Resistenzen bei Tier und Mensch - gemeinsame Forschung in Deutschland

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Significance of environmental contaminations on the development of bacterial resistance against antibacterial agents in indicator animals

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Bundesministerium für Bildung und Forschung

Topics

RESET I

Incorporation of antimicrobial substances in vegetables from manure-fertilized soil and microbiological effects

Significance of dosage and treatment duration on the development of bacterial resistance against antibacterial agents in farm animals

RESET II

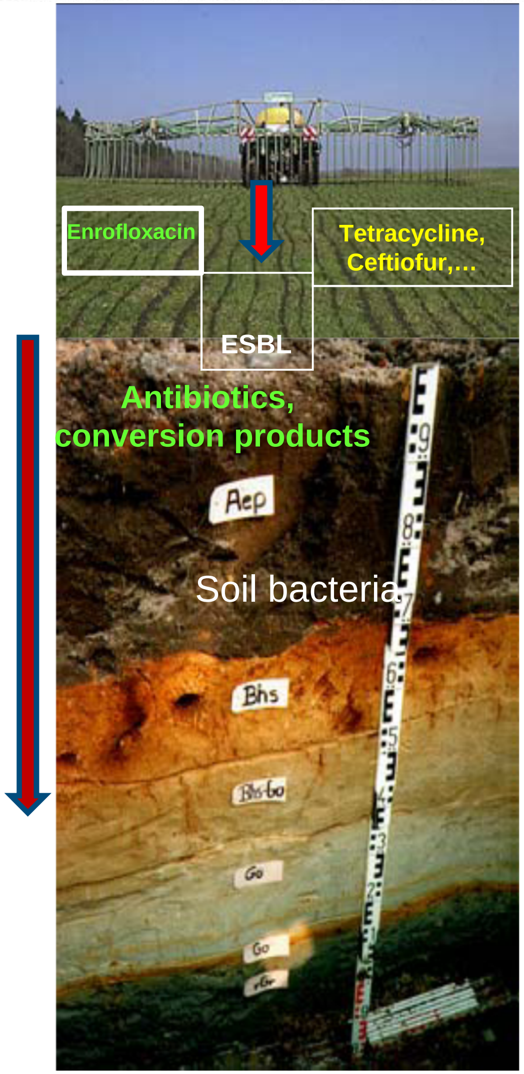
1. **Impact of antibiotics in plants on bacterial susceptibility *in vitro* and *in vivo***
2. **Influence of different oral dosage forms on environmental pollution and bacterial susceptibility**

Background I: first funding period

Tracing the pathways/exposure routes of veterinary antibiotics in agricultural systems

…from the source (animal production – excrements) - via fertilization of soil by liquid manure - transfer into crop plants ?

- **Model studies under farming conditions (approach to agricultural practise)**
- **Hydroponic model systems**

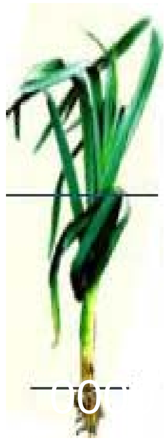


Seeding of white cabbage and leeks

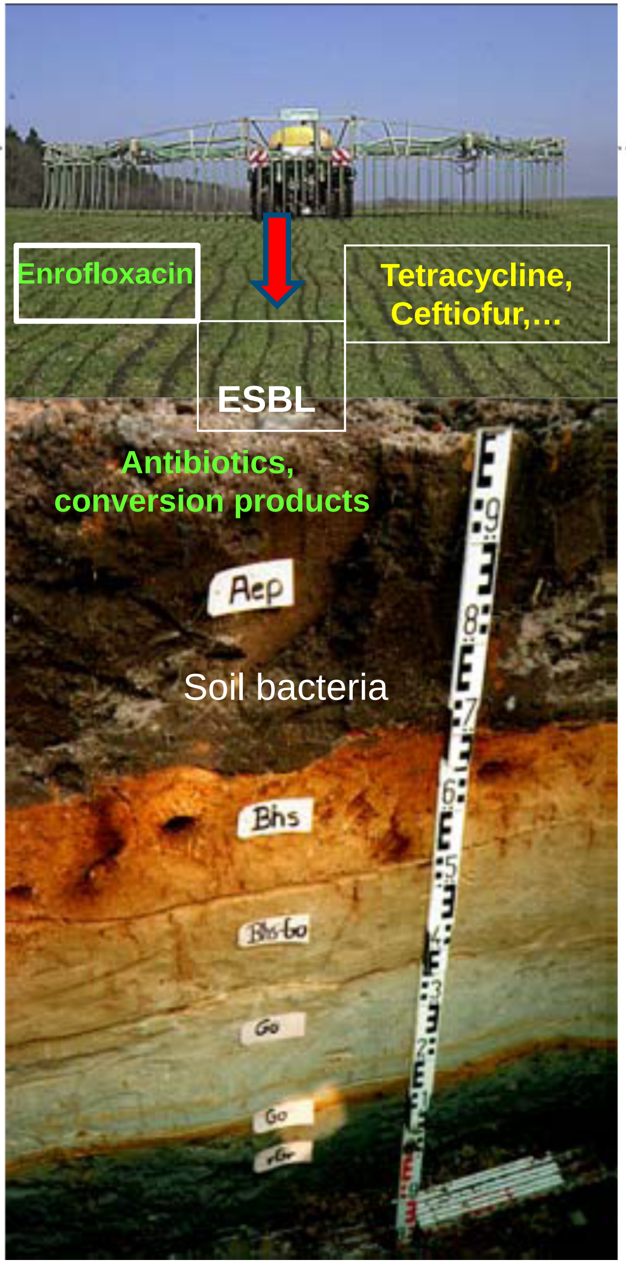
seedlings

14 days after planting: Fertilization in grooves next to the crop row

Cabbage field after harvesting: Sampling in Z-configuration



Trace-Analysis: Antibiotic residues, microbiology



Slurry dispersion on fields:

Impact of antibiotics and bacteria into soil

- **ESBL- *E. coli* survive in soil and on plants until harvest (4-5 months)**

- **Transfer of antibiotics from soil into edible parts of vegetable:**

Enrofloxacine, Tetracycline,..

Effects of low AB-concentrations in food and feed? Risk for consumers?

(Acc. to G.Hamscher, 2004)

Antibiotic contaminated food plants - a risk to consumers?

Comment of the Federal Institute for Risk Assessment (BfR)*

⇒"... that on the basis of the available data to the amount of AB residues (...) in food plants is from a toxicological point of view no risk for the consumer to be expected."

⇒"... the risk of resistance development in bacteria, which may be of importance for the humans, by long-term exposure to small amounts of antibiotics on plant foods .... about uptake and retention… in plant feed and food ....is conclusively not assessable."

