattening pig: ø = 2.8 log CFU/g feces) and group Ctat (contact pigs; G2 and G4, weaner: ø = 3.3 log CFU/g feces; fattening pig: ø = 2.2 log CFU/g feces). The Poisson hurdle model with random effects indicated significant differences in the count part, i.e. in quantitative detection of QNSE significant differences between groups were observed in weaners and fattening pigs (highlighted with an asterisk in Table 3). In the zero-part, i.e. detection of QNSE versus no detection of QNSE, no significant differences were observed between the three groups in weaners. In fattening pigs, values showed large standard errors and therefore were not plausible. Concerning the means and count parts between weaners and fattening pigs, there is a significant increase in log colony forming units per gram feces in group Trt and Ctrl. Group Ctat showed

		group		
		Trt	**Ctat**	**Ctrl**
weaner	**mean**	2.1	3.3	1.7
	x_0	72	72	40
	count part (CI 95%)	1.888 (1.877-1.899)*	3.757 (3.754-3.760)*	1.707 (1.689-1.725)*
	zero part (CI 95%)	1.522 (1.013-2.033)	1.519 (1.009-2.029)	1.497 (0.991-2.003)
fattening pig	**mean**	2.8	2.2	1.9
	x_0	51	66	43
	count part (CI 95%)	3.378 (3.373-3.383)*	2.800 (2.791-2.809)*	1.936 (1.922-1.949)*
	zero part (CI 95%)	4.3E-04# (8.6E-05-2.2E-03)	1.1E-03# (2.2E-04-5.2E-03)	1.3E+04# (3.1E+03-5.7E+04)

Table 3 Hurdle models: x_0 = number of samples with zero QNSE detected, mean, hurdle models with count and zero part and confidence intervals (CI 95%) in log CFU/g feces. Trt = treated group (G1 and G3), Ctat = contact group (G2 and G4), Ctrl = control group (G5), * = significant values (not overlapping confidence intervals), # = indicates not convertible hurdle models with large and non-useful standard errors

significantly lower counts of log colony forming units per gram feces in fattening pigs compared to weaners. For further information view Table 3.

Minimal inhibitory concentration (MIC) data of fecal samples

Nalidixic acid (NA)

Minimal inhibitory concentration was tested in 254 isolates of which the MIC 50% and 90% of NA were > 256 µg/ml. Except for three isolates all of the piglets' isolates of G3 and G4 achieved MICs between 256 and > 256 µg/ml. Isolates' MICs of G1 and G2 showed a wider range (24 - > 256 µg/ml) in piglets. Observing all ages isolates of G3 (n = 78), G4 (n = 79) and G5 (n = 7) reached the same MIC 50% and 90% (> 256 µg/ml). Further information can be obtained from Table 4.

Out of all fecal isolates 98.4% (250/254) were resistant to nalidixic acid according to CLSI guidelines. Four isolates (1.6%) showed intermediate resistant results (Table 5).

group and age	concentrations of nalidixic acid in µg/ml								TOTAL	MIC 50%	MIC 90%
	24	48	64	96	128	192	256	>256			
G1 all ages	2	0	0	5	3	6	13	20	**49**	**256**	**>256**
G1 piglet2w	0	0	0	4	3	1	6	3	17	256	>256
G1 piglet4w	1	0	0	1	0	4	1	9	16	>256	>256
G1 weaner	1	0	0	0	0	0	1	4	6	>256	>256
G1 fattening pig	0	0	0	0	0	1	5	4	10	>256	>256
G2 all ages	1	0	1	4	4	6	11	14	**41**	**256**	**>256**
G2 piglet2w	0	0	1	2	2	3	3	7	18	256	>256
G2 piglet4w	1	0	0	2	2	1	1	4	11	192	>256
G2 weaner	0	0	0	0	0	2	4	0	6	256	256
G2 fattening pig	0	0	0	0	0	0	3	3	6	>256	>256
G3 all ages	1	1	0	0	0	0	14	62	**78**	**>256**	**>256**
G3 piglet2w	1	0	0	0	0	0	1	35	37	>256	>256
G3 piglet4w	0	1	0	0	0	0	5	23	29	>256	>256
G3 weaner	0	0	0	0	0	0	0	4	4	>256	>256
G3 fattening pig	0	0	0	0	0	0	8	0	8	256	256
G4 all ages	0	0	1	0	0	0	35	43	**79**	**>256**	**>256**
G4 piglet2w	0	0	1	0	0	0	16	20	37	>256	>256
G4 piglet4w	0	0	0	0	0	0	11	21	32	>256	>256
G4 weaner	0	0	0	0	0	0	0	1	1	>256	>256
G4 fattening pig	0	0	0	0	0	0	8	1	9	256	>256
G5 all ages	0	0	0	0	0	0	1	6	**7**	**>256**	**>256**
G5 weaner	0	0	0	0	0	0	1	4	5	>256	>256
G5 fattening pig	0	0	0	0	0	0	0	2	2	>256	>256
TOTAL	**4 (1.6%)**	**1 (0.4%)**	**2 (0.8%)**	**9 (3.5%)**	**7 (2.8%)**	**12 (4.7%)**	**74 (29.1%)**	**145 (57.1%)**	**254 (100%)**	**>256**	**>256**

Table 4 Nalidixic acid MIC distribution by group and age: Numbers indicate the number of strains exhibiting the corresponding MIC value. Yellow and red areas indicate the intermediate and resistant isolates, respectively. Breakpoints were obtained from the CLSI guidelines 2017 for human breakpoints. MIC 50% and MIC 90% represent the concentration of nalidixic acid (µg/ml) inhibiting growth of 50% or 90% of strains, respectively. Piglet2w = two-week old piglet, piglet4w = four-week old piglet

group	CLSI		EUCAST		decreased susceptibility	TOTAL
	I (CI 95%)	R (CI 95%)	WT (CI 95%)	M (CI 95%)	DS	
G1	4.1%, n = 2 (0.8 - 12.5%)	95.9%, n = 47 (87.5 - 99.2%)	0.0%, n = 0 (0.0 - 5.0%)	100.0%, n = 49 (95.0 - 100.0%)	4.1%, n = 2 (0.8 - 12.5%)	49 (19.3%)
G2	2.4%, n = 1 (0.2 - 10.9%)	97.6%, n = 40 (89.1 - 99.8%)	0.0%, n = 0 (0.0 - 6.0%)	100.0%, n = 41 (94.0 - 100.0%)	2.4%, n = 1 (0.2 - 10.9%)	41 (16.1%)
G3	1.3%, n = 1 (0.1 - 5.9%)	98.7%, n = 77 (94.1 - 99.9%)	0.0%, n = 0 (0.0 - 3.2%)	100.0%, n = 78 (96.8 - 100.0%)	1.3%, n = 1 (0.1 - 5.9%)	78 (30.7%)
G4	0.0%, n = 0 (0.0 - 3.2%)	100.0%, n = 79 (96.8 - 100.0%)	0.0%, n = 0 (0.0 - 3.2%)	100.0%, n = 79 (96.8 - 100.0%)	0.0%, n = 0 (0.0 - 3.2%)	79 (31.1%)
G5	0.0%, n = 0 (0.0 - 29.3%)	100.0%, n = 7 (70.7 - 100.0%)	0.0%, n = 0 (0.0 - 29.3%)	100.0%, n = 7 (70.7 - 100.0%)	0.0%, n = 0 (0.0 - 29.3%)	7 (2.8%)
TOTAL	**1.6%, n = 4 (0.5 - 3.8%)**	**98.4%, n = 250 (96.2 - 99.5%)**	**0%, n = 0 (0.0 - 1.0%)**	**100%, n = 254 (99.0 - 100.0%)**	**1.6%, n = 4 (0.5 - 3.8%)**	**254 (100%)**

Table 5 Nalidixic acid MICs interpretation: Proportions (%) and the corresponding confidence intervals in brackets. N = number of strains. Yellow and red areas indicate the intermediate and resistant isolates according to the CLSI guidelines 2017 for human breakpoints. WT and M indicate the numbers of strains classified as wildtype or mutant strain according to the EUCAST guidelines 2019. Strains with decreased susceptibility are strains lying between the wildtype's MIC (EUCAST) and the resistant MIC (CLSI).

Ciprofloxacin (CIP)

MIC 50% and 90% of all the tested isolates were 0.38 and > 32 µg/ml ciprofloxacin. In isolates of G3 a markedly higher MIC 50% (8 µg/ml) was observed compared to G1, G2, G4 and G5 (0.125 - 0.25 µg/ml). Considering age categories piglets' and weaners' MIC 50% is more beneficial in isolates of G1, G2 and G5 compared to G3 and G4. In fattening pigs MIC 50% of all groups (G1-G5) decreased to 0.19 µg/ml. Overall 128 isolates were susceptible to ciprofloxacin (50.4%). The major part of isolates susceptible to ciprofloxacin (103/128, 80.5%) showed MICs lying between 0.125 and 0.19 µg/ml (Table 7, see next page). According to the wildtype's MIC (0.064 µg/ml) by EUCAST decreased susceptibility to ciprofloxacin was observed in 133 isolates (133/134, 99.3%). Ciprofloxacin resistant isolates were mostly represented in G3 and G4 (60/120, 50.0% and 36/120, 30.0%). Only seven quinolone resistant isolates were observed in G5, two isolates showed intermediate and complete resistance against ciprofloxacin (MIC = 3 and 4 µg/ml). All seven isolates originated from the same farm on which the last FQ usage was carried out three months ago. Further information can be obtained from Table 6.

