Use of Antibiotics in Agriculture – Consequences for the Environment

After their use in animal husbandry, sulphonamide antibiotics are spread with the liquid manure onto the farmland. Despite an initially rapid decrease of the concentration in the soil, residual amounts remain detectable for months. In addition, rain can wash the antibiotics out of the soil into neighboring water bodies. A further problem is the presence of antibiotic resistant bacteria in the liquid manure. To what extent the use of antibiotics promotes the development and distribution of resistant bacteria is still unclear.

Antibiotics were hailed in the first half of the twentieth century as a miracle cure against bacterial infections. First used in human medication, they are today also indispensable in animal husbandry (see box "Use of antibiotics in animal husbandry"), and around 40 tonnes are applied in Swiss agriculture annually. However, their use involves two basic risks:

The first is that antibiotics, in part still active, in part in a derivative product form, enter the environment through animal excrements which are applied onto the agricultural land as liquid and solid manure. Thus, as much as a few hundred grams of antibiotics per hectare and year might be dispersed. Rain washes the antibiotics into the neighboring water bodies, but the precise fate of the antibiotics in the environment is still little understood. The second risk is that the antibiotics promote the development of resistant bacteria in the animals under treatment. Through the process of natural selection, mutations can create new resistance genes, and these, together with those already present in the bacterial community, can be passed on to other bacterial strains and species, resulting in a rapid dispersal of the resistance genes. If the resistance gene gets transferred to pathogenic bacteria, the results can be fatal, as these bacteria can no longer be combated by available antibiotics. The World Health Organization WHO ranks the problem of development and transfer of resistance genes as particularly serious, and sees the need for urgent action. To date, it is still unclear how great the risk of resistance development and dispersal is for the agricultural environment.

In a joint research project, we are, therefore, studying both the behavior of antibiotics in the environment and – by means of selected resistance genes as probes – the presence of resistant bacteria in agricultural soil. We are specifically interested in knowing whether a relationship exists between the use of antibiotics in agriculture and the appearance of resistance genes in the environment. Our project is part of the Swiss National Fund Research Program 49 "Antibiotics Resistance" [1].

Field Study Under Swiss Agricultural Practice

Starting point of our investigation was the consideration to carry out a field trial under practical conditions. Therefore, we spread liquid manure polluted with antibiotics on two perennial grassland lots of each 0.35 ha. The applications were made at the start of the vegetation period, on 24 March 2003, and after the first cut on 8 May 2003. The manure was applied using the band spreader technique (Photo 1). It was delivered from a pig feeder farm where the sulphonamide antibiotic sulphamethazine had been used as stabling prophylaxis (see box "Special case: sulphonamide antibiotics"). The sulphamethazine concentration in the fresh manure was 15 mg/kg – a heavy but realistic load [2].

Over a time period of four months prior and subsequent to application of the manure, soil samples were taken from the plots (Photo 2). Using a meteorological station directly on the lots, different parameters were recorded continuously, the most important of which was rainfall (Photo 3). Since both pastures were situated alongside a small stream, it was possible to determine the input of antibiotics into the stream wa-

Special Case: Sulphonamide Antibiotics

There were a number of reasons for using liquid manure containing the sulphonamide antibiotic sulphamethazine in our field study:

- **Sulphonamides are common in veterinary medication (e.g. in stabling prophylaxis). Mainly one active substance (sulphamethoxazole) from this group is used in human medicine.**
- **Sulphonamides are only moderately metabolized in animal organisms and relatively quickly excreted [2]. It is exceptional that the metabolites in the manure revert almost entirely to the original active agent form [3]. For our investigation, it is particularly important that the sulphonamides endure in the environment and are, therefore, detectable over a long time period.**
- **At EAWAG methods have been developed for the determinatiom of sulphamethazine in liquid manure [4], in soil [5] and in water [unpublished].**

Use of Antibiotics in Animal Husbandry

Antibiotics used in animal husbandry originate essentially from the same substance groups as human antibiotics: penicillins, tetracyclines, sulphonamides, macrolides, aminoglycosides, and fluorochinolone.

In veterinary medicine, antibiotics are used in the treatment of individual sick animals and in preventive treatment for the entire stock of animals. The procedure of preventive dosing the entire herd with antibiotics once a disease makes an appearance, for example when piglets or calves suffer from diarrhea or respiratory disease, is known as metaphylaxis. Prophylaxis, on the other hand, is applied before any of the animals fall ill, and is used, for example, to prevent infection during stabling of fattening animals from different farms.

