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Treatment of pigs with enrofloxacin via different oral dosage forms – environmental contaminations and resistance development of *Escherichia coli*

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ABSTRACT

Background: Antibacterial agents play important roles in the treatment of bacterial infections. However, the development of antimicrobial resistance (AMR) and carry-over of substances into the environment are several problems arising during oral treatment of bacterial infections. We assessed AMR development in commensal *Escherichia coli* (*E. coli*) in enrofloxacin treated and untreated animals. In addition, we examined fluoroquinolone in the plasma and urine of treated and untreated animals, and in sedimentation dust and aerosol.
Methods: In each trial, six pigs were treated with enrofloxacin via powder, granulate or pellet forms in two time periods (days 1–5 and 22–26). Four pigs served as untreated controls. The minimum inhibitory concentration (MIC) was determined to evaluate AMR development. Analysis of enro- and ciprofloxacin was performed with high performance liquid chromatography.
Results: Non-wildtype *E. coli* (MIC > 0.125 µg/mL) was detected in the pellet treated group after the first treatment period, whereas in the other groups, non-wildtype isolates were found after the second treatment period. *E. coli* with MIC > 4 µg/mL was found in only the pellet trial. Untreated animals showed similar susceptibility shifts several days later. Bioavailability differed among the treatment forms (granulate > pellet > powder). Enro- and ciprofloxacin were detected in aerosols and sedimentation dust (granulate, powder > pellet).
Conclusions: This study indicates that the kind of the oral dosage form of antibiotics affects environmental contamination and AMR development in commensal *E. coli* in treated and untreated pigs.

Keywords: Dust; aerosol; environmental pollution; swine; antibiotic resistance

INTRODUCTION

Antibacterial agents play important roles in the treatment of bacterial infections in humans and animals [1]. Successful therapy with these agents is difficult because of increasing antimicrobial resistance (AMR) in various bacteria. The antibiotics used in veterinary medicine are mainly those used in human medicine. Because of the rising resistance

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Conflict of Interest
The authors declare no conflicts of interest.

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problems and the ability of bacteria with pathogenic potential to infect both humans and animals, some antibiotics are classified as "highest priority critically important antimicrobials for human medicine" [2].

Most antibiotics in veterinary medicine are used for food-producing animals [1], e.g., enrofloxacin (ENR), which was developed for veterinary use only [3]. ENR is well tolerated and absorbed after subcutaneous, intramuscular or oral administration; and is mainly metabolized to its active metabolite, ciprofloxacin (CIP). CIP shows the same spectrum of activity as ENR and is used in, for example, the treatment of urinary tract infections in humans [4-7]. Up to 80% of fluoroquinolones are excreted in unchanged form or as metabolites via the urine [8].

In Germany, ENR is approved for oral or parenteral treatment of bacterial gastrointestinal and respiratory infections in pigs [9]. ENR treatment has been found to cause the development of resistant bacteria in treated animals, and ENR in fecal residues pollutes the environment [10-12]. Antibacterial treatments delivered via feed can cause high environmental contamination, owing to the loss of the substance during the feeding process [13,14]. Although in livestock production, antibiotic agents are often administered via feed or water [15,16], little is known about the effects of different oral dosage forms on environmental pollution and the development of AMR.

Thus, the objective of the present study was to determine the influence of different oral dosage forms of enrofloxacin on carry-over to the environment and the development of AMR in commensal fecal *Escherichia coli* (*E. coli*) in pigs.

MATERIALS AND METHODS

Drugs

ENR was purchased from Molekula GmbH Munich, Germany, and used to prepare the different oral dosage forms. For analytical procedures, ENR and CIP (reference standard, 99.8%, Bayer AG, Wuppertal, Germany) were used. Ofloxacin (Sigma, Germany) was used as an internal standard.

Pharmaceutical formulations

Granulated piglet standard feed (granulate Primo Pro, Deuka, Germany) was used to produce the different oral dosage forms (**Supplementary Fig. 1**). For all formulations, the standard diet was mechanically crushed into powder and supplemented with ENR for treatment with 2.5 mg ENR/kg bw. For pellet production (Institute for Animal Nutrition, University of Veterinary Medicine Hannover Foundation), ENR containing powder was pelletized with a pelletizing machine under high pressure. The pellets had a size of 4 mm (diameter) and were 0.7–0.9 cm in length. Granulate manufacturing was performed by the Institute for Particle Technology (iPAT), TU Braunschweig, as described by Stahl et al. [14].

Animals

Thirty clinically healthy weaned pigs (males and females 3–5 weeks old), weighing 7–12 kg at the beginning of the experiments, were included in the study. They were purchased from the Farm for Education and Research, University of Veterinary Medicine Hannover Foundation in Ruthe, Sarstedt (Germany), and were all from the same breeding program (db77® × db. Viktoria).

The animals had never been treated with fluoroquinolones before the start of the experiments. Nevertheless, they were preselected at their holding of origin to ensure that they had no resistant *E. coli* in their commensal gut flora. Only pigs with no coliform growth on a supplemented endo-agar plate with 0.125 µg/mL ENR were used. After 1 week of acclimation, the test was repeated.

This study was approved by the Lower Saxony State office for Consumer Protection and Food Safety with file mark 31.12-42502-04-11/0388.

Experimental design

In each experiment, ten pigs were randomly divided into two groups. Both groups were housed in the same stable in separate bays without direct contact with the other group, under standardized conditions (23 ± 2°C, 24–59% relative humidity, day-night cycle of 12 h) (**Fig. 1**). The treated group (n = 6) received ENR via feed, whereas the other group (n = 4) was an untreated control group (sentinels). During the treatment periods (days 1–5 and 22–26), the treated pigs received medicated feed once per day at the recommended dose of 2.5 mg ENR/kg bw [9] as the test substance via powder, granulate or pellets. The animals were weighed, and the amount of medicated feed was bodyweight adjusted. Although the animals were housed in groups, they were separated for the feeding procedure: the trough was separated with spacer bars to avoid incorrect dosing. All animals were given free access to tap water.

Before housing, the stable was cleaned with a high-pressure cleaner and disinfected with Venno Oxygen (Menno Chemie-Vertrieb GmbH, Norderstedt, Germany). To ensure that the stable was free from ENR resistant *E. coli* (MIC > 0.125 µg/mL), we collected environmental samples.

Protective clothing (gloves, disposable overalls and overshoes) was worn by the researchers to prevent the spread of microorganisms. Separate equipment was used to clean the bays for each group. Control animals were always fed first.