→ Combined effects by incorporated antibiotics and microbial contamination?

* Release No. 019/2010 of 15 BfR February 2010

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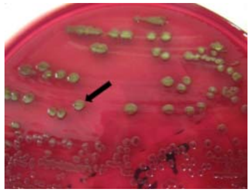
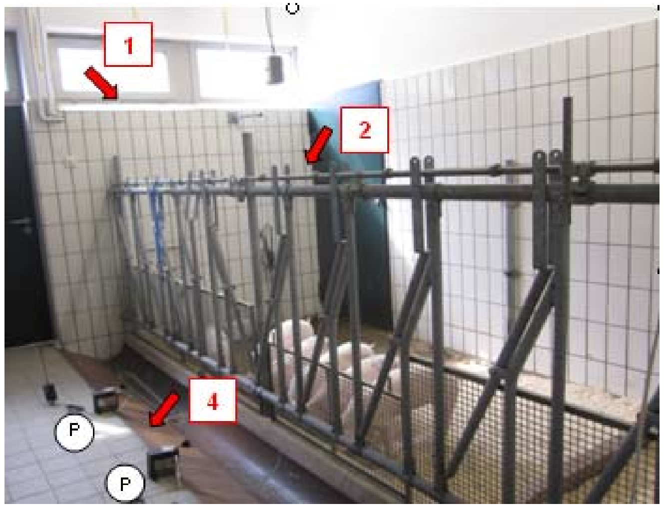
Background II: first funding period

- **Studies on the susceptibility of intestinal *E. coli* after oral (enrofloxacin) and parenteral (ceftiofur) antibiotic treatment in poultry and pigs**



- **Bioavailability determination of antibiotics (plasma)**
- **Determination of environmental antibiotic burden after treatment (dust and aerosol)**



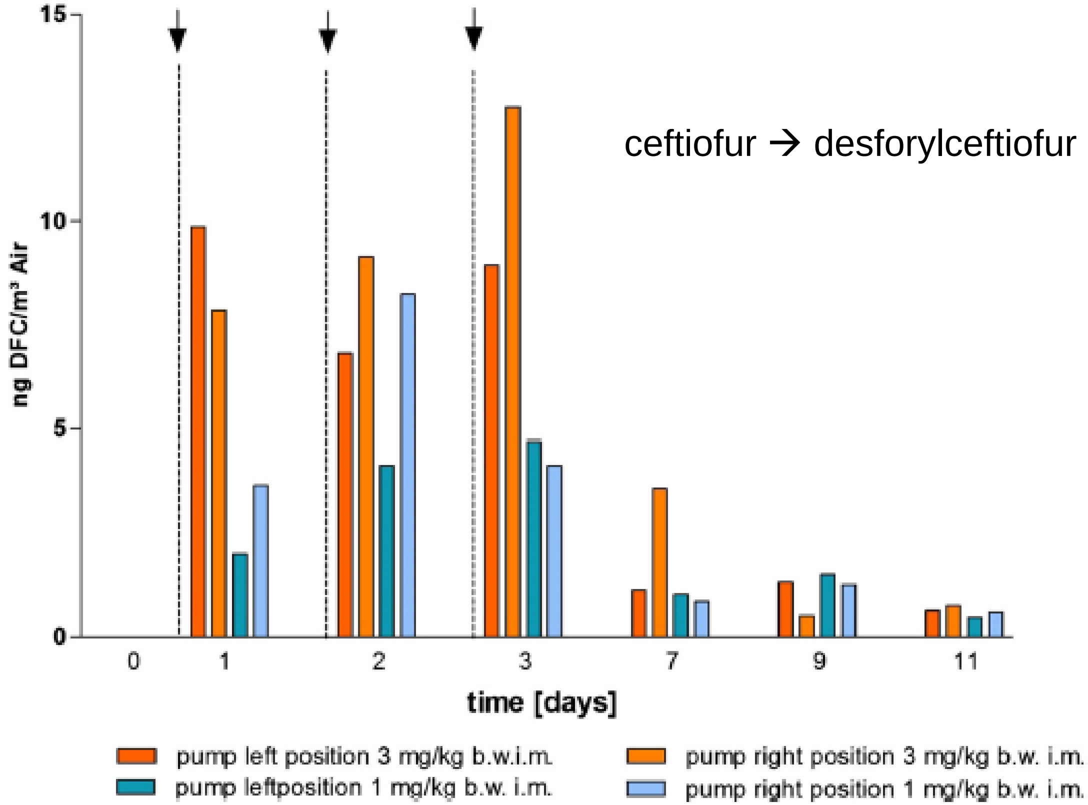
Scherz 2013, Zessel 2012

Environmental contamination after parenteral administration of ceftiofur

Antibiotic residues in the stable after antibiotic treatment with enrofloxacin (poultry, oral administration) and ceftiofur (pigs, parenteral administration) correlate with drug dosage

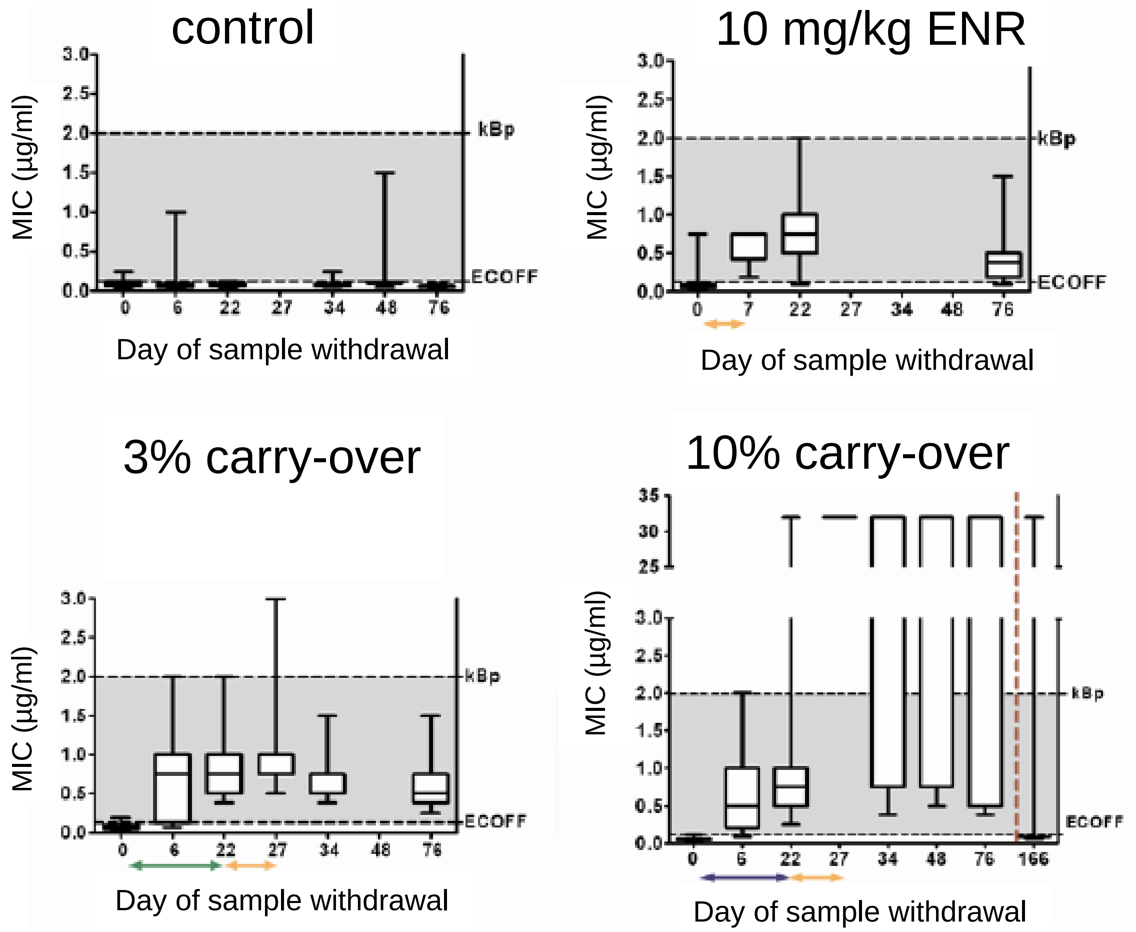
→ is a high environmental pollution associated with shifts in bacterial susceptibility of untreated animals?