Resistance gene	Origin
sul (I)	*Escherichia coli* (non-pathogenic)
sul (II)	*E. coli*
sul (III)	*E. coli*
tet (B)	*E. coli*
tet (C)	*E. coli*
tet (H)	*Pasteurella multocida* (opportunistic pathogen)
tet (M)	*Enterococcus faecalis* (opportunistic pathogen)
tet (O)	*Campylobacter coli* (pathogenic)
tet (Q)	*Bacteroides thetaiotaomicron* (non-pathogenic, found in human gastro-intestinal tracts)
tet (S)	*Listeria monocytogenes* (opportunistic pathogen)
tet (T)	*Streptococcus pyogenes* (opportunistic pathogen)
tet (W)	*Butyrivibrio monocytogenes* (anaerobic rumen bacterium)
tet (Y)	aus Schweinegülle isoliertes Plasmid (unknown bacterium)
tet (Z)	*Corynebacterium glutamicum* (soil bacterium)

Tab. 1: Investigated resistance genes and their origin. The bacteria from which the different resistance genes were first isolated and sequenced are shown. All resistance genes could subsequently be detected in other bacterial strains; *tet* (B), for example, was found up to now in 18 different strains. In most cases the presence of one of the various tetracycline or sulphonamide resistance genes was sufficient to make the carrier bacterium resistant to tetracyclines or sulphonamides, respectively. Opportunistic pathogens do not always cause disease, only for immunologically weakened patients.

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Photographs EAWAG

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ter. For this purpose, we built a measuring station 500 m downstream, which continuously measured the waterflow and automatically took water samples (Photo 4).

At EAWAG, we determinated the sulphamethazine concentrations in the soil (Photo 5) as well as in groundwater and stream water samples. In addition, the manure and soil samples were analyzed at the University of Utrecht by means of molecular biological methods for the presence of 14 different antibiotic resistant genes (Photo 6). 11 of the investigated genes are tetracycline resistance genes, the remainder are oriented against sulphonamides (see Tab. 1). With the applied technique, changes in the presence of the studied resistance genes are detectable providing qualitative or at best semi-quanitative information.

Higher Antibiotic Load in Soil After Manuring

Figure 1 shows the sulphamethazine content of the soil before and after the two manure applications. The sulphamethazine concentrations are expressed as average values over the entire lot, whereas local values can be as much as five times higher due to the heterogeneity. Before the first manuring, no sulphamethazine could be detected in the soil. After the manuring, the concentration leapt up and then fell over time. One day after the manuring, only 10% of the extracted amount was found in the soil pore water. The rest of the sulphamethazine was sorbed to soil particles or had been transformed. Only a few days later, sulphamethazine concentration in the soil had

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decreased further. During the next weeks, concentration remained relatively stable, so that the values at the time of the second manuring had not returned to zero. After the second application, the sulphamethazine concentration increased again.

13 of the 14 Resistance Genes Detectable

In the liquid manure used, we could detect 13 of the 14 tested resistance genes. We found at most 12 of the 14 genes (Fig. 1) in the soil samples. As opposed to sulphamethazine, various resistance genes were already present in the soil before the first manure application. 8 and 11 genes were clearly detectable in the two lots. Additional 1–4 genes gave only weak signals, indicating a probable low amount in the soil.

After manuring, the intensity of the resistance gene signals increased and 10–12 of the 14 resistance genes were clearly detectable over weeks (Fig. 1). We assume that these additional genes derive from the microflora of the manure.

Weather Determines the Fate of the Antibiotics

Interestingly, the weather after the two manure applications was very different. Whereas the first manuring was followed by a dry week without any rain and a precipitation poor April (60% of the long-term average), the week after the second application was very wet. This had a decisive effect on the fate of the sulphamethazine in the environment.

We found that the total sulphamethazine content of the soil increased less strongly after the second manure application with subsequent rain than after the first application without rain (Fig. 1). This was also the case for the sulphamethazine concentrations in the pore water: after the first manuring without rain, they were nearly twice as high (approx. 65 µg/l) as after the second manuring with rain (approx. 35 µg/l). In addition, a higher sulphamethazine concentration could be detected in the stream water

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Perennial pasture was used as the test field.

after the second manure application. It reached a maximum of 4 µg sulphamethazine per liter water (Fig. 2) and was slightly raised during the subsequent rainy period, while the concentration peaks became gradually less pronounced, the more time passed after the application. In contrast, the sulphamethazine concentration in the stream water after the first manuring at the end of March was considerably lower.