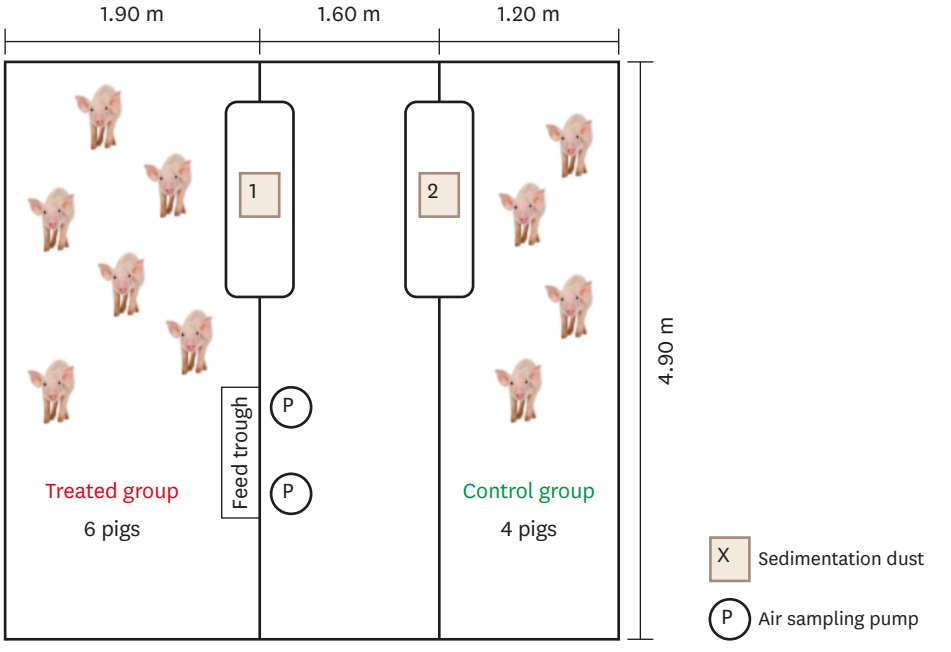

Fig. 1. Animal housing. Schematic plan of the stable with sampling points for sedimentation dust (1 and 2) and aerosol (P); treated animals were housed in a group of n = 6, and untreated animals were housed in a group of n = 4.

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Table 1. Sampling scheme

Treatment		x	x	x	x	x						x	x	x	x	x						
Fecal samples	x								x	Then every 7 d		x								x		x
Dust and aerosol samples	x		x		x		x			Then every 3–4 d			x		x		x					
Day (d)	0	1	2	3	4	5	6	7	8	9–20	21	22	23	24	25	26	27	28	29	30–56	57	END

Sampling times and days of treatment with 2.5 mg/kg bw enrofloxacin.
d = day.

Table 1 shows a schematic timeline for all experimental trials.

Isolation of *E. coli* from rectal fecal samples and MIC-determination

Rectal fecal samples were collected before the first treatment, then once per week (**Table 1**).

As described in Römer et al. [17], 1 g of feces was serially diluted in 9 mL physiological saline solution from 10^{-1} to 10^{-5}. Then 100 µL of 10^{-2} to 10^{-5} dilution was streaked out on antibiotic free endo-agar in duplicate, and a 10^{-2} to 10^{-3} dilution was streaked on antibiotic containing agar with 0.125 µg ENR/mL. In addition, 100 µL of 10^{-2} dilution was cultivated in 5 mL Luria-Bertani broth. After 24 h of incubation (37°C), 100 µL of the overnight culture was plated on endo-agar with 4 µg ENR/mL in duplicate.

If bacterial growth was observed on agar plates, ten *E. coli*-typical single colonies on the antibiotic free and ENR containing agar were dabbed with a cotton swab, and a fractionated smear was made on Columbia agar with 7% sheep's blood and incubated for 24 h (37°C). To calculate the amount of bacteria (colony forming units, CFU) as a percentage, we set the sum of ten colonies/animals per group as 100% (≙ colonies in the treated group and 40 colonies in the untreated group).

To confirm the colonies as *E. coli*, we used LMX-broth modified by Manafi and Ossmer (Merck KGaA, Germany) and Kovcas' indole reagent (Sifin GmbH Berlin, Germany).

The MIC was determined with epsilometer tests (Mic Test Strips, Liofilchem, Italy) according to the manufacturer's protocol, as described by Scherz et al. [18].

For interpretation, an epidemiological cut-off (ECOFF) of 0.125 µg/mL ENR [19] and a clinical breakpoint (cbp) of 4 µg/mL ENR [20] were used. Isolates with MIC values below the ECOFF were defined as susceptible wildtype (WT). All isolates with an MIC above the ECOFF were classified as low-level-fluoroquinolone-resistant (non-wildtype; nWT). Isolates with values > 4 µg/mL ENR, corresponding to the cbp, were classified as clinically resistant (high-level resistant nWT) [21,22].

Blood samples

Blood samples were collected from the *vena cava cranialis* (in EDTA tubes). Samples from control animals were collected every 24 h in the morning on days 1 to 5. In treated animals, blood was collected every day of the treatment period at 1.5 and 7 hours after treatment. The plasma was separated via centrifugation for 10 min (3,000 × g, 4°C) and stored at −20°C.

Urine samples

Urine of untreated animals was sampled before treatment and on days 3, 5 and 7. The urine of treated animals was collected over a period of 10 h on day 1. Samples were stored protected from light at −20°C.

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Sedimentation dust and aerosol collection

Sedimentation dust was collected from predefined makrolon surface areas at two locations in the stable (height of 1.5 m) (**Fig. 1**). One sampling area was next to the treated group, and the other was directly in front of the bay of untreated animals.

Aerosol samples were collected with two air sampling pumps (PCXR8, SKC Inc., USA) positioned in front of the trough of the treated animals. The pumps were turned on for 8 hours with an air flow of approximately 3.5 L/min. The airborne particles were collected with a glass fiber microfilter (25 mm, Whatman, GE Heatlthcare UK Limited, UK). Environmental samples were stored protected from light at −20°C.

Analytical procedures and sample preparation

The samples were prepared and analyzed via high performance liquid chromatography as described by Scherz et al. [18].

All dust samples were spiked with 300 ng internal standard (ofloxacin). For extraction on a shaker, 3 mL dichloromethane (DCM; Poch S.A., Poland) was added (12 min aerosol, 20 min sedimentation dust). Sedimentation dust samples were centrifuged (4°C, 5,580 × g, 10 min). DCM was transferred to a glass vial and evaporated at 40°C under air flow. For analysis, the samples were reconstituted in 30 µL mobile phase (85% citrate buffer, pH 3.0, and 15% acetonitrile).