ceftiofur → desforylceftiofur



Beyer et al. 2015

Influence of subtherapeutic ENR-dosages on bacterial resistance of commensal *E. coli* in poultry



Subtherapeutic dosages of enrofloxacin result in MIC-increases in commensal intestinal *E. coli*

→ **Cabbage feeding**
→ **MIC-shifts?**

10 mg/kg b.w.; day 1-6/22-27
1.0 mg/kg b.w.; day 1-22
0.3 mg/kg b.w.; day 1-22

Scherz 2013

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Enrofloxacin (ENR)

RESET II: Topics

Part 1. Antibiotics in plants and their impact on bacterial resistance

- *in vitro*
- *in vivo*

Part 2. Influence of different oral dosage forms on environmental pollution and bacterial susceptibility

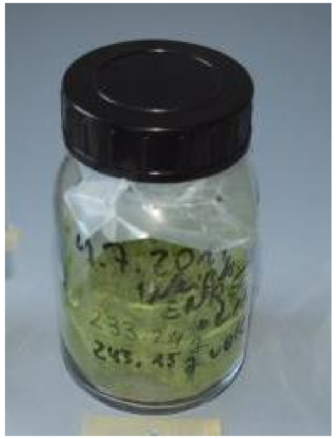
→ powder

→ pellets

→ granulate

Cabbage processing

- collection of leaves
- fresh leaf material
  bacterial inhibition tests
- freeze drying
- grinding

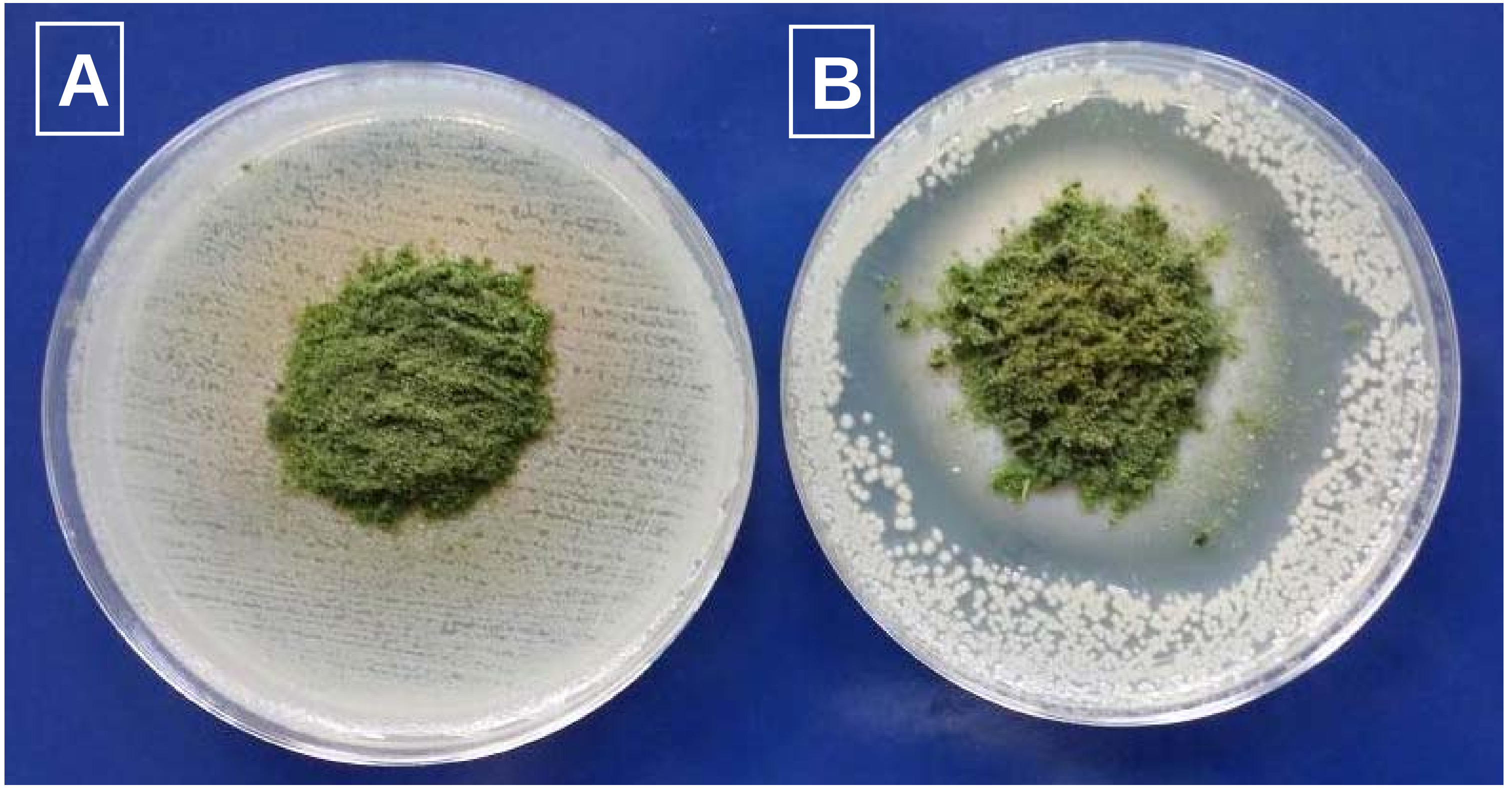


- ENR determination (LC-MS/MS)
- bacterial inhibition tests
- resistance development (MIC shift)
- animal feeding experiments in mice and poultry