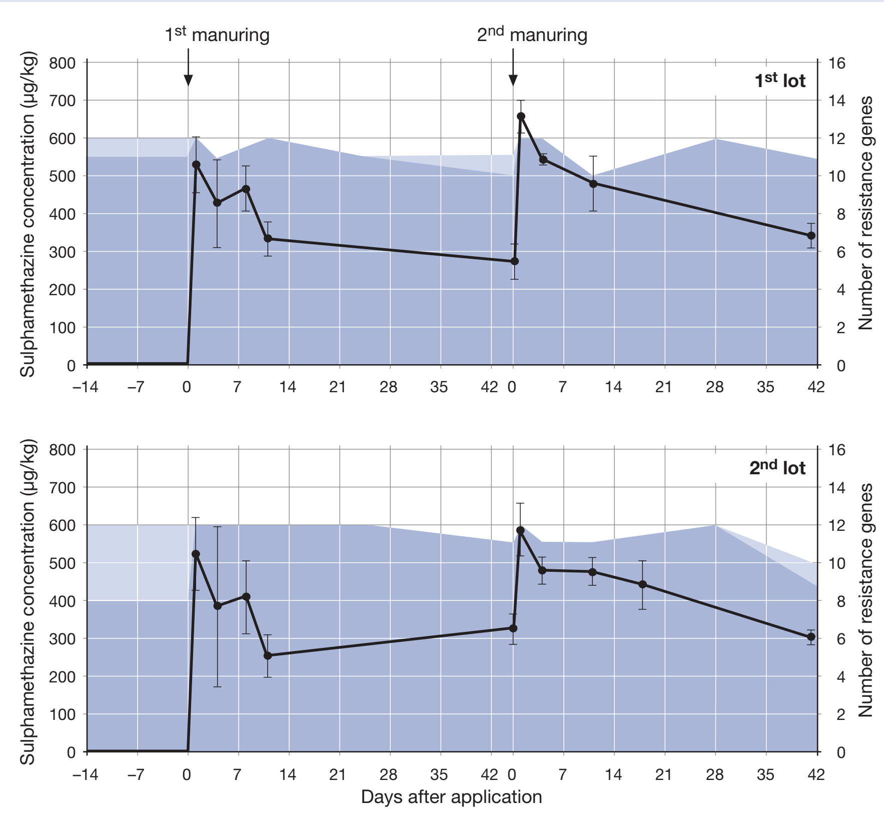

Fig. 1: Concentrations of sulphamethazine (black curve, average values plus standard deviation) and number of resistance genes (dark shaded area = clearly detectable resistance genes, light shaded area = additional, weakly detectable resistance genes) in soil samples of the two perennial pasture lots. On 24 March 2003 and 8 May 2003, sulphamethazine-containing liquid manure (15 mg/kg) was spread onto the fields.

Further Investigation Required

Our field study shows that the sulphonamide antibiotics can still be found in the soil months after the manure applications. The concentrations found in the soil clearly exceeded the so-called trigger value of 100 µg per kg of soil. The trigger value is defined as the threshold limit for the approval of new veterinary medications. If exceeded, environmental impacts must be evaluated in detail [6]. Results from other studies indicate that sulphonamides can have an effect on soil organisms: at a sulphonamide concentration of 1 mg/kg the enzyme activity of soil bacteria changed [7] leading to a reduction of soil respiration [8]. In our field study, these concentrations were locally clearly exceeded due to the heterogeneity in the soil. In addition, we could demonstrate that soil bacteria react with increased tolerance to sulphonamide antibiotics above concentrations of 10 mg/kg [9]. Therefore, it is crucial to investigate the effects of such environmental concentrations closer and to find out more about the bioavailability of sulphonamide in soil.

Our measurements allowed us to confirm the presence of resistance genes in both the manure and the soil. The presence of resistance genes in the environment is also proved by other studies [10, 11]. However, it still remains unclear whether additional antibiotic resistance genes are introduced with the manure into the soil. To be able to answer this question definitively, we need to quantify the genes. In addition, it is important to investigate whether the resistance genes are washed out of the soil by rain into the water bodies, leading to further dispersal of the resistance genes. And finally, it must be clarified whether an increased presence of resistance genes in the environment influences the appearance of resistance in pathogens.

Thus, it is currently not possible to carry out a proper evaluation of the risks that exist in the use of antibiotics in animal husbandry. Too much detailed information is missing. Nevertheless, our investigations lead us to the conclusion that antibiotics should be used carefully and with more awareness of the inherent risks.

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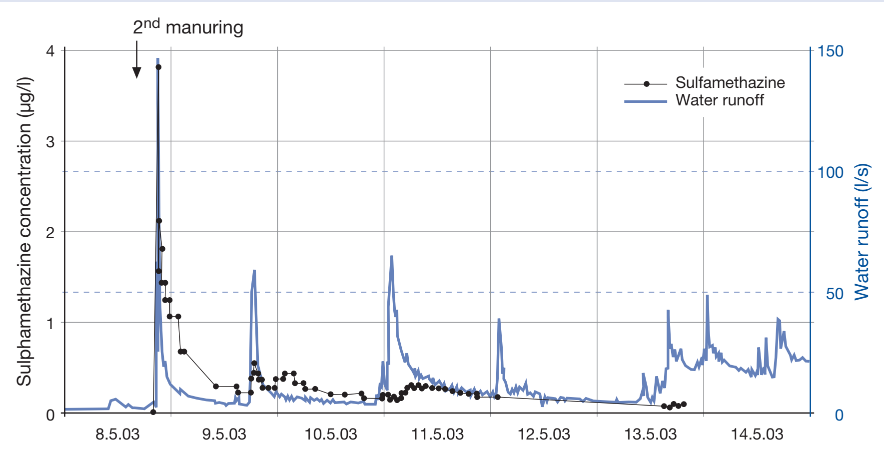

Fig. 2: Water runoff and sulphamethazine concentration in the stream during a rainy period after the second manure application on 8 May 2003.