For the urine samples, 500 µL urine was spiked with 3 mg (treated group) or 0.3 mg (sentinels) ofloxacin and 50 µL NaOH (0.1 N), and this was followed by extraction with 1 mL DCM (shaken for 5 min) and centrifugation (3 min, 23,000 × g). The organic phase was transferred into a glass vial. Extraction was performed once more with 0.8 mL DCM with the residue of the first extraction. The organic phases from both extractions were evaporated (40°C, air flow). Subsequently, 500 µL mobile phase was used for reconstitution.

For plasma, 300 µL plasma was spiked with 300 ng ofloxacin, and extraction was performed with 1.2 mL DCM (5 min shaking). After centrifugation (5 min, 4°C, 23,000 × g), the organic phase was transferred to a glass vial and evaporated (air flow, 40°C). Then 300 µL mobile phase was used for reconstitution.

A 100 µL volume of the sample (sedimentation dust 30 µL) was injected into the high performance liquid chromatograph (508-autosampler; Beckmann, Germany) via a 126-solvent system pump a flow rate of 1 mL/min. Fractionation of the test substances was performed on a CC 250/4 NUCLEODUR 100-5 C18e, 25 cm column with a LiChri-ART 4-4, LiChrospher 100 RP-18e, 5 µm precolumn at 40°C. Detection was performed with a fluorescence detector (RF-551, Shimadzu Torrance, USA; excitation at 280 nm and emission at 450 nm). The limit of detection was 0.2 ng/mL, and the limit of quantification was 0.6 ng/mL.

Statistical analysis

The statistical analysis of the MIC results was performed in SAS software, version 9.3 TS Level 1 M3 (SAS Institute Inc., USA, 2012). For pairwise comparison, the Wilcoxon test was used; the joint reflection of logarithmic MIC values including all dosage forms was performed with multifactorial variance analysis. The significance level was set at 5% ($p < 0.05$).

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RESULTS

Susceptibility of commensal E. coli isolates from feces

Before the experiments, only WT-*E. coli* isolates were found in all fecal samples (**Fig. 2**).

The highest susceptibility shifts of *E. coli* occurred during pellet feeding. First low-level resistant nWT-isolates were detectable on day 8, whereas clinically resistant nWT-*E. coli* were observed from days 29–57.

In the powder and granulate trials, nWT-*E. coli* was detected on day 36, whereas in the powder experiment, very few tested isolates were found.

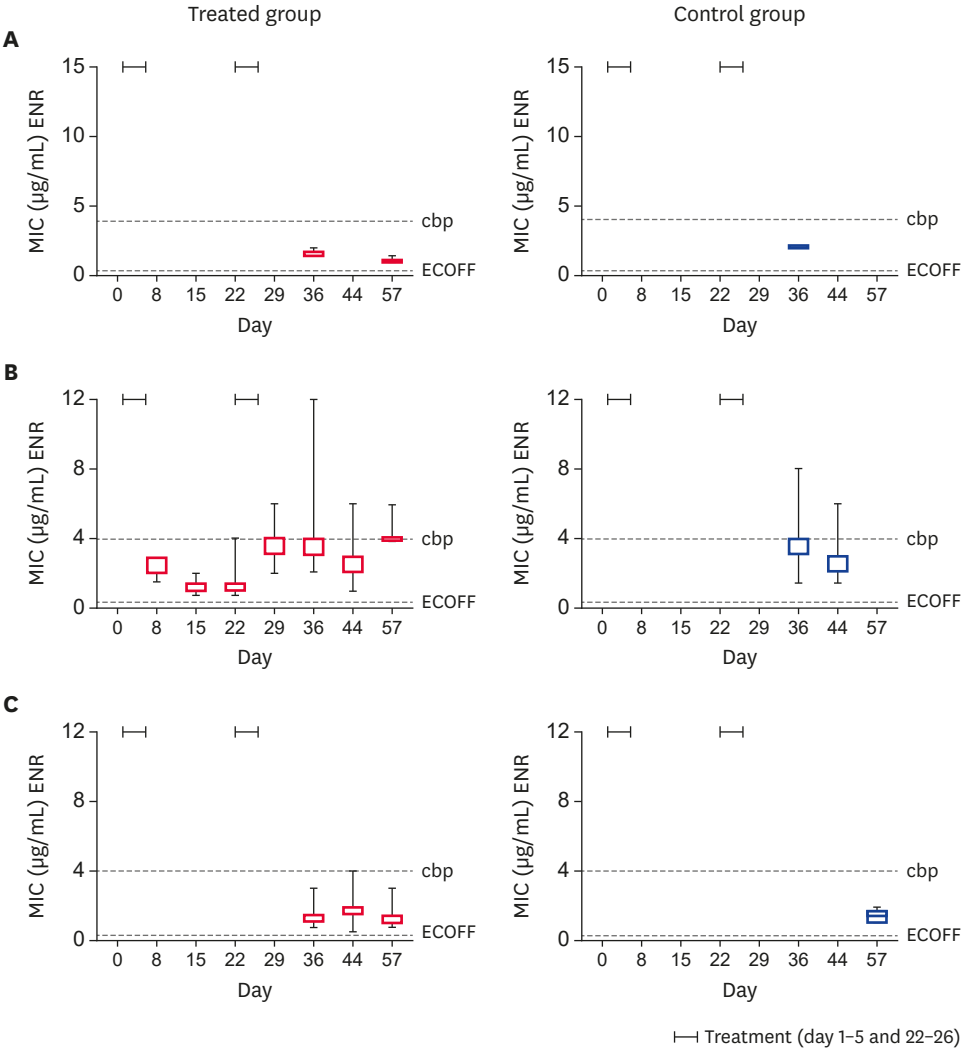

Fig. 2. Susceptibility of fecal *E. coli*. MIC values of treated and untreated control animals in the different experimental setups. (A) Powder, (B) Pellet, (C) Granulate.
treatment was performed on days 1–5 and 22–26; data are shown as box and whiskers (minimum to maximum).
MIC, minimum inhibitory concentration; ENR, enrofloxacin; cbp, clinical breakpoint (4 µg/mL ENR); ECOFF, epidemiological cut-off value (0.125 µg/mL ENR).

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Similar results were found in the control animals. In the powder trial, only one nWT-*E. coli*-isolate was found over the entire time period. In the granulate trial, several nWT-*E. coli* isolates were found on day 57 in one of four pigs, whereas in the pellet trial, clinically resistant nWT-E. coli were found on days 36 and 44. Single MIC values of nWT-*E. coli* are shown in **Supplementary Tables 1-6**.