collection of xylem sap

- ENR determination (LC-MS/MS)
- bacterial inhibition tests

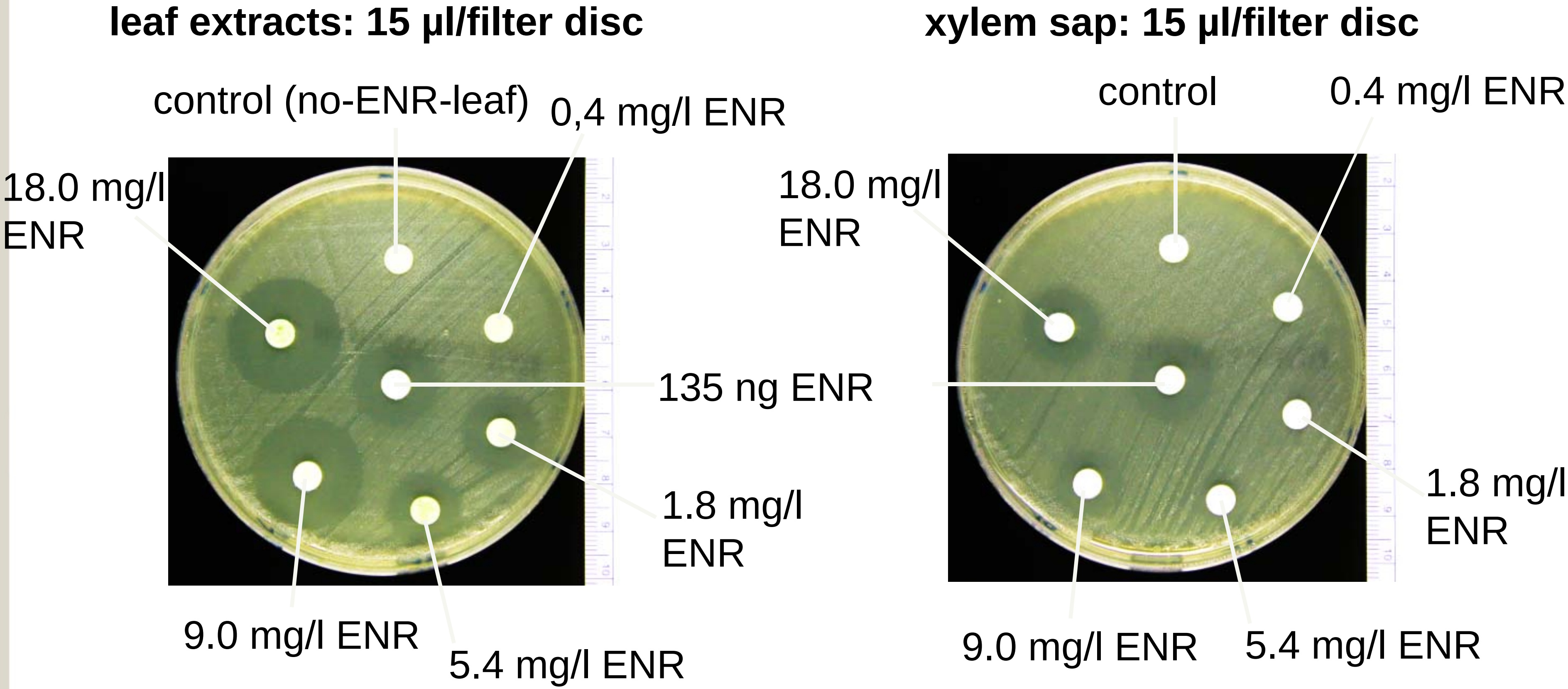
ENR release out of plant material?



A: Cabbage without ENR on Mueller-Hinton-agar (*E.coli* (MIC: 0.015 mg/l))
B: Cabbage with ENR (58 µg/g) on Mueller-Hinton-agar (*E.coli* (MIC: 0.015 mg/l))

Leaf extracts and xylem sap inhibit *E. coli* growth

- **bacterial inhibition test on nutrient rich agar plates**
- **plating of *E. coli* culture and addition of cabbage extracts**

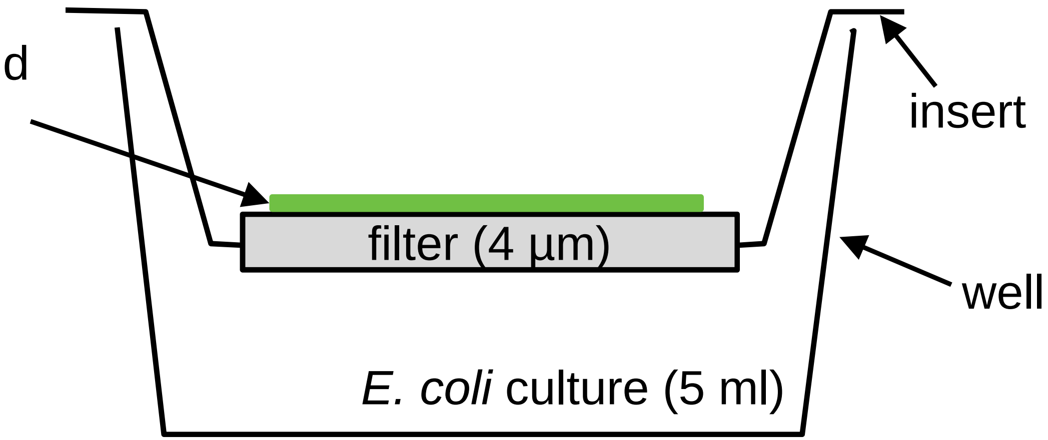


Incubation of *E. coli* with ENR-containing cabbage

➢ **bacterial resistance development?**

cabbage:

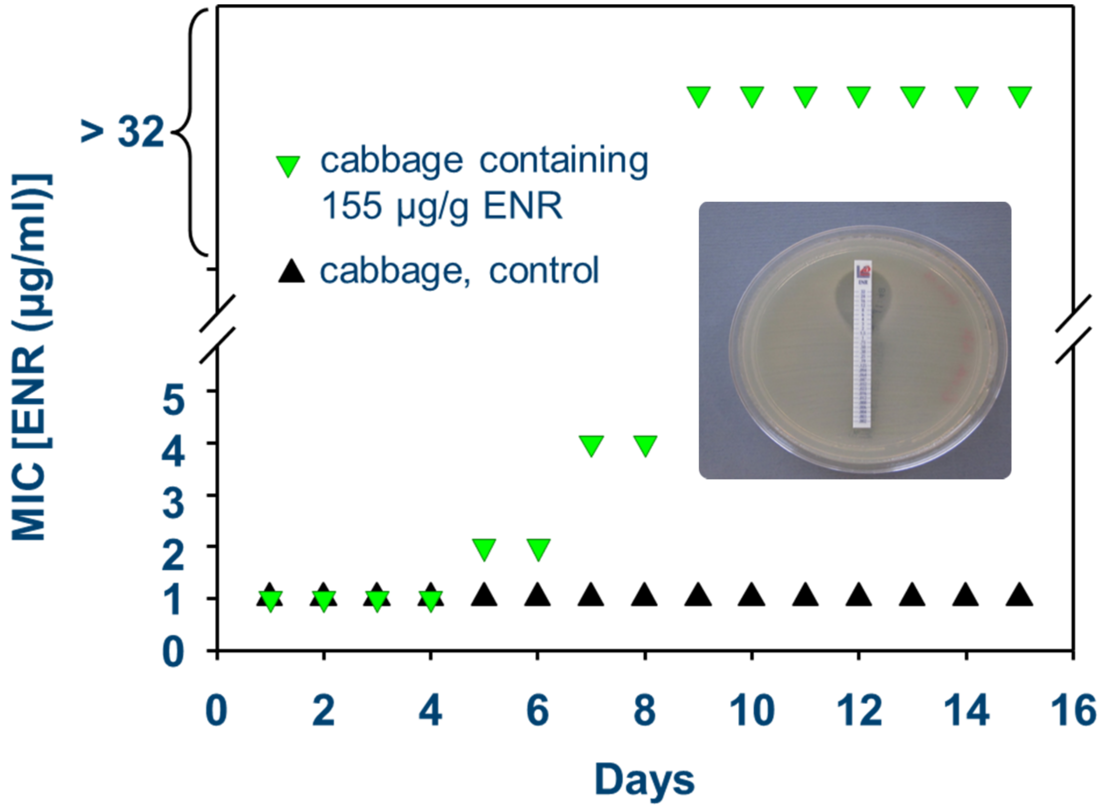
- freeze dried + ground
- 155 µg/g ENR
- 1 g per insert



15 days: every 24 h withdrawal of an aliquot from the *E. coli* culture

MIC shift assay: plating on agar plates containing increasing concentrations of ENR and Etest®

Incubation of *E. coli* with enrofloxacin-containing cabbage leads to a shift in the minimum inhibitory concentration (MIC) of ENR



1g of cabbage with 155 µg/g ENR, incubation with *E. coli* culture

MIC shift assay: plating on agar plates containing increasing concentrations of ENR and Etest®

Animal feeding experiments I

Mice

- feeding of ENR containing cabbage, 0.1 mg ENR/kg b.w. over 21 days followed by 5 days therapeutic dosage
- Samples of faeces and urine (cage)

Results

- detection of ENR in urine of mice in amounts of 0.4-2.7 ng/ml urine
- no MIC shift in *E. coli* isolates from mice faeces, only wild type isolates
- no development of resistance in commensal *E. coli* of mice

(MIC: 0.032 - 0.064 µg/ml)

Animal feeding experiments II

Chicken

- ENR over 21 days in subtherapeutic concentration, then ENR in therapeutic dosage for 5 days
- Samples of faeces and blood at several days
- Establishment of HPLC-method to determine ENR in faeces and blood samples of chicken

Results

- MIC of commensal intestinal *E.coli* → first MIC shift on day 6 in the cabbage group
- Content of ENR in faeces and blood→ analysis is still in progress

Summary part 1

Poster: Langenkaemper et al.

- **cabbage has a considerable capacity for uptake of ENR in hydroponic culture**
- **ENR that went through the chain -cabbage uptake, freeze drying, and grinding- is still active as an antibiotic substance (inhibition effect on growth of *E. coli* culture, MIC shift)**
- **mice receiving low doses of ENR via cabbage did not develop resistant commensal *E. coli*, ENR was detected in mice urine**
- **in chicken receiving low doses of ENR via cabbage a MIC-shift was detectable, analysis of bioavailability is after establishment of the determination method in progress now**

RESET II: Topics

Part 1. Antibiotics in plants and their impact on bacterial resistance

- *in vitro*
- *in vivo*

Part 2. Influence of different oral dosage forms on environmental pollution and bacterial susceptibility

→ **powder**

→ **pellets**

→ **granulate**

Aims part 2

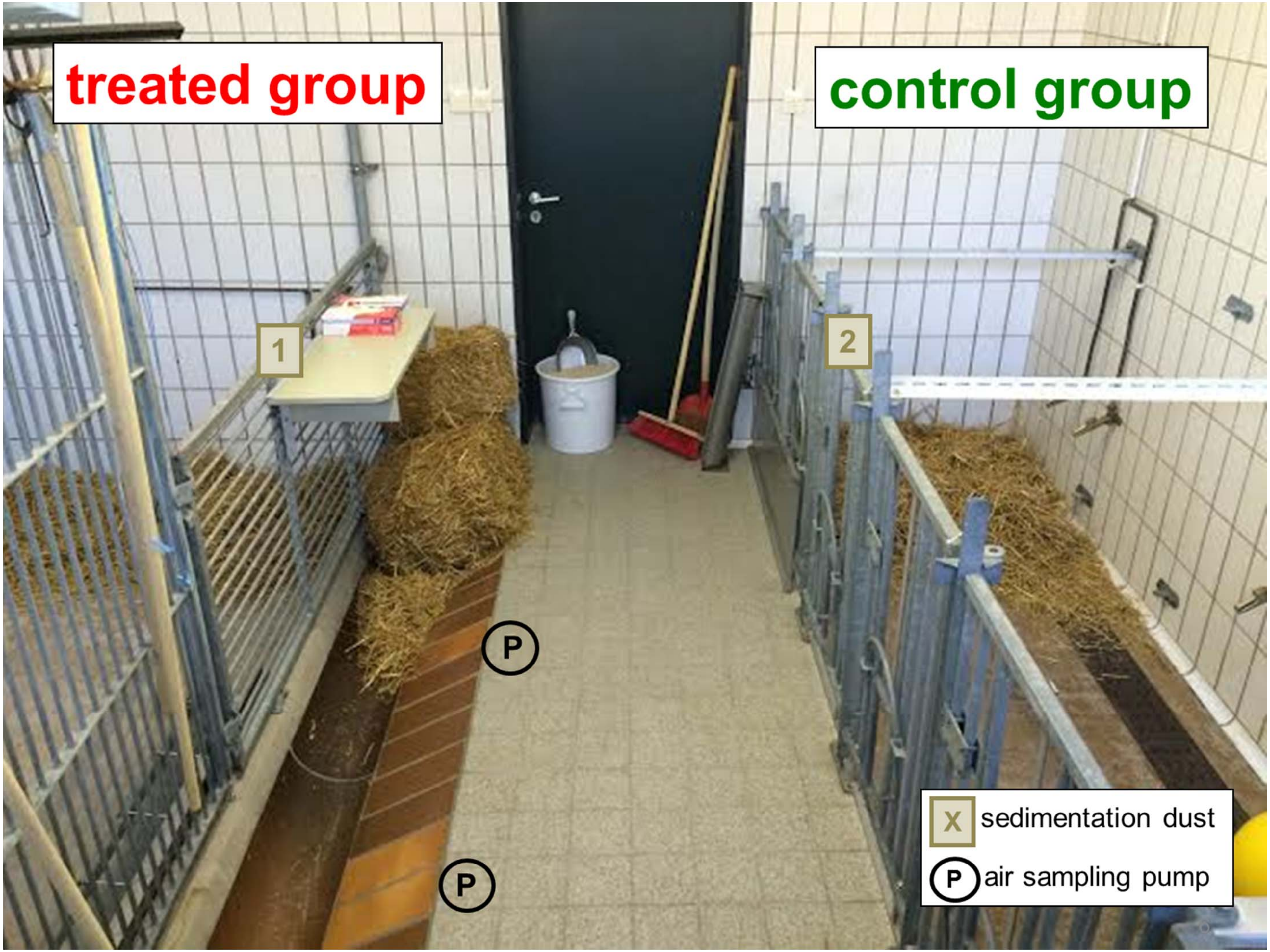
Influence of different oral dosage forms with antibiotics…