group	CLSI			EUCAST		decreased susceptibility	TOTAL
	S (CI 95%)	I (CI 95%)	R (CI 95%)	WT (CI 95%)	M (CI 95%)	DS	
G1	69.4%, n = 34 (55.5 - 80.5%)	6.1%, n = 3 (2.1 - 16.5%)	24.5%, n = 12 (14.6 - 38.1%)	2.1%, n = 1 (0.2 - 9.2%)	97.9%, n = 48 (90.8 - 99.8)	73.5%, n = 36 (60.0 - 84.3%)	49 (19.3%)
G2	70.8%, n = 29 (55.5 - 82.4%)	2.4%, n = 1 (0.4 - 12.6%)	26.8%, n = 11 (15.7 - 41.9%)	0.0%, n = 0 (0.0 - 6.0%)	100.0%, n = 41 (94.0 - 100.0%)	73.2%, n = 30 (58.3 - 84.9%)	41 (16.1%)
G3	23.1%, n = 18 (15.1 - 33.6%)	0.0%, n = 0 (0.0 - 4.7%)	76.9%, n = 60 (66.4 - 84.9%)	0.0%, n = 0 (0.0 - 3.2%)	100.0%, n = 78 (96.8 - 100.0%)	23.1%, n = 18 (14.8 - 33.3%)	78 (30.7%)
G4	53.2%, n = 42 (42.3 - 63.8)	1.3%, n = 1 (0.2 - 6.8)	45.6%, n = 36 (35.0 - 56.5%)	0.0%, n = 0 (0.0 - 3.2%)	100.0%, n = 79 (96.8 - 100.0%)	54.4%, n = 43 (43.4 - 65.1%)	79 (31.1%)
G5	71.4%, n = 5 (35.9 - 91.8%)	14.3%, n = 1 (2.6 - 51.3%)	14.3%, n = 1 (2.6 - 51.3%)	0.0%, n = 0 (0.0 - 29.3%)	100.0%, n = 7 (70.7 - 100.0%)	85.7%, n = 6 (49.9 - 98.5%)	7 (2.8%)
TOTAL	**50.4%, n = 128 (44.3 - 56.5%)**	**2.4%, n = 6 (1.2 - 5.1%)**	**47.2%, n = 120 (41.2 - 53.4%)**	**0.4%, n = 1 (0.0 - 1.9%)**	**99.6%, n = 253 (98.1 - 100.0%)**	**52.4%, n = 133 (46.2 - 58.5%)**	**254 (100%)**

Table 6 Ciprofloxacin MICs interpretation: Proportions (%) and the corresponding 95% confidence intervals in brackets (). N = number of strains. Light green, yellow and red areas indicate the sensible, intermediate and resistant isolates according to the CLSI guidelines 2017 for human breakpoints. WT and M indicate the numbers of strains classified as wildtype or mutant strain according to the EUCAST guidelines 2019. Strains with decreased susceptibility are strains lying between the wildtype's MIC (EUCAST) and the resistant MIC (CLSI).

group and age	concentrations of ciprofloxacin in µg/ml														TOTAL	MIC 50%	MIC 90%
	0.047	0.094	0.125	0.19	0.25	0.38	3	4	6	8	12	24	32	>32			
G1 all ages	1	5	22	6	0	0	3	3	3	2	0	0	1	3	**49**	**0.125**	**8**
G1 piglet2w	0	1	11	2	0	0	1	0	0	0	0	0	0	2	17	0.125	>32
G1 piglet4w	0	4	4	1	0	0	2	2	1	0	0	0	1	1	16	0.19	>32
G1 weaner	1	0	2	1	0	0	0	1	1	0	0	0	0	0	6	0.19	6
G1 fattening pig	0	0	5	2	0	0	0	0	1	2	0	0	0	0	10	0.19	8
G2 all ages	0	8	14	6	1	0	1	3	2	1	0	0	2	3	**41**	**0.125**	**32**
G2 piglet2w	0	2	7	2	0	0	1	2	1	0	0	0	0	3	18	0.19	>32
G2 piglet4w	0	5	2	0	1	0	0	1	0	0	0	0	2	0	11	0.125	32
G2 weaner	0	0	4	2	0	0	0	0	0	0	0	0	0	0	6	0.125	0.19
G2 fattening pig	0	1	1	2	0	0	0	0	1	1	0	0	0	0	6	0.19	8
G3 all ages	0	0	6	11	1	0	0	1	19	12	0	2	9	17	**78**	**8**	**>32**
G3 piglet2w	0	0	2	0	0	0	0	1	4	10	0	2	7	11	37	24	>32
G3 piglet4w	0	0	2	6	0	0	0	0	14	2	0	0	1	4	29	6	>32
G3 weaner	0	0	0	0	0	0	0	0	1	0	0	0	1	2	4	>32	>32
G3 fattening pig	0	0	2	5	1	0	0	0	0	0	0	0	0	0	8	0.19	0.25
G4 all ages	0	5	13	20	2	2	1	0	18	4	1	0	0	13	**79**	**0.25**	**>32**
G4 piglet2w	0	5	5	8	0	2	0	0	2	4	1	0	0	10	37	0.38	>32
G4 piglet4w	0	0	7	5	1	0	1	0	15	0	0	0	0	3	32	6	32
G4 weaner	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	6	6
G4 fattening pig	0	0	1	7	1	0	0	0	0	0	0	0	0	0	9	0.19	0.25
G5 all ages	0	0	2	3	0	0	1	1	0	0	0	0	0	0	**7**	**0.19**	**4**
G5 weaner	0	0	1	2	0	0	1	1	0	0	0	0	0	0	5	0.19	4
G5 fattening pig	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0.19	0.19
TOTAL	**1 (0.4%)**	**18 (7.1%)**	**57 (22.4%)**	**46 (18.1%)**	**4 (1.6%)**	**2 (0.8%)**	**6 (16.5%)**	**8 (3.1%)**	**42 (16.5%)**	**19 (7.5%)**	**1 (0.4%)**	**2 (0.8%)**	**12 (4.7%)**	**36 (14.2%)**	**254 (100%)**	**0.38**	**>32**

Table 7 Ciprofloxacin MIC distribution by group and age: Numbers indicate the number of strains exhibiting the corresponding MIC value. Light green, yellow and red areas indicate the sensible, intermediate and resistant isolates, respectively. Breakpoints were obtained from the CLSI guidelines 2017 for human breakpoints. MIC 50% and MIC 90% represent the concentration of ciprofloxacin (µg/ml) inhibiting growth of 50% or 90% of strains, respectively. Piglet2w = two-week old piglet, piglet4w = four-week old piglet

NA and CIP susceptibility proportions (groups and age categories)

Nalidixic acid susceptibility proportions were similar in weaners and fattening pigs of G1 to G4 and Trt (S = 0.0%, 95% CI = 0.0 - 85.4%, R = 100.0%, 95% CI = 14.6 - 100.0%), Ctat and Ctrl (S = 0.0%, 95% CI = 0.0 - 66.7%, R = 100.0%, 95% CI = 33.3 - 100.0%). Ciprofloxacin susceptibility proportions in weaners of G2 (S = 100.0%, 95% CI = 66.9 - 100.0%, R = 0.0%, 95% CI = 0.0 - 33.1%) and G3 (S = 0.0%, 95% CI = 0.0 - 44.5%, R = 100.0%, 95% CI = 55.5 - 100.0%) differed significantly but in group Trt, Ctat and Ctrl (S = 40.0 - 85.7%, 95% CI = 15.3 - 98.5%, R = 14.3 - 60.0%, 95% CI = 1.5 - 84.7%) no significantly different susceptibility proportions were observed. In fattening pigs of G1 to G5 (S = 66.7 - 100.0%, 95% CI = 28.6 - 100.0%, R = 0.0 - 33.3%, 95% CI = 0.0 - 71.4%) and Trt, Ctat and Ctrl (S = 83.3 - 100.0%,

	G1	G2	G3	G4	G5
NA weaner	S = 0.0%, n = 0 (0.0 - 33.1%) R = 100.0%, n = 6 (66.9 - 100.0%)	S = 0.0%, n = 0 (0.0 - 33.1%) R = 100.0%, n = 6 (66.9 - 100.0%)	S = 0.0%, n = 0 (0.0 - 44.5%) R = 100.0%, n = 4 (55.5 - 100.0%)	S = 0.0%, n = 0 (0.0 - 85.4%) R = 100.0%, n = 1 (14.6 - 100.0%)	S = 0.0%, n = 0 (0.0 - 38.0%) R = 100.0%, n = 5 (62.0 - 100.0%)
NA fattening pig	S = 0.0%, n = 0 (0.0 - 21.8%) R = 100.0%, n = 10 (78.2 - 100.0%)	S = 0.0%, n = 0 (0.0 - 33.1%) R = 100.0%, n = 6 (66.9 - 100.0%)	S = 0.0%, n = 0 (0.0 - 26.3%) R = 100.0%, n = 8 (73.7 - 100.0%)	S = 0.0%, n = 0 (0.0 - 23.8%) R = 100.0%, n = 9 (76.2 - 100.0%)	S = 0.0%, n = 0 (0.0 - 66.7%) R = 100.0%, n = 2 (33.3 - 100.0%)
CIP weaner	S = 66.7%, n = 4 (28.6 - 92.4%) R = 33.3%, n = 2 (7.6 - 71.4%)	S = 100.0%, n = 6 (66.9 - 100.0%) R = 0.0%, n = 0 (0.0 - 33.1%)	S = 0.0%, n = 0 (0.0 - 44.5%) R = 100.0%, n = 4 (55.5 - 100.0%)	S = 0.0%, n = 0 (0.0 - 85.4%) R = 100.0%, n = 1 (14.6 - 100.0%)	S = 60.0%, n = 3 (20.9 - 90.6%) R = 40.0%, n = 2 (9.4 - 79.1%)
CIP fattening pig	S = 70.0%, n = 7 (39.4 - 90.8%) R = 30.0%, n = 3 (9.2 - 60.6%)	S = 66.7%, n = 4 (28.6 - 92.4%) R = 33.3%, n = 2 (7.6 - 71.4%)	S = 100.0%, n = 8 (73.7 - 100.0%) R = 0.0%, n = 0 (0.0 - 26.3%)	S = 100.0%, n = 9 (76.2 - 100.0%) R = 0.0%, n = 0 (0.0 - 23.8%)	S = 100.0%, n = 2 (33.3 - 100.0%) R = 0.0%, n = 0 (0.0 - 66.7%)
	Trt	**Ctat**	**Ctrl**		
NA weaner	S = 0.0%, n = 0 (0.0 - 21.8%) R = 100.0%, n = 10 (78.2 - 100.0%)	S = 0.0%, n = 0 (0.0 - 29.3%) R = 100.0%, n = 7 (70.7 - 100.0%)	S = 0.0%, n = 0 (0.0 - 38.0%) R = 100.0%, n = 5 (62.0 - 100.0%)		
NA fattening pig	S = 0.0%, n = 0 (0.0 - 12.9%) R = 100.0%, n = 18 (87.1 - 100.0%)	S = 0.0%, n = 0 (0.0 - 15.2%) R = 100.0%, n = 15 (84.8 - 100.0%)	S = 0.0%, n = 0 (0.0 - 66.7%) R = 100.0%, n = 2 (33.3 - 100.0%)		
CIP weaner	S = 40.0%, n = 4 (15.3 - 69.7%) R = 60.0%, n = 6 (30.3 - 84.7%)	S = 85.7%, n = 6 (49.9 - 98.5%) R = 14.3%, n = 1 (1.5 - 50.1%)	S = 60.0%, n = 3 (20.9 - 90.6%) R = 40.0%, n = 2 (9.4 - 79.1%)		
CIP fattening pig	S = 83.3%, n = 15 (61.8 - 95.1%) R = 16.7%, n = 3 (4.9 - 38.2%)	S = 86.7%, n = 13 (63.6 - 97.2%) R = 13.3%, n = 2 (2.8 - 36.4%)	S = 100.0%, n = 2 (33.3 - 100.0%) R = 0.0%, n = 0 (0.0 - 66.7%)		
	part of SPS	**not part of SPS**			
NA weaner	S = 0.0%, n = 0 (0.0 - 17.3%) R = 100.0%, n = 13 (82.7 - 100.0%)	S = 0.0%, n = 0 (0.0 - 23.8%) R = 100.0%, n = 9 (76.2 - 100.0%)			
NA fattening pig	S = 0.0%, n = 0 (0.0 - 14.4%) R = 100.0%, n = 16 (85.6 - 100.0%)	S = 0.0%, n = 0 (0.0 - 12.3%) R = 100.0%, n = 19 (87.7 - 100.0%)			
CIP weaner	S = 61.5%, n = 8 (35.0 - 83.6%) R = 38.5%, n = 5 (16.4 - 65.0%)	S = 44.4%, n = 4 (17.2 - 74.6%) R = 55.6%, n = 5 (25.4 - 82.8%)			
CIP fattening pig	S = 68.7%, n = 11 (44.4 - 87.0%) R = 31.3%, n = 5 (13.0 - 55.6%)	S = 100.0%, n = 19 (87.7 - 100.0%) R = 0.0%, n = 0 (0.0 - 12.3%)			