Number of *E. coli* isolates

The number of *E. coli* isolates found in the different experimental trials is shown in **Table 2**.

In all treated groups, a strong decline of the amount of WT-*E. coli* was observed directly after the first treatment period (day 8). After the second treatment period (day 29), a massive decrease was observed in the powder and granulate treated groups, whereas only a slight decrease was observed in the pellet treated group. In treated animals, the highest amounts of nWT-*E. coli* were found in the pellet trial, compared with the powder and pellet trials.

In control animals, the amount of WT-*E. coli* slightly decreased in the powder and pellet trials between days 8 and 29 (**Table 2**), whereas in the granulate trial, no decrease was observed. The highest amounts of nWT-*E. coli* were found in untreated animals in the pellet trial, followed by the granulate and the powder trials.

Plasma levels of ENR and CIP

Differing plasma levels of ENR and CIP were found in treated animals in the different experimental groups (**Fig. 3**). In total, ENR administration via granulate resulted in the highest ENR values (maximum 277.2 ng/mL). In the pellet fed group, the maximum concentration was 194.5 ng/mL, and that in the powder fed group was 109.5 ng/mL. The CIP concentrations showed the opposite trend (powder 50.1 ng/mL > pellet 36.4 ng/mL > granulate 25.1 ng/mL). Area under the curve calculations of ENR for 120 h revealed the

Table 2. Amount of fecal *E. coli*

Groups	CFU in %					
	0 μg/mL ENR (WT-*E. coli*)			0.125 μg/mL ENR (nWT-*E. coli*)		
	Powder	Pellet	Granulate	Powder	Pellet	Granulate
Control group (day)						
0	100	100	100	0	0	0
8	82.5	100	100	0	0	0
15	100	90	100	0	0	0
22	87.5	87.5	100	0	0	0
29	100	90	100	0	0	0
36	100	100	100	2.5	62.5	0
43	100	100	100	0	72.5	0
57	100	100	100	0	0	20
Treated group (day)						
0	100	100	100	0	0	0
8	5	15	0	0	42	0
15	100	92	83	0	88	0
22	100	100	77	0	87	0
29	5	93	0	0	62	0
36	100	100	97	7	100	93
43	100	100	100	0	100	87
57	100	87	100	8	5	100

CFU in percentages on non-supplemented endo-agar plates (0 μg/mL ENR) and supplemented endo-agar plates with 0.125 μg/mL ENR in untreated control animals and treated animals (2.5 mg ENR/kg bw on days 1–5 and 22–26); the sum of ten colonies/plate/group was set as 100%; the reduced colony amount on 0 μg/mL ENR endo-agar plates is highlighted in gray; growth on 0.125 μg/mL ENR endo-agar plates is highlighted in blue.
CFU, colony forming units; ENR = enrofloxacin; WT, wildtype; nWT, non-wildtype.

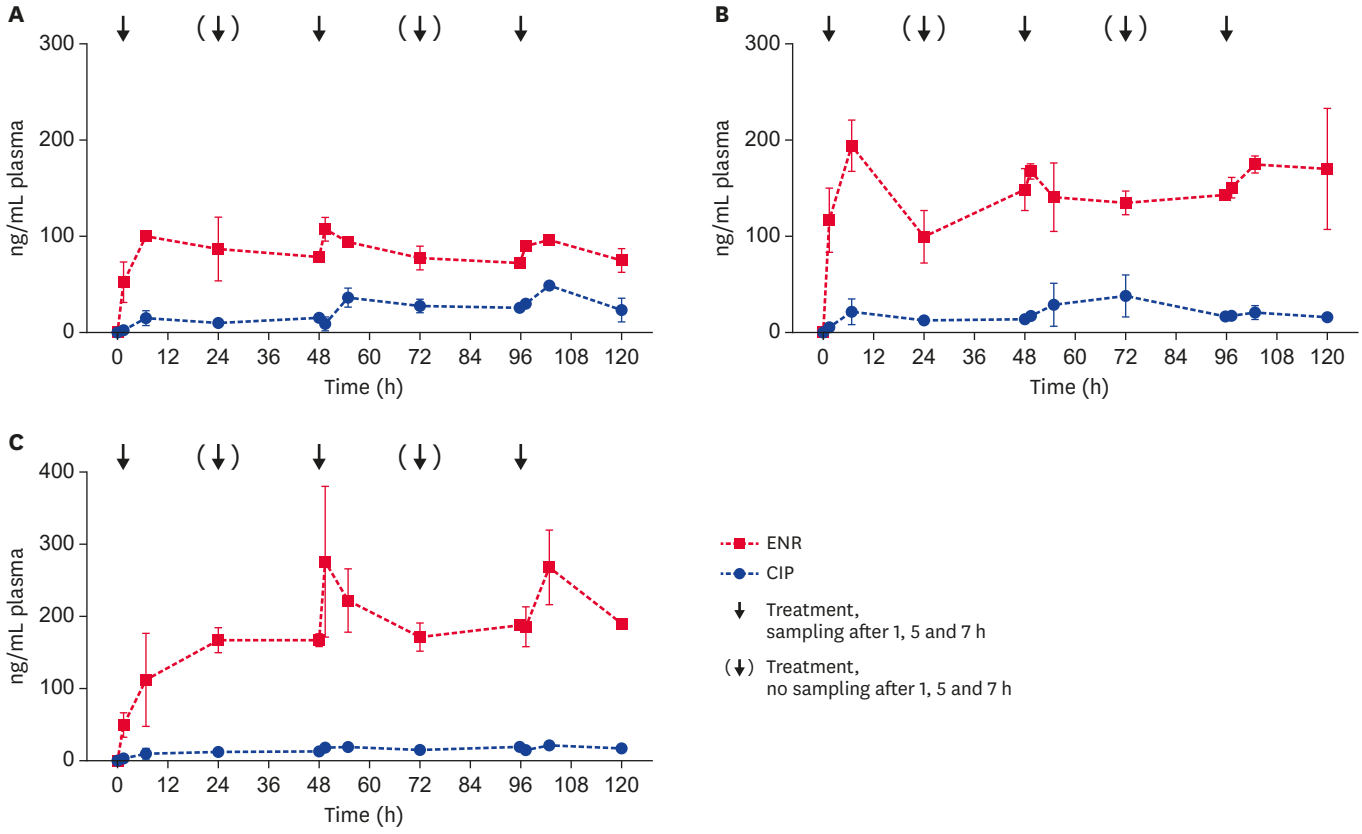

Fig. 3. Fluoroquinolone amounts in plasma. Amounts of ENR and CIP in the plasma of treated animals in different experimental setups. (A) Powder, (B) Pellets, (C) Granulate. Arrows indicate treatment with 2.5 mg/kg bw ENR. Data are shown as mean ± SD (n = 3).
ENR, enrofloxacin; CIP, ciprofloxacin.

highest values for granulate (21.65 μg×t/mL), followed by pellet (17.25 μg×t/mL) and powder (10.25 μg×t/mL).