1. … on the susceptibility of commensal *E. coli* in treated pigs
2. … on the carry over in the environment
3. **… on the development of resistant *E. coli* in non treated pigs kept in the same stable**

→ powder

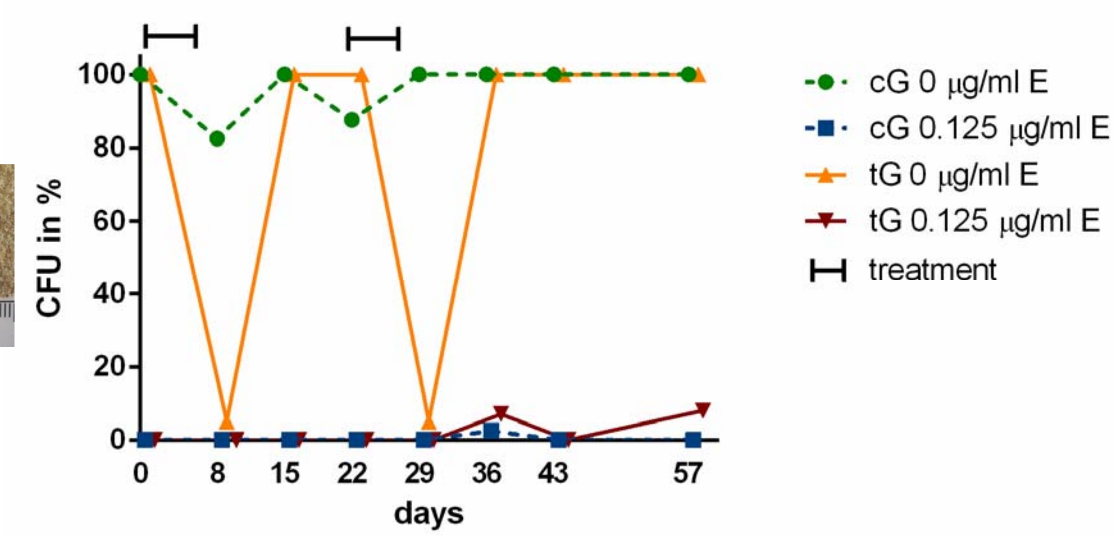
→ pellets

→ granulate


treated group
control group
1
2
P
P
X sedimentation dust
P air sampling pump

Bacterial susceptibility

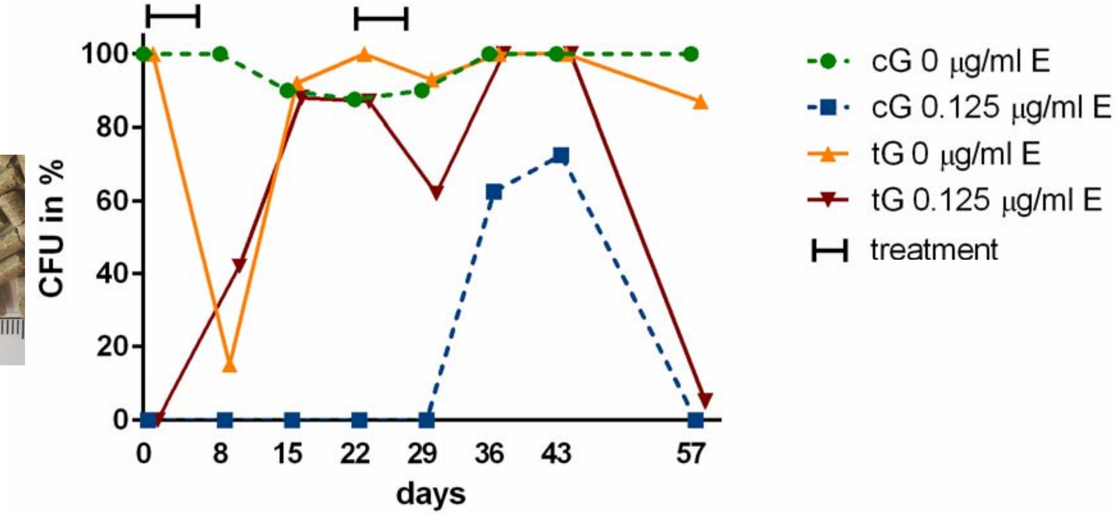
powder



cG = control group (sentinel)
tG = treated group

CFU= colony forming units
E = enrofloxacin
I—I = treatment period

pellet



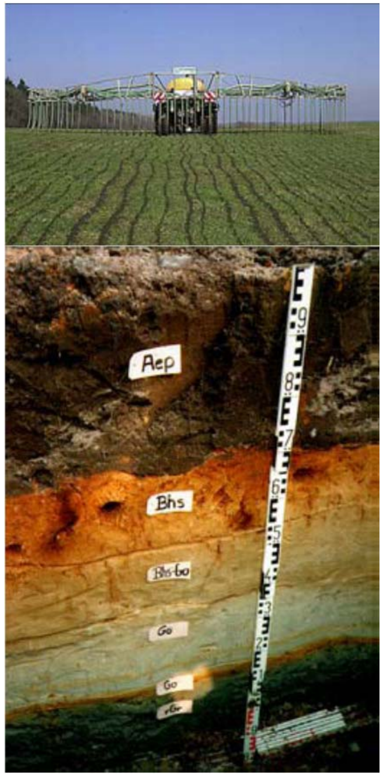
Diss. 2017; Hagedorn

Results and summary part 2

- **similar oral bioavailability of ENR in all dosage forms**
- **greatest MIC shift during pellet feeding, followed by granulate feeding (pellet > granulate >> powder)**
- **higher values of enro- and ciprofloxacin in sedimentation dust during oral treatment via powder and granulate**
- **no correlation between dust pollution and development of bacterial resistance**
- **MIC shift in sentinels detectable (granulate & pellet)**

Results & Outlook

•Risk for untreated animals
•Environmental pollution
•Different dosage forms?