Table 8 Minimal inhibitory concentrations (µg/ml) of nalidixic acid (NA) and ciprofloxacin (CIP) are classified as sensible (S) and resistant (intermediate and resistant summarized = R) isolates according to CLSI guidelines 2017 for human breakpoints with the corresponding 95% confidence intervals in brackets (), n = number of isolates originating from weaners and fattening pigs, SPS = sow-pool-system

95% CI = 33.3 - 100.0%, R = 0.0 - 16.7%, 95% CI = 0.0 - 66.7%) no differences were observed (Table 8).
NA and CIP susceptibility proportions (connection to sow-pool-system)

Fattening pigs originating from farms connected to a sow-pool-system had a significantly higher ciprofloxacin resistance rate (R = 31.3%, 95% CI = 13.0 - 55.6%) compared to fattening pigs of farms without SPS-connection (R = 0.0%, 95% CI = 0.0 - 12.3%). For further information see table 8.

Demographic data of environmental samples

130 wipe samples (67 dust and 63 pen samples) and 49 slurry samples were tested (179 environmental samples) and used for further analysis. Of all environmental samples 14.0% (25/179) showed growth of QNSE. Floor and wall wipes reached the highest detection rate (23.8%, 15/63), followed by slurry (18.4%, 9/49) and dust (1.5%, 1/67). In the farrowing unit 29.4% (10/34) of the environmental samples contained QNSE. From farrowing to fattening unit the detection rate declined (33.3% to 10.7%). After individual cleaning in the fattening unit, in four of 50 environmental samples (8.0%) QNSE were detected. The environmental samples of the farrowing unit reached the highest means (ø = 3.1 log CFU/g feces, ø = 3.7 log CFU/g feces) (Table 9).

		environmental samples			TOTAL
		dust	floor and wall wipes	slurry	
FARU	2w p.p.	0.0% (0/8) CI = 0.0 - 26.3% x_0 = 8	66.7% (4/6) CI = 28.6 - 92.4% x_0 = 2 ø = 3.0 m = 3.0	50.0% (2/4) CI = 12.2 - 87.8% x_0 = 2 ø = 3.6 m = 2.0	33.3% (6/18) CI = 15.2 - 56.3% x_0 = 12 ø = 3.1 m = 0.0
	4w p.p.	11.1% (1/9) CI = 1.2 - 41.5% x_0 = 8 ø = 3.9 m = 0.0	42.9% (3/7) CI = 13.8 - 76.6% x_0 = 4 ø = 3.2 m = 0.0		25.0% (4/16) CI = 9.0 - 49.1% x_0 = 12 ø = 3.7 m = 0.0
RU		0.0% (0/13) CI = 0.0 - 23.8% x_0 = 13	7.7% (1/13) CI = 0.8 - 30.8% x_0 = 12 ø = 0.9 m = 0.0	30.8% (4/13) CI = 11.3 - 57.8% x_0 = 9 ø = 3.0 m = 0.0	12.8% (5/39) CI = 5.0 - 25.9% x_0 = 34 ø = 2.5 m = 0.0
FU		0.0% (0/19) CI = 0.0 - 12.3% x_0 = 19	21.1% (4/19) CI = 7.5 - 42.7% x_0 = 15 ø = 2.0 m = 0.0	11.1% (2/18) CI = 2.3 - 31.2% x_0 = 16 ø = 2.5 m = 0.0	10.7% (6/56) CI = 4.5 - 20.8% x_0 = 50 ø = 2.2 m = 0.0
FU after cleaning		0.0% (0/18) CI = 0.0 - 12.9% x_0 = 18	16.7% (3/18) CI = 4.9 - 38.2% x_0 = 15 ø = 3.0 m = 0.0	7.1% (1/14) CI = 0.7 - 28.9% x_0 = 13 ø = 1.2 m = 0.0	8.0% (4/50) CI = 2.7 - 18.0% x_0 = 46 ø = 2.6 m = 0.0
TOTAL		1.5% (1/67) CI = 0.1 - 6.8% x_0 = 66 ø = 3.0 m = 0.0	23.8% (15/63) CI = 14.6 - 35.4% x_0 = 48 ø = 2.8 m = 0.0	18.4% (9/49) CI = 9.4 - 30.9% x_0 = 40 ø = 2.8 m = 0.0	14.0% (25/179) CI = 9.4 - 19.7% x_0 = 154 ø = 2.9 m = 0.0

Table 9 Detection rates and corresponding 95% confidence intervals (CI), x_0 = number of samples with zero QNSE detected, mean (= ø) and median (= m) log colony forming unit per gram dust or wipe or per milliliter slurry (log CFU/g or log CFU/ml) of QNSE in environmental samples from different farm units, median = 0.0 were expressed in colony forming unit per gram feces (CFU/g), FARU = farrowing unit, 2/4w p.p. = two and four weeks postpartum, RU = rearing unit, FU = fattening unit

Minimal inhibitory concentration (MIC) data of environmental samples

sample	concentrations of nalidixic acid in µg/ml						TOTAL	MIC 50%	MIC 90%
	24	32	64	96	256	>256			
dust	0	0	1	0	0	0	1	64	64
wipes	2	1	0	1	2	9	15	>256	>256
slurry	1	0	0	0	4	4	9	256	>256
TOTAL	**3 (12.0%)**	**1 (4.0%)**	**1 (4.0%)**	**1 (4.0%)**	**6 (24.0%)**	**13 (52.0%)**	**25 (100%)**	**>256**	**>256**

	concentrations of ciprofloxacin in µg/ml												
	0.047	0.125	0.19	0.25	0.38	4	6	8	24	>32			
dust	0	0	0	1	0	0	0	0	0	0	1	0.25	0.25
wipes	1	0	5	0	1	2	1	2	1	2	15	4	>32
slurry	0	4	2	1	0	0	0	0	0	2	9	0.19	>32
TOTAL	**1 (3.1%)**	**4 (12.5%)**	**7 (34.4%)**	**2 (15.6%)**	**1 (3.1%)**	**2 (6.3%)**	**1 (3.1%)**	**2 (6.3%)**	**1 (3.1%)**	**4 (12.5%)**	**25 (100%)**	**0.25**	**>32**

Table 10 Nalidixic acid and ciprofloxacin MIC distribution by sample: Numbers indicate the number of strains exhibiting the corresponding MIC value. Light green, yellow and red areas indicate the sensible, intermediate and resistant isolates, respectively. Breakpoints were obtained from the CLSI guidelines 2017 for human breakpoints. MIC50 and MIC90 represent the concentration of nalidixic acid inhibiting growth of 50% or 90% of strains, respectively.

MIC 50% and 90% of nalidixic acid for all environmental isolates reached the upper measuring limit (> 256 µg/ml). Ciprofloxacin's MIC 90% was > 32 µg/ml but MIC 50% was low (0.25 µg/ml). The MIC range of nalidixic acid and ciprofloxacin were both wide in wipe (n=15) and slurry isolates (n=9) (Table 10). According to CLSI 88.0% (22/25) and 36.0 % (9/25) of environmental isolates were resistant to nalidixic acid and ciprofloxacin, respectively. Most of these isolates were found in wipe samples (13/15 and 8/15). Of the 15 ciprofloxacin susceptible isolates 12 (12/15, 80.0%) showed decreased susceptibility to ciprofloxacin following the EUCAST guidelines (Table 11).