Neither ENR nor CIP was detected in the plasma samples of the control animals at any time.

Urine levels of ENR and CIP

During treatment, the ENR urine concentrations were as high as 3.62 μg/mL in the powder treated group, 0.36 μg/mL in the pellet treated group and 1.04 μg/mL in the granulate treated group (data not shown). In the urine of untreated animals, the detected ENR was in only the nanogram range, whereas CIP was not detected at any time.

ENR and CIP content in sedimentation dust and aerosol

Comparison of the results of sedimentation dust and aerosol analysis indicated that the amounts of ENR and CIP were highest in the powder and granulate experiments, whereas pellet feeding resulted in lower environmental contamination (**Figs. 4** and **5**). Sedimentation dust samples collected next to the treated pigs exhibited higher amounts of ENR in the granulate trial, whereas similar results were found for both locations in the other trials.

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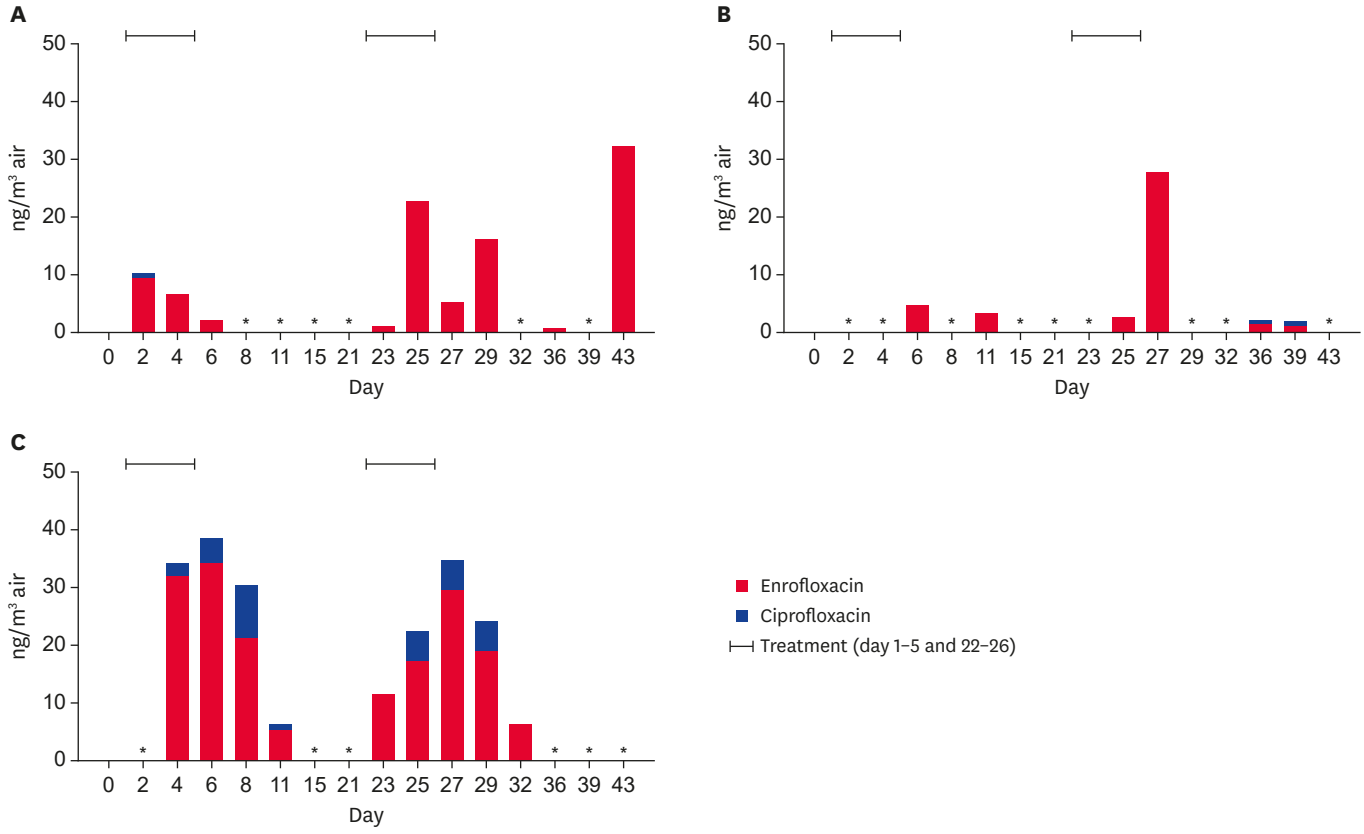

Fig. 4. Fluoroquinolones in aerosol. Enrofloxacin and ciprofloxacin content in aerosol in ng/m³ air (pooled results for two pumps); locations close to the trough of the treated group; *below limit of quantification. (A) Powder, (B) Pellets, (C) Granulate.
d, day.

DISCUSSION

The development of AMR in commensal and pathogenic bacteria poses a global threat in both human and veterinary medicine [23-27]. More than half of all antibiotics used are associated with the production of animals for food. Worldwide, antibiotics are used not only for therapeutic purposes in farm animals, but also for prophylaxis and metaphylaxis; outside the European Union, they are also used in certain regions to promote growth [28-31]. Independently of the administration route, the use of antibiotics leads to the carry-over of active substances into the environment [32,33]. Antibiotic residues persist in stable dust over several decades [34], whereas as many as five different antibiotics with concentrations of 0.2–12.5 mg/kg dust have been found in livestock farms [34]. Because large animal groups are often orally treated [16], an optimized oral formulation with low potential for AMR development in treated and untreated animals is essential in the One Health approach [35]. Stahl et al. [14] have shown that the use of low-dust sulfadiazine-containing oral formulations (pellet and granulate, compared with powder) decreases the carry-over of antibiotics to the environment. However, the effects of antibiotic residues on AMR development in untreated animals was not investigated.