New strategies?!?

Consumers

Thanks…

RESET

collaborative research project

http://www.reset-verbund.de

ESBL and (fluoro)quinolone Resistance in Enterobacteriaceae

Project No. 01KI1313A

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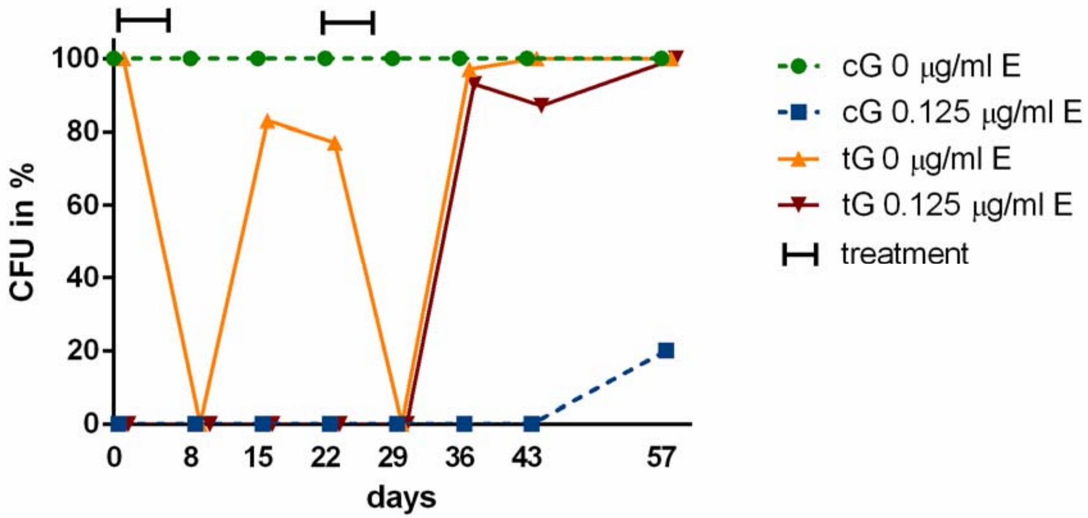
All RESET-members…for the excellent collaboration in the last years!

Granulate



100
80
60
40
20
0
CFU in %
0
8
15
22
29
36
43
57
days
cG 0 µg/ml E
cG 0.125 µg/ml E
tG 0 µg/ml E
tG 0.125 µg/ml E
treatment

MRI
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Paderborn University
Manfred Grote

RESET collaborative research project
http://www.reset-verbund.de

ESBL and (fluoro)quinolone
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Results

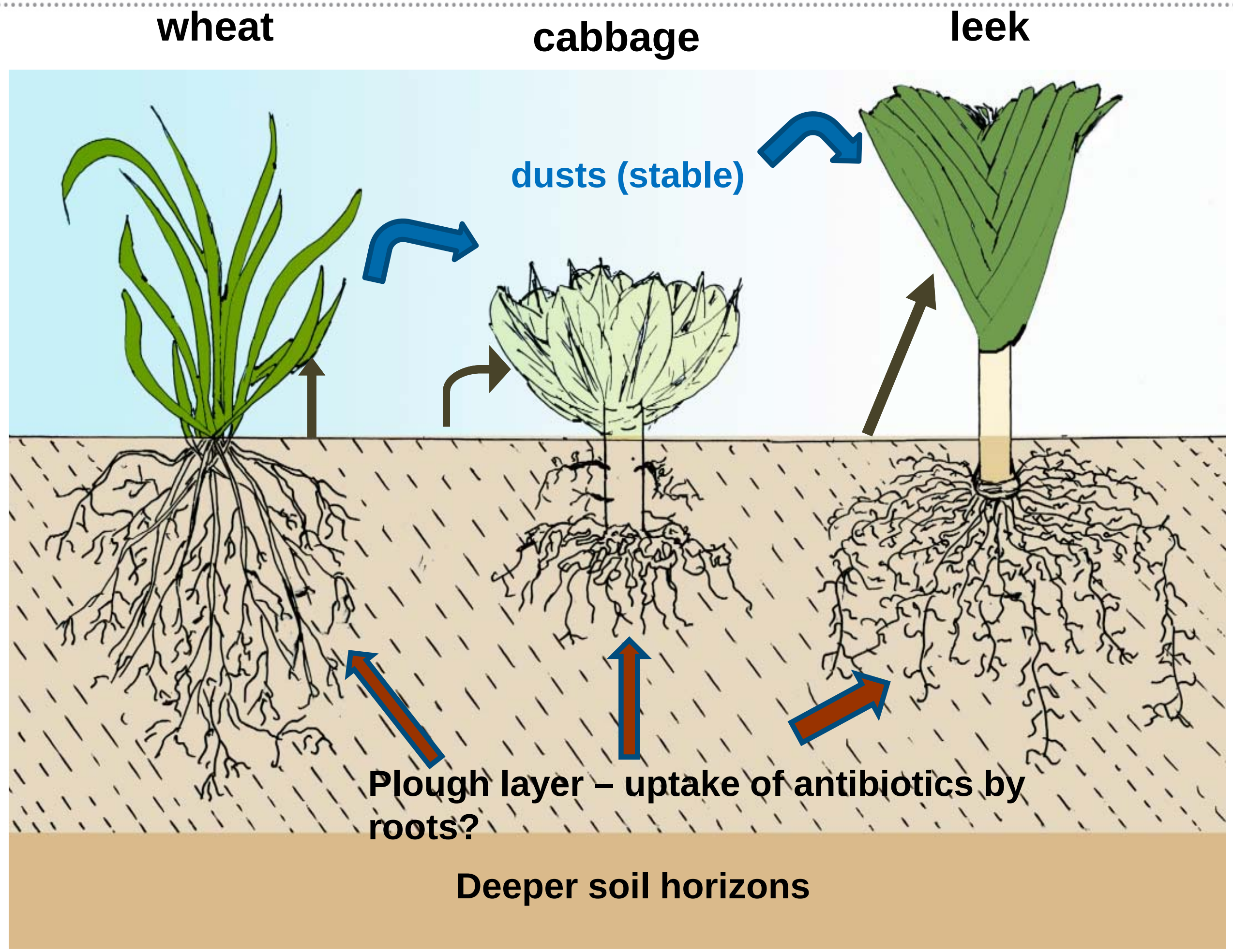
ESBL- *E. coli* survive in soil and on plants until harvest (4-5 months)

Transfer of antibiotics from soil into edible parts of vegetable:

Enrofloxacine, Tetracycline,..