sample	CLSI			EUCAST		decreased susceptibility	TOTAL
	nalidixic acid						
	I		R	WT	M	DS	
dust	0.0%, n = 0 (0.0 - 85.4%)		100.0%, n = 1 (14.6 - 100.0%)	0.0%, n = 0 (0.0 - 85.4%)	100.0%, n = 1 (14.6 - 100.0%)	0.0%, n = 0 (0.0 - 85.4%)	1 (4.0%)
wipes	13.3%, n=2 (2.8 - 36.4%)		86.7%, n = 13 (63.6 - 97.2%)	0.0%, n = 0 (0.0 - 15.2%)	100.0%, n = 15 (84.8 - 100.0%)	0.0%, n = 0 (0.0 - 15.2%)	15 (60.0%)
slurry	11.1%, n=1 (1.2 - 41.5%)		88.9%, n = 8 (58.5 - 98.8%)	0.0%, n = 0 (0.0 - 23.8%)	100.0%, n = 9 (76.2 - 100.0%)	0.0%, n = 0 (0.0 - 23.8%)	9 (36.0%)
TOTAL	**12.0%, n=3 (3.4 - 28.7%)**		**88.0%, n = 22 (71.3 - 96.6%)**	**0.0%, n = 0 (0.0 - 9.5%)**	**100%, n = 25 (90.5 - 100.0%)**	**0.0%, n = 0 (0.0 - 9.5%)**	**25 (100%)**
	ciprofloxacin						
dust	100.0%, n = 1 (20.7 - 100.0%)	0.0%, n = 0 (0.0 - 79.3%)	0.0%, n = 0 (0.0 - 79.3%)	0.0%, n = 0 (0.0 - 85.4%)	100.0%, n = 1 (14.6 - 100.0%)	100.0%, n = 1 (14.6 - 100.0%)	1 (4.0%)
wipes	46.7%, n = 7 (24.8 - 69.9%)	6.6%, n = 1 (1.2 - 29.8%)	46.7%, n = 7 (24.8 - 69.9%)	6.6%, n = 1 (0.7 - 27.2%)	93.4%, n = 14 (82.8 - 99.3%)	26.7%, n = 4 (9.7 - 51.7%)	15 (60.0%)
slurry	77.8%, n = 7 (45.2 - 93.7%)	0.0%, n = 0 (0.0 - 29.9%)	22.2%, n = 2 (6.3 - 54.7%)	0.0%, n = 0 (0.0 - 23.8%)	100.0%, n = 9 (76.2 - 100.0%)	77.8%, n = 7 (45.6 - 95.1%)	9 (36.0%)
TOTAL	**60.0%, n = 15 (40.7 - 76.6%)**	**4.0%, n = 1 (0.7 - 19.5%)**	**36.0%, n = 9 (20.2 - 55.5%)**	**4.0%, n = 1 (0.4-17.3%)**	**96.0%, n = 24 82.7-99.6%)**	**48.0%, n = 12 (29.5-66.9%)**	**25 (100%)**

Table 11 Nalidixic acid and ciprofloxacin MICs interpretation: Proportions (%) and the corresponding 95% confidence intervals in brackets (). N = number of strains. Light green, yellow and red areas indicate the sensible, intermediate and resistant isolates according to the CLSI guidelines 2017 for human breakpoints. WT and M indicate the numbers of strains classified as wildtype or mutant strain according to the EUCAST guidelines 2019. Strains with decreased susceptibility are strains lying between the wildtype's MIC (EUCAST) and the resistant MIC (CLSI).

Discussion

Limitations of the study

Sampling was performed after a recently introduced law revision concerning more restrictive requirements for veterinary prescriptions of FQ on pig farms [8]. Therefore, only a small number of farrowing units (two farrow-to-finish, three farrow and rearing and two farrowing farms) were left meeting our inclusion criteria which could have caused a selection bias. Further, a good mixing of various farm sizes was not achieved sampling farms which were mostly small to medium-sized. In previous studies investigating FQ resistance in pigs animal husbandry and FQ treatment were managed by the study investigators which is why we chose our study to be carried out under field conditions [31, 32, 43]. This included FQ treatment performed by the individual farmers who used different FQ products and different prescriptions (single versus multiple treatments) according to their private veterinarian. Treated and contact animals were either held in the same pen or room. Besides other farm-specific effects, these differences could have had some impact on our results, e.g. different treatment schemes could lead to variable detection rates, counts of quinolone non-susceptible *E. coli* and minimal inhibitory concentrations of ciprofloxacin. Treatment of dams or piglets was mostly performed shortly after birth due to postpartum dysgalactia syndrome (PPDS). Collecting necessary amounts of feces of newly born piglets for the laboratory methods used is almost impossible. Due to these limitations control sampling of piglets before treatment was not performed. Taking rectal swabs would be an alternative for future projects [32]. A previous study investigated the occurrence of QNSE in dams of the piglets included in this study (G1 and G2). In all dams control sampling was performed shortly after entry into the farrowing unit and before any FQ treatment. They found equal detection rates of QNSE (33.3%, 10 out of 30 dams) in both groups (dams FQ treated and dams not FQ treated) before FQ treatment [44]. Import and vertical transmission of quinolone resistant *E. coli* in hatcheries without antibiotic selective pressure was recently published [45]. Enrofloxacin and ciprofloxacin concentrations were measurable in blood serum samples of control weaners grouped together with FQ orally and parenterally treated weaners [32]. Thus, it remains unclear if the source of QNSE in piglets of G1, G2 and G4 were either the piglets' dams or the treated piglets (transmitting QNSE via birth and excretions) or a selective pressure made by FQ residues (excreted via milk, urine and feces) in their environment. After freezing (- 80°C, swabs stored in tryptic soy broth and glycerol) recovery rate of FQ susceptible and resistant *E. coli* was reported to be good but with a significant reduction in number of *E. coli* at a storage temperature of - 20°C (fecal slurries with phosphate buffered saline and glycerol) [46, 47]. In our study native feces was stored in stool tubes at - 20°C because of subsequent processing. According to these two reports we expect that there was a quantitative reduction of *E. coli* in our study. The mildly selective medium used (Rapid-*E. coli* 2 agar plates supplemented with 8 mg/L nalidixic acid) and picking one isolate of each sample might have distorted our results by over- or underestimating the detection of QNSE.

QNSE isolated from fecal samples

QNSE detection rates in fecal samples

The highest QNSE detection rate was found in parenterally and perorally treated pigs (G3: 54.2%). Further, it was the group with the highest number of FQ resistant *E. coli* (n = 60) isolated. These results support the opinion that FQ resistance is positively associated with previous FQ treatment and can be reduced trough restricted FQ use [48-51]. In feces of piglets pertaining to FQ treated dams (G1) QNSE were found. From G1 piglets to G1 weaners the QNSE detection rate decreased significantly (piglets two- and four-week old: 94.4% and 94.1%, 95% CI: 75.6 - 99.4%, weaners: 13.3%, 95% CI: 5.7 - 25.5%). Similar results were observed in a French study investigating flumequine treated sows and their progeny from various farrow-to-finish herds. However, from weaners (age: 60 days, 4.91%) to finishers (age: 150 days, 1.91%) the percentage of quinolone-resistant *E. coli* was still decreasing [52]. This is in contrast to our study revealing a higher QNSE detection rate in G1 fattening pigs (23.8%, 95% CI: 12.9 - 38.2%) compared to G1 weaners (13.3%, 95% CI: 5.7 - 25.5%) but with overlapping 95% CIs. Apart from G1 similar results were seen in G3, G4 and in review of all weaners and fattening pigs (see Table 2). To the author's knowledge, this is the first report describing an increase of QNSE detected in fattening pigs. In our study pigs no additional FQ treatments were performed except for two weaners which received a second FQ treatment. According to our enquiries, the stocks which our study animals were grouped together with in the fattening units previously had received FQ treatment on the breeding farm. Thus, we assume that pigs from these stocks might have also spread QNSE and the herd mixing could have led to this increase leaving more QNSE positive study pigs. Interspecies transfer of nalidixic acid resistant *E. coli* in chickens and cattle was published before [53, 54]. Furthermore, there is a risk for transferring resistant bacteria between countries via purchase of animals. For example, in chickens international transfer of colistin-resistant *E. coli* was reported [20]. No significant differences between study groups could be observed at a specific sampling time (i.e. at the four different ages from farrowing to fattening unit). This was rather surprising to us assuming that different FQ backgrounds (treated vs. contact vs. control animals) would lead to different detection rates. The relatively low number of sampled pigs per group and age might have concealed this effect and could be rechecked by larger group designs. Irrelevant of age control pigs were tested positive for QNSE but the detection rate (G5, 7.8%, 95% CI: 3.5 - 14.7%) was significantly lower compared to pigs of other groups (G1-G4, 36.9 - 54.2%, 95% CI: 28.3 - 62.2%). All seven QNSE isolates (7/90, 7.8%) in the control group originated from pigs of the same farm reporting the last FQ treatment to be three months ago (last treatment: July 2017, sampling: October 2017). In the other control farms no QNSE were found and the last FQ treatment was reported between 30 and 34 months before sampling. This is in contrast to a Swedish and English study finding quinolone resistant bacteria isolated in swine without any prehistory of FQ use. The latter study described that, beside reducing antimicrobial use to a minimum, biosecurity, e.g. purchase of animals, surrounding animal farms and farm hygiene, is an important factor in the existence and spread of antimicrobial resistant microbes [55, 56]. Although results must be compared with caution because of different material and methods performed, QNSE detection rates in our piglets were markedly higher (G1-G4, 72.5 - 100.0%) compared to Belloc et al. (2005) (percentage of quinolone-resistant *E. coli* at

7 and 30 days of age: 14.0 and 14.7%) [52]. The gastrointestinal tract of newborns is first colonized by the mother's vaginal flora and microbes of their environment. The shift from liquid to solid feed and increasing age lead to an alternation of colonizing microbes [57, 58]. Neonatal antibiotic treatment was reported to have a negative influence on the microbial diversity. It decreases the abundance of protective commensal bacteria which promotes the colonization of antibiotic resistant bacteria [59]. These aspects might explain the high detection rates of QNSE in our study piglets and the significant reduction in weaners. QNSE were detected in both contact groups (G2 and G4) from piglets to fattening pigs having in- or direct contact to G1 - and G3 - pigs. Furthermore, at any age the detection rates in contact animals (G2 and G4, Table 2) were sometimes equal or larger compared to treated pigs (G1 and G3, Table 2). Despite the small number of study animals (n = 15) and a different indicator bacterial species (*Campylobacter*) used, comparable detection rates of FQ resistant *Campylobacter* in contact animals were discovered in a Japanese study. After three days of group housing all five contact pigs previously being negative (grouped together with one previously with 5 mg/kg enrofloxacin intramuscularly treated pig which was tested positive for FQ resistant *Campylobacter*) were colonized by FQ resistant *Campylobacter* [60]. This is in contrast to a German experimental trial where four ciprofloxacin-resistant *E. coli* isolates (defined as above the epidemiological cut-off value: MIC > 0.06ug/ml) were detected in each treated and contact group but 47 ciprofloxacin-resistant *E. coli* isolates in the control group. Possible factors that could explain the low detection rate in treated and contact animals in the study described are the experimental environment conditions (free of ciprofloxacin-resistant *E. coli*), choice of study animals (single breeding unit, no antimicrobial use in dams and piglets before), high hygiene and biosecurity standards during the study and small group sizes. Contact animals either get colonized by FQ resistant bacteria by oral uptake via feces and urine or antibiotic residues in excretions which exert a selection for FQ resistant bacteria [32]. Separation of diseased animals and proper hygiene levels are keys to promote healing and prevent infectious diseases from spreading in the animal stock [61]. According to the results above this measurement is also advisable to reduce the risk of transferring resistant bacteria or exposing animals and human beings to antibiotic residues. Seven out of 19 fattening pigs (36.8%) were positive for QNSE after oral treatment with FQ as suckling piglet compared to one out of 8 fattening pigs (12.5%) tested positive for QNSE after intramuscular treatment as suckling piglet. Due to a low power we forewent carrying out any further statistical evaluation of the route of applications (i.m. and p.o.) used in this study. However, previous investigation reported resistance rates to be independent of the route of administration [62, 63]. More studies with larger sample sizes are needed.