Therefore, the present study was conducted to investigate the influence of different oral dosage forms on the carry-over of enrofloxacin to the environment and the development of AMR in commensal *E. coli* in treated and untreated animals. The applied dosage forms

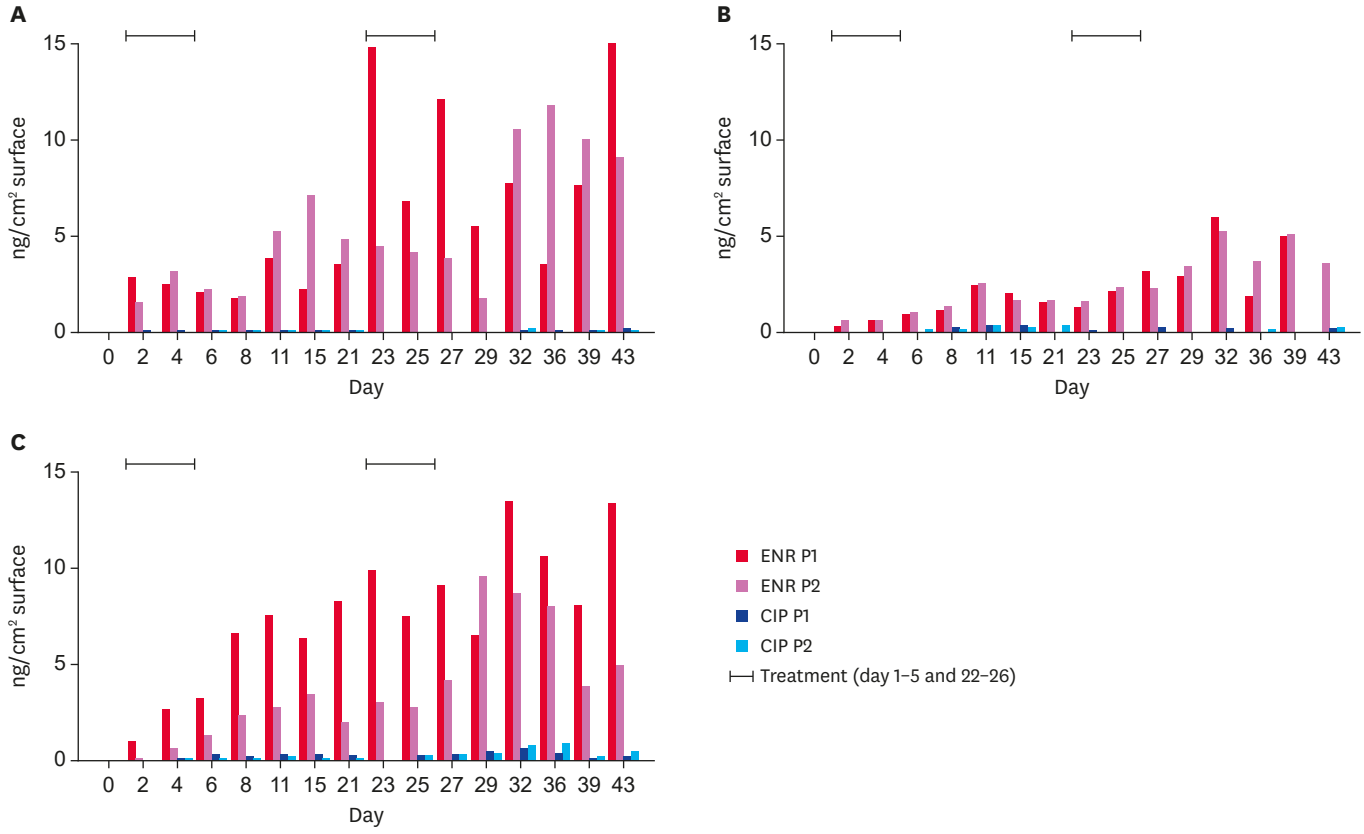

Fig. 5. Fluoroquinolones in sedimentation dust. ENR and CIP content in the sedimentation dust in ng/cm² area (P1: near the treated animals, P2: in front of the untreated animals). (A) Powder, (B) Pellets, (C) Granulate.
d, day; ENR, enrofloxacin; CIP, ciprofloxacin.

resulted in different plasma levels of ENR and CIP; the highest amounts of ENR were found with granulate, followed by pellet and powder treatments. These variations in bioavailability might have been be due to differences in the digestibility or absorption surface of the different dosage forms [16].

The metabolism of ENR to CIP differed among the experimental trials. This finding is notable, given that drug metabolism is mainly influenced by the age and weight of the animals [36]. Because both parameters were similar in all trials, the factors involved in the modification of metabolism remain a matter of speculation.

Another reason for the different ENR plasma concentrations might have been a loss due to dust formation. Similarly to the results for sulfadiazine [14], the greatest environmental contamination with ENR was caused by powder feeding. Because the granulate particles used in the present study were small, considerable dust formation would be expected. In accordance with findings from Stahl et al. [14], pellet feeding was associated with low environmental pollution.

The detection of ENR in the urine of untreated animals, as already shown for sulfonamides [13], demonstrated the importance of avoiding antibiotic carry-over to stables. Thus, such subtherapeutic concentrations of ENR residues in stables are bioavailable and pose a risk of AMR development [18]. In contrast to Stahl et al. [14], we detected no antibiotic agents

in the plasma of untreated animals. This difference might have been due to the higher environmental contamination in the study by Stahl et al. [14], in which as much as 8 μg sulfadiazine was found per m^3 air, whereas in the present study, the maximum ENR amounts were in the range of 30–40 ng/m^3 air. Antimicrobial agents in sedimentation dust and aerosols can also pose a health risk to farmers [37] and should therefore be avoided.

Susceptibility shifts were found in all experimental trials to different degrees. *E. coli* with MICs between the ECOFF and cbp were detected in the granulate and pellet treated groups, whereas clinically resistant isolates were found in the pellet trial.

In the powder trial, only a few resistant isolates were available for evaluation. The antibiotic concentration reached in the intestine might possibly have been too low for the selection of resistant isolates because of high loss into the environment during the feeding process. This result could be explained by the mutant selection window (MSW) hypothesis. The lower limit of the MSW represents the lowest concentration of an antibiotic at which the growth of most WT-bacteria is suppressed. Below this concentration, mutants have no selection advantage over WT-*E. coli* [38,39].

In the pellet trial, resistant nWT-*E. coli* with MIC values between the ECOFF and cbp were detected in the feces in four of six pigs in the treated group on day 8. Simultaneously, only several WT-*E. coli* were cultivated. This finding might also be explained by the MSW hypothesis. However, why the second ENR treatment via pellets did not lead to a significant decreased in cultivable WT-*E. coli* remains an open question. This population of bacteria was unlikely to be persistent, because they were present in too large numbers. Wiuff et al. [12] have observed that ENR treatment in pigs with small numbers of resistant *E. coli* (i.m. and orally, 2.5 mg/kg bw) initially decreased the total number of coliform intestinal bacteria. After 2 days of treatment, the total number had recovered, but the bacteria had been completely replaced by ENR-resistant bacteria. In addition, in a control group, some resistant isolates were found within the first 5 days and subsequently rapidly increased. Only after 10 days did the ENR-resistant phenotypes of both groups decrease again. On day 14, approximately 30% of bacteria were resistant [12].