Effects of low AB-concentrations in food and feed? Risk for consumers?

Possible pathways of antibiotics *(and bacteria)* to plants….



….exposure routes of antibiotics into the food chain

32

….. continuing uptake of low concentrations of antibiotics by contaminated crop plants:

→Promotion of initiation of resistance?

Possible exposure of consumers to antibiotics by contaminated crop plants:

→a risk to consumers?

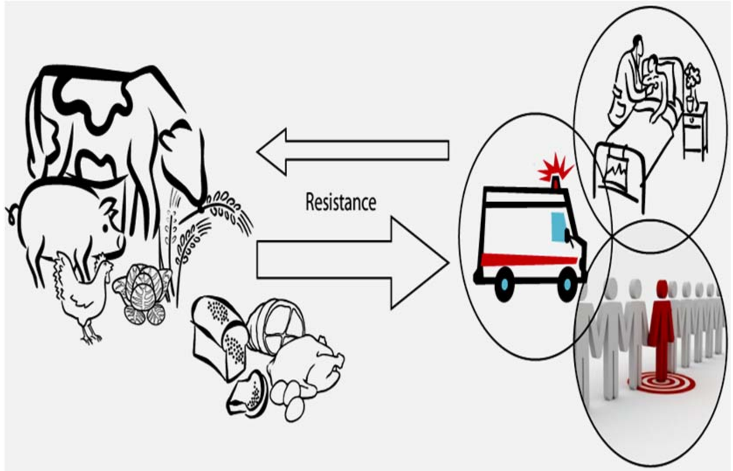
***sub-inhibitory concentration of antibiotics: below the *minimum inhibitory concentration* (MIC)**

Outlook

***In future research must be considered:**

Collateral effects of sub-inhibitoric doses of microbial active contaminants in food and feed,

such as effects on the intestinal microbiome in man and animal.



*According to: T. Looft, T. A. Johnson, H. K. Allen et al.,
In-feed antibiotic effects on the swine intestinal microbiome, PNAS, January 31**, 2012**, 109, 1691 - 1696

Experimentelle Ergebnisse

(Zum Vergleich der Ergebnisse: Folie von L. Kreienbrock, Allgemeiner Vortrag)

Behandlung von Tiergruppen mit Antibiotika führt zu Verschleppung in die direkte Tierumgebung

Gemüsepflanzen (Weißkohl, Porree) nehmen antimikrobiell wirksame Stoffe aus Böden auf

ESBL-*E. coli*, die zur Gülle dotiert worden sind, lassen sich in Boden und Gemüse nachweisen

Die Exposition mit subtherapeutischen AB- Konzentrationen übt einen Selektionsdruck aus

- Antibiotic resistance is one of the biggest threats to global health today.
  It can affect anyone, of any age, in any country
- Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process
- A growing number of infections are becoming harder to treat as the antibiotics used to treat them become less effective
- Antibiotic resistance leads to longer hospital stays, higher medical costs and increased mortality

➢ A post-antibiotic era—in which common infections and minor injuries can kill— is a very real possibility for the 21st century#

Antimicrobial resistance: global report on surveillance.
World Health Organization, 2014

World Health Organization

- **broad-spectrum antibiotic drugs, gyrase and topoisomerase inhibition in bacterial species, prevents bacterial cell division**

- **WHO list of critically important antimicrobials[1]**
- **- reserve antibiotics**
  - veterinary use is only considered acceptable provided that no alternative treatment is available

[1]**Answer to the Request for scientific advice on the impact on public health and animal health of the use of antibiotics in animals,** Antimicrobial Advice ad hoc Expert Group
European Medicines Agency, 2014

Bacterial resistance against fluoquinolones

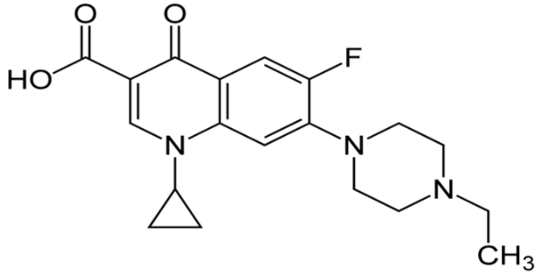
- **mutations of gyrase and topoisomerase genes**
- **efflux mechanisms**
- **plasmid encoded resistance**

- **Sub-inhibitory levels of antibiotic substances**

accelerate the emergence and spread of antibiotic-resistant bacteria among humans and animals[2]

bacterial resistance development at antibiotic concentrations several 100-fold below sub-minimal inhibitory concentrations, e.g. for ciprofloxacin[3]

[1]Sköld 2011 *Antibiotics and antibiotic resistance*. Wiley
[2]Andersson & Hughes 2014 Nature Reviews Microbiology **12**, 465
[3]Sandegren 2014 Upsala J. Med. Sci. **119**, 103



- most frequently used fluoroquinolone in animal production
- biotransformation, i.e. removal of ethyl group: ciprofloxacin[1]
- environmental distribution: is found in animal urine and excrements, waste water, is stable in slurry and soil[1]
- can be taken up by plants such as cucumber[2], lettuce[2] and red cabbage[3] from the soil (µg/kg f.w. range)
- has been detected in supermarket vegetables in China: detection frequency of quinolones >90%, concentrations from 10 to 193 µg/kg d.w.[4]
- leek, white and red cabbage readily take up ENR in hydroponic cultures (up to 6 mg/kg f.w. in leaves)[3, 5]

[1]Rusu et al. 2015 Environ. Chem. Let. **13**, 21 | [2]Lillenberg et al. 2010 Agron. Res. **8**, 807

[3]Chowdhury et al. 2016 J. Verbrauch. Lebensm. **11**, 61 | [4]Wu et al. 2011 Huan. Kexue/Evironm. Sci. **32**, 1703

[5]Grote et al. 2009 J. Verbrauch. Lebensm. **4**,287

Experimental model

- **substance:** enrofloxacin
- **animal:** pig
  → treated group and control group
- **indicator bacteria:** commensal intestinal *E. coli*
  → ECOFF* for enrofloxacin: < 0.125 µg/ml
- **treatment:** via different oral dosage forms

*ECOFF: epidemiological cut-off