Counts of QNSE per gram feces

In the current literature several studies describe quantitative analyses with commensal *E. coli* before, during and after treatment to assess the antibiotic's influence on the gut flora. These studies agree that during and shortly after treatment there is a decrease of *E. coli* but with a recolonization within days or weeks [31, 52, 60, 63, 64]. In our study, with increasing age of the pigs we detected continuously decreasing mean and median counts of QNSE per gram feces. In fact, the content of QNSE decreased by a factor of almost 10^5 between two-week old piglets (mean of

G3: 7.1 log CFU/g feces) and fattening pigs (mean of G3: 2.2 log CFU/g feces). Samples from two-week old piglets of G1 and G3 (mean of G1: 7.0 log CFU/g feces, mean of G3: 7.1 log CFU/g feces) showed the highest counts which met our expectations because they were either FQ treated piglets or piglets of FQ treated sows. Treated pigs were shown before to have higher counts per gram feces dependent on dosing than placebo pigs [65]. This could explain why counts of G1 (piglets of treated sows) were lower compared to G3 expecting to have received a lower dose of FQ via milk than treated piglets. Nonetheless, we were surprised by the number of QNSE detected in these samples with the treatment of piglets and sows being about ten to 14 days ago. In a study measuring FQ resistant *Campylobacter* in weaners (age: 18 days) during and after FQ administration similar amounts (10^5 to 10^7 colony forming units per gram feces) were found five days post treatment [60]. In our study counts in samples from four-week old piglets decreased compared to samples of two-week old piglets but were tenfold higher in piglets of treated sows (G1) and contact piglets (G2) (mean of G1: 6.4 log CFU/g feces, mean of G2: 6.4 log CFU/g feces) compared to treated piglets (G3) and contact piglets (G4) (mean of G3: 5.4 log CFU/g feces, mean of G4: 5.1 log CFU/g feces). The amount of FQ used are dosed according to the animal's bodyweight. Consequently, in farms with FQ treatment in sows larger amounts of FQ were used. This could lead to a larger amount of antibiotic residues in milk, feces and the environment which could explain these high counts in four-week old piglets. In sows independent of FQ treatment QNSE were detected shortly after entry into the farrowing units and before farrowing by Stohler et al. (2019) [44]. In a Swedish study successful vertical transmission of quinolone resistant *E. coli* was described in broiler production by introducing positive breeding birds [45]. In a recent study piglets of dams with detection of ampicillin or azithromycin resistance had a higher chance of being positive for these resistances [62]. This means that transmission of QNSE from positive sows to their progeny could possibly explain the high detection rates in piglets of G1 (treated piglets) and G2 (contact piglets). Another interesting observation were the means of QNSE detected in contact weaners (G2: 3.1 log CFU/g feces, G4: 3.4 log CFU/g feces) being ten- to a hundredfold higher than in treated weaners (G1: 2.3 log CFU/g feces, G3: 1.0 log CFU/g feces). In the count part of the hurdle model contact weaners (Ctat) had significantly higher counts (Ctat; 3.757 log CFU/g feces, 95% CI: 3.754 - 3.760) compared to treated weaners (Trt; 1.888 log CFU/g feces, 95% CI: 1.877 - 1.899). A similar outcome was observed when one weaner being positive for FQ resistant *Campylobacter* after enrofloxacin treatment was grouped together with five negative weaners. After five days in four of five weaners same or higher amounts of FQ resistant Campylobacter were detected [60]. These results lead to the assumption that QNSE could maintain and remain easier in the intestinal floras of contact weaners than in those of treated weaners. The intake of antibiotic residues and therefore the exposure of the intestinal flora to low-dose antibiotic amounts could promote development of resistant bacteria or exchange of antimicrobial resistance genes between bacteria in contact animals. From the qualitative view our results and the results of Burow et al. (2019) suggest that treated animals are more likely to harbor antibiotic resistant bacteria than not treated animals [62]. But in terms of quantity, contact weaners could harbor more antibiotic resistant bacteria than treated weaners. Either in weaners or fattening pigs, the results of the hurdle model showed significantly lower counts in control animals (Ctrl weaner; 1.707 log CFU/g feces, 95% CI: 1.689 - 1.725, Ctrl fattening pig; 1.936 log CFU/g feces, 95% CI: 1.922 - 1.949) compared to treated (Trt weaner; 1.888 log

CFU/g feces, 95% CI: 1.877 - 1.899, Trt fattening pigs; 3.378 log CFU/g feces, 95% CI: 3.373 - 3.383) and contact animals (Ctat weaner; 3.757 log CFU/g feces, 95% CI: 3.754 - 3.760, Ctat fattening pig; 2.800 log CFU/g feces, 95% CI: 2.791 - 2.809). This is in agreement with other investigations with the exception that all control animals were negatively tested for resistant bacteria. An explanation for this finding may be the already mentioned fact, that our study was carried out under field conditions with lower hygienic standards compared to the other experimental studies. Detections rates of weaners were insignificantly lower than those in fattening pigs. Furthermore, no significance was found in the zero part of the hurdle model within the groups (Trt, Ctat and Ctrl) and between weaners and fattening pigs. However, the count part of the hurdle model showed that there is a significant difference in counts of QNSE between weaners and fattening pigs and between all three groups (view Table 3). As described above along with the environment and the additional FQ treatment, which can be ruled out, purchase and integration of pigs with possible FQ contact can reincrease the quantity of QNSE excreted per pig due to horizontal transfer of QNSE or antibiotic residues. Pigs which have not been treated or not being in contact with FQ treated pigs might have the lowest or no excretions of QNSE which can be confirmed by four other surveys [56, 60, 64, 66] but one study describing contradictory results [32].

MIC of QNSE isolated from feces

Looking at the distribution of ciprofloxacin resistant *E.coli* strains (120/254, 47.2%), most of them were found in pigs which were part of G3 (treated pigs, 60/120, 50.0%) and G4 (contact pigs, 36/120, 30.0%), followed by pigs of G1 (treated pigs, 12/120, 10.0%), G2 (contact pigs, 11/120, 9.2%) and G5 (control pigs, 1/120, 0.8%). Resistance proportions significantly differed between G3 (S: 23.1%, 95% CI 15.1 - 33.6%, R: 79.6%, 95% CI: 66.4 - 84.9%) and G1, G2, G4 and G5 (S: 53.2 - 71.4%, 95% CI: 35.9 - 91.8%, R: 14.3 - 45.6%, 95% CI: 2.6 - 56.5%, view Table 6). This meets the results of Römer et al. (2017) comparing *E. coli* growth on enrofloxacin supplemented agar plates between an experimental and control group although MIC values of the two groups did not differ significantly [31]. Seven days after enrofloxacin treatment they found the first non-wildtype-*E. coli* (MIC-values above ECOFF). Due to our study protocol we are not able to chronologically describe the first detection of QNSE or FQ resistant *E. coli*. In the study of Römer et al. (2017) the experimental group was held in the same room with the control group which was tested positive for non-wildtype-*E. coli* (agar with 0.125 mg/L enrofloxacin) only after the second treatment at day 28 and for enrofloxacin-resistant *E. coli* (agar with 4mg/L enrofloxacin) at day 42 [31]. On the contrary, we found QNSE and FQ resistant *E. coli* in both treated and contact piglets (held in the same room or litter) at the first sampling day (two-weeks of age) and again at four-weeks of age (second sampling day). This emphasizes that transmission may be faster under field conditions, e.g. by higher animal density, compared to laboratory standards. Wild-type *E. coli* susceptible to ciprofloxacin were only found before FQ treatment [31]. During our study there was only one QNSE strain with a MIC value below the ECOFF detected which belonged to a weaner of G1, i.e. approximately nine weeks after FQ treatment. By Römer et al. (2017) highest MIC_{CIP} values (6 - 32 mg/L) were measured three to five weeks (day 42 and day 54) after a second treatment of FQ. Highly ciprofloxacin resistant strains were detected in almost all age and croup categories of our trial but