Some clinically resistant *E. coli* were even detected in the pellet trial and were found until the end of the experiment. The reason for the high AMR development with pellet feeding might have been extended digestion time, owing to the shape of the feed particles [16] in comparison to powder or granulate. However, whether these nWT were always the same resistant phenotypes or whether *de novo* synthesis of ENR-resistant *E. coli* occurred remained unclear [39]. In this case, typing of the nWT-*E. coli* with pulse field gel electrophoresis could provide information on their origin [17].

Detection of nWT-*E. coli* in sentinels in all trials correlated with the detection of nWT-isolated in treated animals with a slight time shift, thus indicating a carry-over of resistant bacteria from treated to untreated animals rather than development of resistance caused by antibiotic residues.

Nonetheless, ENR was detected in the urine of some animals and could have led to AMR development [18]. Thus, further analysis of the isolates may reveal airborne *E. coli*-transmission [17].

In conclusion, oral antibiotic treatment via feed results in antibiotic carry-over to the environment and AMR development in commensal *E. coli* in pigs, depending on the oral dosage form. Subsequently, antibiotic residues in the environment and the transmission of resistant bacteria pose potential risks to animal health. The tested granulate combines good characteristics, such as high bioavailability and moderate AMR development, although its environmental pollution is high. Therefore, a substantial need exists for research on feed administration, in accordance with the One Health approach [35].

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Single MIC values of *E. coli*-Isolates in µg/mL of the control group per animal and day of the powder trial; green = colony count on antibiotic free endo-agar plates; orange = colony count on enrofloxacin supplemented endo-agar plates (0.125 µg/mL)

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Supplementary Table 2

Single MIC values of *E. coli*-Isolates in µg/mL of the treated group per animal and day of the powder trial; green = colony count on antibiotic free endo-agar plates; orange = colony count on enrofloxacin supplemented endo-agar plates (0.125 µg/mL)

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Supplementary Table 3

Single MIC values of *E. coli*-Isolates in µg/mL of the control group per animal and day of the pellet trial; green = colony count on antibiotic free endo-agar plates; orange = colony count on enrofloxacin supplemented endo-agar plates (0.125 µg/mL)

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Supplementary Table 4

Single MIC values of *E. coli*-Isolates in µg/mL of the treated group per animal and day of the pellet trial; green = colony count on antibiotic free endo-agar plates; orange = colony count on enrofloxacin supplemented endo-agar plates (0.125 µg/mL)

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Supplementary Table 5

Single MIC values of *E. coli*-Isolates in µg/mL of the control group per animal and day of the granulate trial; green = colony count on antibiotic free endo-agar plates; orange = colony count on enrofloxacin supplemented endo-agar plates (0.125 µg/mL)

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Supplementary Table 6

Single MIC values of *E. coli*-Isolates in µg/mL of the treated group per animal and day of the granulate trial; green = colony count on antibiotic free endo-agar plates; orange = colony count on enrofloxacin supplemented endo-agar plates (0.125 µg/mL)

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Supplementary Fig. 1

Oral dosage forms. Enrofloxacin containing feed; (A) Powder, (B) Pellets, (C) Granulate.

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REFERENCES

1. Ungemach FR, Müller-Bahrdt D, Abraham G. Guidelines for prudent use of antimicrobials and their implications on antibiotic usage in veterinary medicine. Int J Med Microbiol. 2006;296 Suppl 41:33-38. PUBMED | CROSSREF
2. World Health Organization. *Critically Important Antimicrobials for Human Medicine, 6th Revision*. Geneva: World Health Organization; 2019, 1-45.
3. Scheer M. Untersuchungen zur antibakteriellen Aktivität von Baytril. Vet Med Nachr. 1987;2:90-99.
4. Eliopoulos GM, Gardella A, Moellering RC Jr. *In vitro* activity of ciprofloxacin, a new carboxyquinoline antimicrobial agent. Antimicrob Agents Chemother. 1984;25(3):331-335. PUBMED | CROSSREF
5. Wingender W, Graefe KH, Gau W, Förster D, Beermann D, Schacht P. Pharmacokinetics of ciprofloxacin after oral and intravenous administration in healthy volunteers. Eur J Clin Microbiol. 1984;3(4):355-359. PUBMED | CROSSREF
6. Tyczkowska K, Hedeen KM, Aucoin DP, Aronson AL. High-performance liquid chromatographic method for the simultaneous determination of enrofloxacin and its primary metabolite ciprofloxacin in canine serum and prostatic tissue. J Chromatogr A. 1989;493(2):337-346. PUBMED | CROSSREF
7. Flammer K, Aucoin DP, Whitt DA. Intramuscular and oral disposition of enrofloxacin in African grey parrots following single and multiple doses. J Vet Pharmacol Ther. 1991;14(4):359-366. PUBMED | CROSSREF
8. Lomaestro BM, Bailie GR. Absorption interactions with fluoroquinolones. 1995 update. Drug Saf. 1995;12(5):314-333. PUBMED | CROSSREF
9. Vetidata. Drug: Enrofloxacin [Internet]. Leipzig: Vetidata; https://vetidata.de/. Updated 2019. Accessed 2019 May.
10. Burow E, Käsbohrer A. Risk factors for antimicrobial resistance in escherichia coli in pigs receiving oral antimicrobial treatment: a systematic review. Microb Drug Resist. 2017;23(2):194-205. PUBMED | CROSSREF
11. Hamscher G, Pawelzick HT, Sczesny S, Nau H, Hartung J. Antibiotics in dust originating from a pig-fattening farm: a new source of health hazard for farmers? Environ Health Perspect. 2003;111(13):1590-1594. PUBMED | CROSSREF