most of them were collected from two- and four-week old piglets being sampled around one to three weeks after their treatment [31]. Similar peak times were observed by Delsol et al. (2004) and Belloc et al. (2005) [52, 64]. In the study of Burow et al. (2018) detection times were different. First ciprofloxacin non-wildtype *E.coli* isolates were detected at day 56 (app. seven weeks after treatment) in orally treated pigs and their contact pigs [32]. Surprisingly, control animals were tested positive much earlier during treatment days (day 1) up to 42 days after treatment. However, highest MIC values of ciprofloxacin did not exceed 2 µg/ml in the described study. Observing ciprofloxacin's MIC 50% and MIC 90%, we noticed some gradual decreases with age in all five groups. Huang et al. (2014) also showed a decrease in the average MICs of experimental pigs from day 30 (stopping the oral treatment) until day 60 [48]. Control pigs did not exceed an average MIC of 1 µg/ml. Without any further drug selection pressure improving fitness may take over a more important role in bacteria. *In vitro* FQ resistant *Salmonellae* showed prolonged generation times and disability to maintain in the gut flora of chickens compared to wildtype strains. Without antibiotic selection pressure *in vitro* and *in vivo* fitness costs reversed leading to a slight decrease in FQ resistance [67]. However, there exist fitness-compensatory mutations and additional resistance mutations which can improve the fitness and therefore maintain or increase the level of minimal inhibitory concentration [68]. Apart from initial FQ treatment in our study no additional FQ treatment was performed except for two weaners. In the absence of further FQ selection pressure and further fitness improvements these factors might explain some gradual decreases of MICs with increasing age category. In weaners of G2 (S = 100.0%, 95% CI = 66.9 - 100.0%, R = 0.0%, 95% CI = 0.0 - 33.1%) and G3 (S = 0.0%, 95% CI = 0.0 - 44.5%, R = 100.0%, 95% CI = 55.5 - 100.0%, view Table 8) ciprofloxacin susceptibility proportions differed significantly. In groups of Trt and Ctrl this was not reproducible. Treated pigs having a higher risk of carrying ciprofloxacin resistant strains met our expectations due to the current literature [31, 51, 62, 65, 69]. Further, we would have expected G5 to have significantly different susceptibility proportions compared to G1 to G4. The low number of strains isolated in the different groups of weaners and fattening pigs might have made it impossible to observe such a difference. Other studies with more strains per group are needed to test differences in susceptibility proportions. Being part of a SPS seems to inherit a higher risk of having FQ resistant strains in fattening pigs compared to fattening pigs originating from farms not connected to a SPS. As mentioned before sows carried QNSE and FQ resistant *E. coli* which were sampled shortly after entry into the farrowing unit and before the FQ treatment [44, 52]. This means that the transmission of QNSE and FQ resistant *E. coli* is promoted by a system of moving sows from one unit to another. In the current literature we did not find any comparable studies which investigated the occurrence of bacterial resistances in pigs of SPS and without SPS connection.

QNSE isolated from environmental samples

QNSE detection rates in environmental samples

Of all 179 environmental samples 25 (14.0%) were tested positive for QNSE. Most of the positive samples were floor and wall wipes (15/63, 23.8%), followed by slurry samples (9/49, 18.4%) and one dust sample (1/67, 1.5%). FQ treatment was performed in the farrowing units where in one third of the environmental samples

(10/34, 29.4%) QNSE were observed. A recently published study in Switzerland used similar sampling and laboratory techniques and revealed much higher proportions of environmental samples (pen wall samples: 51.9%, liquid manure: 70.4% and dust: 45.2%) being positive for quinolone resistant *E. coli*. Farrowing to rearing farms were also affected the most besides a much higher detection rate (25/26, 96.2%) [70]. The influence of season was investigated before with contradictory outcomes concerning its effect [56, 71, 72]. Further, weather conditions, e.g. rain causing a dilution effect, could have had an influence by causing a lower detection rate in slurry samples. It was rather surprising to us that in this experimental trial only 1 of 67 dust samples maximally stored for half a year contained QNSE. In comparison, a German study detected FQ resistant *E. coli* in 54 of 119 dust samples of which some were more than 20 years old. Different sampling and store conditions might explain this disparity [24]. Besides the animals themselves, farmers and veterinarians spend a lot of time in barns where they can inhale dust or dust can be released into the environment by ventilation systems [73]. More investigation is needed to evaluate the level of risk for transferring QNSE via farm dust. In almost one fifth of the slurry samples QNSE were detected (9/49, 18.4%). Farmers fertilize their crop fields using liquid manure. In recent years a lot of research was invested in this upcoming topic revealing antibiotic residues and antibiotic resistance genes are distributed bearing the risk of contaminating harvest production for livestock and human beings [20-24]. To reduce or eliminate pathogenic and antibiotic resistant bacteria in the livestock's environment cleaning and disinfection are crucial [61, 74]. This differed widely having fattening units which have never cleaned and disinfected their pens and fattening units with a good cleaning and disinfection plan after every emptying. Out of the three floor and wall wipes being positive for QNSE two originated from fattening farms performing cleaning on a regular basis but without disinfection afterwards. This emphasizes the need of cleaning and disinfection and practicing it correctly. After all, only a reduction of bacteria can be achieved and not a complete elimination.

Number of QNSE in environmental samples

In the semiquantitative approach of slurry samples on average 2.8 log CFU/ml were detected. In dust and floor and wall wipes the average count was 3.0 log CFU/g and 2.8 log CFU/g, respectively. In disagreement with the survey of von Ah et al. (2019) counts of quinolone-resistant colonies were the highest in slurry samples (11'831 CFU/ml = 4.1 log CFU/ml) followed by dust (111 CFU/g = 2.0 log CFU/ml) and pen wall samples (40 CFU/g = 1.6 log CFU/g) [70]. Similar to fecal samples highest counts of environmental samples were detected in the farrowing units (3.1 and 3.7 log CFU/g). This underlines that the highest detection rate and count of QNSE must be clearly linked to institutions where FQ treatment is performed because the chance of finding FQ residues is high [73]. Nevertheless, QNSE were detected in the fattening units' environment but from this set of data we cannot determine the source of QNSE.

MIC of QNSE in environmental samples

MIC distribution of nalidixic acid and ciprofloxacin were most favourable in dust but only one strain was detected which limits the significance of this information (view Table 10). This is in contrast to the studies of Schulz et al. (2016) and Römer et al.

(2017) in which they observed growth of *E. coli* on plates supplemented with 2 µg/ml ciprofloxacin and 4 µg/ml enrofloxacin, respectively [24, 31]. MIC 50% of wipe samples (4 ug/ml) was higher compared to MIC 50% of slurry samples (0.19 ug/ml). From these results we could assume that MIC distribution might be more favourable in slurry than in floor and wall wipes. Nevertheless, only few strains were detected in environmental samples leaving wide and overlapping confidence intervals in resistance proportions between dust, floor and wall wipes and slurry. Comparing MICs of fecal samples with the MICs of environmental samples we observed a slightly better resistance situation in environmental samples (FQ resistant *E. coli* in environmental samples: 36.0%, 95% CI: 20.2 - 55.5%, FQ resistant *E. coli* in fecal samples: 47.2%, 95% CI: 41.2 - 53.4%) but with overlapping 95% CIs. Studies testing a greater amount of environmental strains are needed to verify this tendency.

Outlook

In this study we compared farms with a regular FQ usage and farms without any FQ usage for the past three to 34 months. For future research it would be of interest to compare farms using low and high amounts of FQ with farms which have never used FQ before. Furthermore, the total number of FQ treatments and FQ treated pigs per farm should be included into the study protocol for evaluation which of these two factors would be of more influence in FQ resistance situation. This information could be useful for future consulting of pig farm management. These aspects were investigated before concerning tetracycline resistance which had an effect on the probability of detecting resistant isolates [75]. FQs have been reported to promote the selection of resistances against other antibiotics in *E. coli* and *Campylobacter coli* [32, 64, 76]. Thus, a more extensive testing for other antibiotic resistances would be beneficial to see if besides FQ resistances other selection processes are promoted. For further studies measurements of FQ residues and genetic background of detected strains would be helpful to gain information about origin and spread of individual *E. coli* strains [31, 32]. Additionally, sampling the environment before the entry of animals and sampling piglets before any treatment could rule out preexisting contamination with antibiotic residues, QNSE or FQ resistant *E. coli*.

Conclusion

In this study we investigated the presence of QNSE in pigs treated with FQs, pigs from FQ treated sows, pigs being in indirect or direct contact with the pigs mentioned before and pigs from farms where FQs have not been used for several months or years. Additionally, the environment around the pigs was screened. Two-hundred and fifty-four of 621 fecal samples were tested positive for QNSE (40.9%). According to age most of the QNSE-positive samples were found in two- (109/116, 94.0%) and four-week old piglets (88/104, 85.6%). QNSE were also present in fecal samples of pigs originating from farms which stopped FQ use months or years ago (7/90, 7.8%). The detection rate (G1, G2, G3, and G4 vs. G5) and counts of QNSE (Trt and Ctat vs. Ctrl) were significantly lower in control pigs compared to treated and contact pigs. Almost half of the isolated QNSE-strains (126/254, 49.6%) were intermediate or resistant to ciprofloxacin (≥ 3 µg/ml ciprofloxacin respectively). Fattening pigs of SPS-farms showed a significantly higher ciprofloxacin resistance rate compared to fattening pigs of non-SPS-farms. In the environment 14.0% of samples (25/179) contained QNSE of which most were isolated of floor and wall wipes. Quantitatively most QNSE were present in the farrowing units. 40.0% (10/25) of the isolates were either intermediate or resistant to ciprofloxacin. From these results we can clearly see that FQ resistant bacteria in pigs are associated with FQ use in farrowing units but that there are no boarders to FQ resistant bacteria when it comes to contact animals and the environment. Further, restricted or non-use of FQs is not the only act it needs to minimize or eliminate FQ resistant bacteria in pig farming. Further research in the spread of FQ resistant bacteria and its promoting factors are necessary. Adapting a special management of antibiotically treated pigs in farms, restricted transport and purchase are also of concern. Especially in the environment, e.g. farm dust and slurry, new approaches are needed to reduce the contamination with FQ resistant bacteria and transfer into food chain.