12. Wiuff C, Lykkesfeldt J, Svendsen O, Aarestrup FM. The effects of oral and intramuscular administration and dose escalation of enrofloxacin on the selection of quinolone resistance among *Salmonella* and coliforms in pigs. Res Vet Sci. 2003;75(3):185-193.
PUBMED | CROSSREF
13. Kietzmann M, Markus W, Chavez J, Bollwahn W. Drug residues in untreated swine. Dtsch Tierarztl Wochenschr. 1995;102(11):441-442.
PUBMED
14. Stahl J, Zessel K, Schulz J, Finke JH, Müller-Goymann CC, Kietzmann M. The effect of miscellaneous oral dosage forms on the environmental pollution of sulfonamides in pig holdings. BMC Vet Res. 2016;12(1):68.
PUBMED | CROSSREF
15. Merle R, Hajek P, Käsbohrer A, Hegger-Gravenhorst C, Mollenhauer Y, Robanus M, et al. Monitoring of antibiotic consumption in livestock: a German feasibility study. Prev Vet Med. 2012;104(1-2):34-43.
PUBMED | CROSSREF
16. Kietzmann M, Bäumer W. Oral medication via feed and water -- pharmacological aspects. Dtsch Tierarztl Wochenschr. 2009;116(6):204-208.
PUBMED
17. Römer A, Scherz G, Reupke S, Meißner J, Wallmann J, Kietzmann M, et al. Effects of intramuscularly administered enrofloxacin on the susceptibility of commensal intestinal *Escherichia coli* in pigs (sus scrofa domestica). BMC Vet Res. 2017;13(1):378.
PUBMED | CROSSREF
18. Scherz G, Stahl J, Glünder G, Kietzmann M. Effects of carry-over of fluoroquinolones on the susceptibility of commensal *Escherichia coli* in the intestinal microbiota of poultry. Berl Munch Tierarztl Wochenschr. 2014;127(11-12):478-485.
PUBMED
19. EUCAST. Antimicrobial wild type distributions of microorganisms. https://mic.eucast.org/Eucast2/regShow.jsp?Id=9596. Updated 2017. Accessed June 2021.
20. Baptista B, Delgado M, Costa M, Mendonc N, Duarte Correia JH, Canic M, et al. *In vitro* activity of enrofloxacin, marbofloxacin, orbifloxacin and ciprofloxacin against clinical Escherichia coli strains isolated from food-producing animals in Portugal. J Vet Pharmacol Ther. 2009;29:76.
21. Bagel S, Hüllen V, Wiedemann B, Heisig P. Impact of gyrA and parC mutations on quinolone resistance, doubling time, and supercoiling degree of Escherichia coli. Antimicrob Agents Chemother. 1999;43(4):868-875.
PUBMED | CROSSREF
22. Chang SK, Wei HW, Lo DY, Kuo HC. Antimicrobial resistance of *Escherichia coli* isolates from canine urinary tract infections. J Vet Med Sci. 2015;77(1):59-65.
CROSSREF
23. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015;40(4):277-283.
PUBMED
24. Lutz EA, McCarty MJ, Mollenkopf DF, Funk JA, Gebreyes WA, Wittum TE. Ceftiofur use in finishing swine barns and the recovery of fecal *Escherichia coli* or *Salmonella* spp. resistant to ceftriaxone. Foodborne Pathog Dis. 2011;8(11):1229-1234.
PUBMED | CROSSREF
25. Chantziaras I, Smet A, Haesebrouck F, Boyen F, Dewulf J. Studying the effect of administration route and treatment dose on the selection of enrofloxacin resistance in commensal *Escherichia coli* in broilers. J Antimicrob Chemother. 2017;72(7):2142.
PUBMED | CROSSREF
26. Wagner BA, Straw BE, Fedorka-Cray PJ, Dargatz DA. Effect of antimicrobial dosage regimen on *Salmonella* and *Escherichia coli* isolates from feeder swine. Appl Environ Microbiol. 2008;74(6):1731-1739.
PUBMED | CROSSREF
27. Mazurek J, Pusz P, Bok E, Stosik M, Baldy-Chudzik K. The phenotypic and genotypic characteristics of antibiotic resistance in *Escherichia coli* populations isolated from farm animals with different exposure to antimicrobial agents. Pol J Microbiol. 2013;62(2):173-179.
PUBMED | CROSSREF
28. Aarestrup FM. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. Int J Antimicrob Agents. 1999;12(4):279-285.
PUBMED | CROSSREF
29. Witte W, Klare I. *Antibiotikaresistenz Bei Bakteriellen Infektionserregern*. Berlin: Springer Verlag; 1999.

30. Schwarz S, Kehrenberg C, Walsh TR. Use of antimicrobial agents in veterinary medicine and food animal production. Int J Antimicrob Agents. 2001;17(6):431-437.
PUBMED | CROSSREF

31. Looft T, Johnson TA, Allen HK, Bayles DO, Alt DP, Stedtfeld RD, et al. In-feed antibiotic effects on the swine intestinal microbiome. Proc Natl Acad Sci U S A. 2012;109(5):1691-1696.
PUBMED | CROSSREF

32. Beyer A, Baumann S, Scherz G, Stahl J, von Bergen M, Friese A, et al. Effects of ceftiofur treatment on the susceptibility of commensal porcine *E.coli*--comparison between treated and untreated animals housed in the same stable. BMC Vet Res. 2015;11(1):265.
PUBMED | CROSSREF

33. Schulz J, Kemper N, Hartung J, Janusch F, Mohring SA, Hamscher G. Analysis of fluoroquinolones in dusts from intensive livestock farming and the co-occurrence of fluoroquinolone-resistant *Escherichia coli*. Sci Rep. 2019;9(1):5117.
PUBMED | CROSSREF

34. Schulz J, Ruddat I, Hartung J, Hamscher G, Kemper N, Ewers C. Antimicrobial-resistant *Escherichia coli* survived in dust samples for more than 20 years. Front Microbiol. 2016;7:866.
PUBMED | CROSSREF

35. Shallcross LJ, Davies SC. The World Health Assembly resolution on antimicrobial resistance. J Antimicrob Chemother. 2014;69(11):2883-2885.
PUBMED | CROSSREF

36. Studzinski T. The post-natal changes in minimal metabolic rate in the pig. J Physiol. 1972;224(2):305-316.
PUBMED | CROSSREF

37. Donham KJ, Zavala DC, Merchant J. Acute effects of the work environment on pulmonary functions of swine confinement workers. Am J Ind Med. 1984;5(5):367-375.
PUBMED | CROSSREF

38. Zhou J, Dong Y, Zhao X, Lee S, Amin A, Ramaswamy S, et al. Selection of antibiotic-resistant bacterial mutants: allelic diversity among fluoroquinolone-resistant mutations. J Infect Dis. 2000;182(2):517-525.
PUBMED | CROSSREF

39. Drlica K. The mutant selection window and antimicrobial resistance. J Antimicrob Chemother. 2003;52(1):11-17.
PUBMED | CROSSREF