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Annex

Isolate	Sample	Unit	SPS	Group	QNSE CFU/g or ml	QNSE log CFU/g or ml	MIC_{NA}	MIC_{CIP}
1	Fecal	Farrowing	+	1	32'000'000	7.5	256	0.125
2	Fecal	Farrowing	+	1	42'000'000	7.6	256	0.125
3	Fecal	Farrowing	+	1	1'330'000	6.1	96	0.094
4	Fecal	Farrowing	+	1	970'000	6.0	>256	>32
5	Fecal	Farrowing	+	1	10'480'000	7.0	>256	>32
6	Fecal	Farrowing	+	1	14'880'000	7.2	256	0.125
7	Fecal	Farrowing	+	1	4'180'000	6.6	256	0.125
8	Fecal	Farrowing	+	1	4'420'000	6.6	256	0.19
9	Fecal	Farrowing	+	1	680'000	5.8	128	0.125
10	Fecal	Farrowing	+	1	3'160'000	6.5	256	0.19
11	Fecal	Farrowing	+	1	28'800'000	7.5	96	0.125
12	Fecal	Farrowing	+	1	6'130'000	6.8	192	0.125
13	Fecal	Farrowing	+	1	16'160'000	7.2	128	0.125
14	Fecal	Farrowing	+	1	180'000	5.3	>256	3
15	Fecal	Farrowing	+	1	2'000'000	6.3	96	0.125
16	Fecal	Farrowing	+	1	3'440'000	6.5	96	0.125
17	Fecal	Farrowing	+	1	21'680'000	7.3	128	0.125
18	Fecal	Farrowing	+	2	85'000	4.9	>256	6
19	Fecal	Farrowing	+	2	1'500'000	6.2	256	0.19
20	Fecal	Farrowing	+	2	70'000	4.8	256	0.125
21	Fecal	Farrowing	+	2	1'000	3.0	>256	>32
22	Fecal	Farrowing	-	2	47'000	4.7	128	0.125
23	Fecal	Farrowing	+	2	1'000	3.0	256	0.125
24	Fecal	Farrowing	+	2	3'940'000	6.6	96	0.094
25	Fecal	Farrowing	+	2	450'000	5.7	>256	>32
26	Fecal	Farrowing	+	2	23'280'000	7.4	>256	>32
27	Fecal	Farrowing	+	2	260'000	5.4	192	0.125
28	Fecal	Farrowing	+	2	220'000	5.3	>256	4
29	Fecal	Farrowing	+	2	3'220'000	6.5	128	0.125
30	Fecal	Farrowing	+	2	24'000'000	7.4	96	0.125
31	Fecal	Farrowing	+	2	87'000	4.9	>256	4
32	Fecal	Farrowing	+	2	4'880'000	6.7	64	0.094
33	Fecal	Farrowing	+	2	720'000	5.9	192	0.19
34	Fecal	Farrowing	+	2	1'380'000	6.1	192	0.125
35	Fecal	Farrowing	+	2	35'000'000	7.5	>256	3
36	Fecal	Farrowing	+	3	35'000	4.5	>256	8
37	Fecal	Farrowing	+	3	900	3.0	24	0.125
38	Fecal	Farrowing	+	3	43'200'000	7.6	>256	8
39	Fecal	Farrowing	+	3	1'556'000	6.2	>256	>32
40	Fecal	Farrowing	+	3	100'000	5.0	>256	8
41	Fecal	Farrowing	+	3	72'800'000	7.9	>256	8
42	Fecal	Farrowing	+	3	3'700'000	6.6	>256	>32
43	Fecal	Farrowing	+	3	7'000	3.8	>256	>32
44	Fecal	Farrowing	+	3	1'000'000	6.0	>256	8
45	Fecal	Farrowing	+	3	700'000	5.8	>256	>32
46	Fecal	Farrowing	+	3	1'000'000	6.0	>256	8
47	Fecal	Farrowing	+	3	5'500'000	6.7	>256	>32
48	Fecal	Farrowing	+	3	1'400'000	6.1	>256	>32
49	Fecal	Farrowing	+	3	194'400'000	8.3	>256	>32
50	Fecal	Farrowing	+	3	29'700'000	7.5	>256	8
51	Fecal	Farrowing	-	3	1'600'000	6.2	>256	32
52	Fecal	Farrowing	-	3	16'600'000	7.2	>256	>32
53	Fecal	Farrowing	-	3	7'440'000	6.9	>256	24
54	Fecal	Farrowing	-	3	12'300'000	7.1	>256	32
55	Fecal	Farrowing	-	3	6'000	3.8	>256	32
56	Fecal	Farrowing	-	3	4'000	3.6	>256	32
57	Fecal	Farrowing	-	3	33'200'000	7.5	>256	24
58	Fecal	Farrowing	-	3	34'400'000	7.5	>256	32
59	Fecal	Farrowing	-	3	5'400'000	6.7	>256	32
60	Fecal	Farrowing	-	3	62'400'000	7.8	>256	32
61	Fecal	Farrowing	-	3	1'000'000	6.0	>256	8
62	Fecal	Farrowing	-	3	3'500'000	6.5	>256	6
63	Fecal	Farrowing	-	3	60'000	4.8	>256	8
64	Fecal	Farrowing	-	3	100'000	5.0	>256	6
65	Fecal	Farrowing	-	3	100'000	5.0	>256	4
66	Fecal	Farrowing	-	3	200'000	5.3	256	0.125
67	Fecal	Farrowing	-	3	140'000	5.1	>256	6
68	Fecal	Farrowing	-	3	800'000	5.9	>256	6
69	Fecal	Farrowing	-	3	100'000	5.0	>256	>32
70	Fecal	Farrowing	-	3	40'000	4.6	>256	>32
71	Fecal	Farrowing	-	3	50'000	4.7	>256	8
72	Fecal	Farrowing	-	3	20'000	4.3	>256	>32

73	Fecal	Farrowing	+	4	1’900’000	6.3	>256	8
74	Fecal	Farrowing	+	4	1’870’000	6.3	256	0.19
75	Fecal	Farrowing	+	4	30’000	4.5	>256	6
76	Fecal	Farrowing	+	4	8’000	3.9	64	0.19
77	Fecal	Farrowing	+	4	2’000	3.3	256	0.19
78	Fecal	Farrowing	+	4	1’380’000	6.1	256	0.19
79	Fecal	Farrowing	+	4	90’000	5.0	>256	>32
80	Fecal	Farrowing	+	4	60’000	4.8	>256	0.38
81	Fecal	Farrowing	+	4	3’000	3.5	>256	0.38
82	Fecal	Farrowing	+	4	30’000	4.5	>256	>32
83	Fecal	Farrowing	+	4	5’240’000	6.7	>256	>32
84	Fecal	Farrowing	+	4	1’090’000	6.0	>256	>32
85	Fecal	Farrowing	+	4	4’100’000	6.6	>256	>32
86	Fecal	Farrowing	+	4	740’000	5.9	>256	8
87	Fecal	Farrowing	+	4	60’000	4.8	>256	>32
88	Fecal	Farrowing	-	4	40’000	4.6	256	0.094
89	Fecal	Farrowing	-	4	6’080’000	6.8	256	0.19
90	Fecal	Farrowing	-	4	1’440’000	6.2	256	0.19
91	Fecal	Farrowing	-	4	110’000	5.0	256	0.125
92	Fecal	Farrowing	-	4	20’000	4.3	256	0.094
93	Fecal	Farrowing	-	4	100’000	5.0	256	0.094
94	Fecal	Farrowing	-	4	800’000	5.9	256	0.094
95	Fecal	Farrowing	-	4	30’000	4.5	>256	>32
96	Fecal	Farrowing	-	4	20’000	4.3	>256	0.094
97	Fecal	Farrowing	-	4	500’000	5.7	256	0.125
98	Fecal	Farrowing	-	4	430’000	5.6	256	0.19
99	Fecal	Farrowing	-	4	2’000	3.3	256	0.125
100	Fecal	Farrowing	-	4	25’000	4.4	>256	12
101	Fecal	Farrowing	-	4	330’000	5.5	256	0.125
102	Fecal	Farrowing	-	4	1’000	3.0	256	0.125
103	Fecal	Farrowing	-	4	40’000	4.6	>256	>32
104	Fecal	Farrowing	-	4	25’000	4.4	>256	8
105	Fecal	Farrowing	-	4	30’000	4.5	256	0.19
106	Fecal	Farrowing	-	4	6’000	3.8	>256	6
107	Fecal	Farrowing	-	4	7’000	3.8	>256	>32
108	Fecal	Farrowing	-	4	440’000	5.6	>256	>32
109	Fecal	Farrowing	-	4	40’000	4.6	>256	8
110	Fecal	Farrowing	+	1	240’000	5.4	192	0.094
111	Fecal	Farrowing	+	1	9’000	4.0	>256	0.125
112	Fecal	Farrowing	+	1	220’000	5.3	>256	4
113	Fecal	Farrowing	-	1	56’000	4.7	24	0.19
114	Fecal	Farrowing	+	1	6’880’000	6.8	192	0.094
115	Fecal	Farrowing	+	1	3’220’000	6.5	>256	32
116	Fecal	Farrowing	+	1	1’480’000	6.2	192	8
117	Fecal	Farrowing	+	1	61’000	4.8	>256	0.125
118	Fecal	Farrowing	+	1	15’000’000	7.2	>256	>32
119	Fecal	Farrowing	+	1	11’000	4.0	>256	3
120	Fecal	Farrowing	+	1	3’440’000	6.5	96	0.094
121	Fecal	Farrowing	+	1	119’000	5.3	256	0.125
122	Fecal	Farrowing	+	1	89’000	4.9	>256	6
123	Fecal	Farrowing	+	1	12’440’000	7.1	192	0.094
124	Fecal	Farrowing	+	1	22’000	4.3	>256	3
125	Fecal	Farrowing	+	1	60’000	4.8	>256	0.125
126	Fecal	Farrowing	+	2	830’000	5.9	256	0.125
127	Fecal	Farrowing	+	2	2’240’000	6.4	>256	0.094
128	Fecal	Farrowing	+	2	3’840’000	6.6	96	0.094
129	Fecal	Farrowing	+	2	500	2.7	>256	4
130	Fecal	Farrowing	+	2	5’780’000	6.8	192	0.094
131	Fecal	Farrowing	+	2	3’520’000	6.5	>256	32
132	Fecal	Farrowing	+	2	4’000	3.6	128	0.125
133	Fecal	Farrowing	+	2	4’240’000	6.6	128	0.094
134	Fecal	Farrowing	+	2	5’120’000	6.7	>256	32
135	Fecal	Farrowing	+	2	250’000	5.4	96	0.094
136	Fecal	Farrowing	+	2	1’450’000	6.2	24	0.25
137	Fecal	Farrowing	+	3	1’000’000	6.0	>256	0.19
138	Fecal	Farrowing	+	3	2’976’000	6.5	>256	8
139	Fecal	Farrowing	+	3	200	2.3	>256	>32
140	Fecal	Farrowing	+	3	7’000	3.8	>256	6
141	Fecal	Farrowing	+	3	224’000	5.4	48	0.19
142	Fecal	Farrowing	+	3	74’000	4.9	>256	6
143	Fecal	Farrowing	+	3	62’000	4.8	256	0.19
144	Fecal	Farrowing	+	3	480’000	5.7	256	0.19
145	Fecal	Farrowing	+	3	14’000	4.1	>256	9
146	Fecal	Farrowing	+	3	59’000	4.8	>256	>32
147	Fecal	Farrowing	+	3	1’000’000	6.0	256	0.19
148	Fecal	Farrowing	+	3	1’000’000	6.0	>256	0.19

149	Fecal	Farrowing	+	3	3’000	3.5	>256	>32
150	Fecal	Farrowing	+	3	1’000’000	6.0	>256	6
151	Fecal	Farrowing	-	3	300	2.5	256	0.125
152	Fecal	Farrowing	-	3	200	2.3	>256	6
153	Fecal	Farrowing	-	3	3’700	3.6	>256	6
154	Fecal	Farrowing	-	3	69’000	4.8	>256	6
155	Fecal	Farrowing	-	3	23’000	4.4	>256	6
156	Fecal	Farrowing	-	3	30’000	4.5	>256	6
157	Fecal	Farrowing	-	3	4’000	3.6	>256	6
158	Fecal	Farrowing	-	3	11’000	4.0	>256	6
159	Fecal	Farrowing	-	3	5’000	3.7	>256	6
160	Fecal	Farrowing	-	3	784’000	5.9	>256	6
161	Fecal	Farrowing	-	3	2’180’000	6.3	>256	6
162	Fecal	Farrowing	-	3	33’000	4.5	>256	0.125
163	Fecal	Farrowing	-	3	100	2.0	256	>32
164	Fecal	Farrowing	-	3	800	2.9	>256	6
165	Fecal	Farrowing	-	3	22’000	4.3	>256	6
166	Fecal	Farrowing	+	4	269’000	5.4	>256	6
167	Fecal	Farrowing	+	4	55’000	4.7	>256	6
168	Fecal	Farrowing	+	4	1’000	3.0	>256	3
169	Fecal	Farrowing	+	4	201’000	5.3	>256	6
170	Fecal	Farrowing	+	4	21’000	4.3	>256	6
171	Fecal	Farrowing	+	4	427’000	5.6	>256	6
172	Fecal	Farrowing	+	4	2’000	3.3	>256	0.125
173	Fecal	Farrowing	+	4	6’000	3.8	>256	6
174	Fecal	Farrowing	+	4	5’000	3.7	>256	6
175	Fecal	Farrowing	+	4	1’628’000	6.2	>256	>32
176	Fecal	Farrowing	+	4	741’000	5.9	>256	>32
177	Fecal	Farrowing	+	4	496’000	5.7	>256	0.25
178	Fecal	Farrowing	-	4	57’000	4.8	>256	>32
179	Fecal	Farrowing	-	4	19’000	4.3	256	0.19
180	Fecal	Farrowing	-	4	50’000	4.7	256	0.125
181	Fecal	Farrowing	-	4	4’000	3.6	256	0.125
182	Fecal	Farrowing	-	4	18’000	4.3	256	0.19
183	Fecal	Farrowing	-	4	1’000	3.0	256	0.125
184	Fecal	Farrowing	-	4	400	2.6	256	0.19
185	Fecal	Farrowing	-	4	1’000	3.0	256	0.125
186	Fecal	Farrowing	-	4	400	2.6	256	0.19
187	Fecal	Farrowing	-	4	200	2.3	256	0.125
188	Fecal	Farrowing	-	4	14’000	4.1	256	0.19
189	Fecal	Farrowing	-	4	31’000	4.5	>256	6
190	Fecal	Farrowing	-	4	30’000	4.5	>256	6
191	Fecal	Farrowing	-	4	2’000	3.3	>256	6
192	Fecal	Farrowing	-	4	2’800	3.4	>256	6
193	Fecal	Farrowing	-	4	6’000	3.8	>256	6
194	Fecal	Farrowing	-	4	100	2.0	256	0.125
195	Fecal	Farrowing	-	4	200	2.3	>256	6
196	Fecal	Farrowing	-	4	148’000	5.2	>256	6
197	Fecal	Farrowing	-	4	317’000	5.5	>256	6
198	Fecal	Rearing	-	1	100	2.0	>256	0.19
199	Fecal	Rearing	+	1	4’500	3.7	256	0.125
200	Fecal	Rearing	+	1	300	2.5	24	0.047
201	Fecal	Rearing	+	1	100	2.0	>256	6
202	Fecal	Rearing	+	1	100	2.0	>256	4
203	Fecal	Rearing	+	1	4’400	3.6	>256	0.125
204	Fecal	Rearing	+	2	400	2.6	256	0.19
205	Fecal	Rearing	-	2	400	2.6	256	0.19
206	Fecal	Rearing	+	2	4’700	3.7	192	0.125
207	Fecal	Rearing	+	2	100	2.0	256	0.125
208	Fecal	Rearing	+	2	1’300	3.1	256	0.125
209	Fecal	Rearing	+	2	39’600	4.6	192	0.125
210	Fecal	Rearing	+	3	100	2.0	>256	>32
211	Fecal	Rearing	+	3	100	2.0	>256	6
212	Fecal	Rearing	-	3	100	2.0	>256	>32
213	Fecal	Rearing	-	3	100	2.0	>256	32
214	Fecal	Rearing	+	4	100’000	5.0	>256	6
215	Fecal	Rearing	-	5	100	2.0	>256	3
216	Fecal	Rearing	-	5	800	2.9	256	0.125
217	Fecal	Rearing	-	5	100	2.0	>256	4
218	Fecal	Rearing	-	5	200	2.3	>256	0.19
219	Fecal	Rearing	-	5	1’100	3.0	>256	0.19
220	Fecal	Fattening	+	1	3’700	3.6	>256	0.125
221	Fecal	Fattening	-	1	100	2.0	256	0.19
222	Fecal	Fattening	+	1	200	2.3	>256	8
223	Fecal	Fattening	+	1	100	2.0	256	0.125
224	Fecal	Fattening	+	1	900	3.0	>256	8

225	Fecal	Fattening	+	1	15'000	4.2	256	0.125
226	Fecal	Fattening	+	1	100	2.0	256	0.125
227	Fecal	Fattening	+	1	100	2.0	>256	6
228	Fecal	Fattening	+	1	400	2.6	192	0.19
229	Fecal	Fattening	+	1	15'000	4.2	256	0.125
230	Fecal	Fattening	+	2	100	2.0	>256	0.19
231	Fecal	Fattening	+	2	500	2.7	256	0.19
232	Fecal	Fattening	+	2	100	2.0	256	0.094
233	Fecal	Fattening	+	2	7'100	3.9	>256	6
234	Fecal	Fattening	+	2	100	2.0	>256	8
235	Fecal	Fattening	+	2	100	2.0	256	0.125
236	Fecal	Fattening	+	3	100	2.0	256	0.19
237	Fecal	Fattening	-	3	200	2.3	256	0.19
238	Fecal	Fattening	-	3	100	2.0	256	0.125
239	Fecal	Fattening	-	3	100	2.0	256	0.19
240	Fecal	Fattening	-	3	300	2.5	256	0.19
241	Fecal	Fattening	-	3	200	2.3	256	0.19
242	Fecal	Fattening	-	3	3'100	3.5	256	0.25
243	Fecal	Fattening	-	3	200	2.3	256	0.125
244	Fecal	Fattening	-	4	200	2.3	256	0.19
245	Fecal	Fattening	-	4	500	2.7	256	0.25
246	Fecal	Fattening	-	4	100	2.0	>256	0.19
247	Fecal	Fattening	-	4	100	2.0	256	0.19
248	Fecal	Fattening	-	4	600	2.8	256	0.19
249	Fecal	Fattening	-	4	300	2.5	256	0.19
250	Fecal	Fattening	-	4	100	2.0	256	0.125
251	Fecal	Fattening	-	4	200	2.3	256	0.19
252	Fecal	Fattening	-	4	2'500	3.4	256	0.19
253	Fecal	Fattening	-	5	3'700	3.6	>256	0.125
254	Fecal	Fattening	-	5	200	2.3	>256	0.19
255	Dust	Farrowing	+	1, 2	70'000	4.8	64	0.25
256	Wipe	Farrowing	-	1, 2	2'000	3.3	96	0.19
257	Wipe	Farrowing	+	1, 2	200	2.3	>256	4
258	Wipe	Farrowing	-	3, 4	2'000	3.3	>256	24
259	Wipe	Farrowing	-	3, 4	2'000	3.3	>256	8
260	Wipe	Farrowing	+	1, 2	5'350	3.7	>256	4
261	Wipe	Farrowing	+	1, 2	5'900	3.8	24	0.047
262	Wipe	Farrowing	-	3, 4	200	2.3	256	0.19
263	Wipe	Rearing	+	3, 4	100	2.0	>256	8
264	Wipe	Fattening	+	3, 4	600	2.8	>256	>32
265	Wipe	Fattening	-	3, 4	200	2.3	>256	>32
266	Wipe	Fattening	-	3, 4	200	2.3	256	0.19
267	Wipe	Fattening	-	3, 4	1'000	3.0	>256	0.19
268	Wipe	Fattening-CL	+	1, 2	400	2.6	24	0.38
269	Wipe	Fattening-CL	-	1, 2	14'200	4.2	32	0.19
270	Wipe	Fattening-CL	+	1, 2	5'100	3.7	>256	6
271	Slurry	Farrowing	+	3, 4	200	2.3	>257	>32
272	Slurry	Farrowing	+	3, 4	16'000	4.2	>257	>32
273	Slurry	Rearing	+	1, 2	3'000	3.5	24	0.25
274	Slurry	Rearing	+	1, 2	100	2.0	256	0.125
275	Slurry	Rearing	+	1, 2	1'000	3.0	256	0.125
276	Slurry	Rearing	+	3, 4	8'000	3.9	>256	0.125
277	Slurry	Fattening	+	1, 2	4'000	3.6	256	0.125
278	Slurry	Fattening	-	3, 4	2'000	3.3	256	0.19
279	Slurry	Fattening	-	3, 4	200	2.3	>256	0.19

Key:
QNSE CFU/g or ml: Quinolone non-susceptible *E. coli* counts in colony forming units per gram feces/dust/wipe or per milliliter slurry
QNSE log CFU/g or ml: quinolone non-susceptible *E. coli* counts in log10 colony forming units per gram feces/dust/wipe or per milliliter slurry
MIC_{NA} : minimal inhibitory concentration of nalidixic acid in microgram per milliliter (µg/ml)
MIC_{CIP}: minimal inhibitory concentration of ciprofloxacin in microgram per milliliter (µg/ml)
SPS+ : connected to a sow-pool-system
SPS-: not connected to a sow-pool-system
Fattening-CL: Fattening unit after individual cleaning procedure

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