DETERMINATION OF ANTIBIOTIC RESIDUES IN LEACHATE OF CONVENTIONAL AND ORGANIC DAIRY FARMS

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SUMMARY

The scientific interest in antibiotic substances in the environment has increased. The assumed quantity of antibiotics excreted by animal husbandry reaches thousands of tons per year. The environment is contaminated by these compounds on different pathways. To verify if animal husbandry can be a source of antibiotic residues showing up in the environment, manure and leachate samples from dairy farms were analysed. Neither in liquid manure nor in leachate were analysed antibiotics detected. As the main concern regarding the use of antibiotics is the development of resistant bacteria strains, further studies are implicitly required.

Keywords: agriculture, antibiotics, leachate, manure, residues

OBJECTIVES

More than 50 pharmaceutical compounds, or their metabolites, respectively, have been isolated from ground- and even drinking water (MÜCKTER, 2006). Even though the analysed concentrations are on a low level in areas next to drinking water resources, a possible impact on human and animal health can not be excluded. Therefore, and due to the recent achievements in the field of modern analytical techniques, the scientific interest in antimicrobially active compounds in the environment has increased during the last decade. There are three risks deriving from immoderate appliance of antibiotics resulting in environmental contamination with original substances or derivatives: the indirect impact on human and animal health via resistant micro-organisms, the direct organic damage and the influences on the biotic environment are a matter of concern.

In human as well as in veterinary medicine, antibiotics are used to treat and prevent disease. However, they are not completely eliminated in the organism, as they are bioactive substances, acting highly effectively at low doses and excreted after a short time of residence. Antibiotics are optimised with regard to their pharmacokinetics in the organisms: organic accumulation is, as in other pharmaceutics, objectionable and thus, they are excreted as parent compounds or metabolites (KÜMMERER et al., 2000; THIELE-BRUHN, 2003). Excretion rates are dependant on the substance, the mode of application, the excreting species and time after administration, but it has been shown that rates vary between 40% to 90% for tetracyclines and sulphonamides (BERGER et al., 1986; HALLER et al., 2001; HALLING-SØRENSEN, 2001). Administered medicaments, their metabolites or degradation products reach the aquatic environment by the application of manure or slurry to areas used agriculturally, or by pasture-reared animals excreting directly on the land, followed by surface run-off, driftage or leaching in deeper layers of earth (Figure 1).

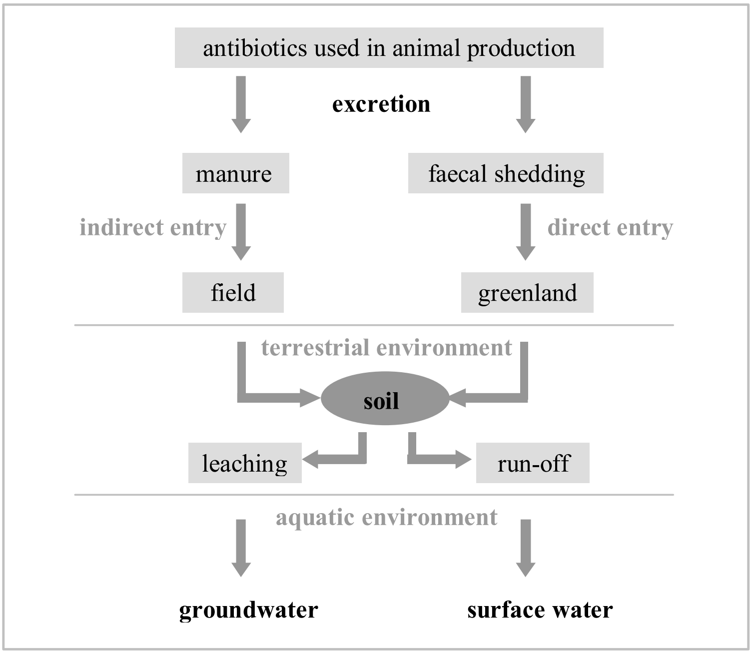

Figure 1. Exposure pathways of veterinary antibiotics in the environment

Concentration limits of antibiotics in the environment are not regulated, even though the growing concern has been taken into account with the prescription of environmental risk assessment of veterinary pharmaceuticals in the U.S and Europe (EMEA, 1997; THIELE-BRUHN, 2003). Risk assessment is realised by the calculation of predicted environmental concentrations (PEC) and comparison with predicted, biological non-effective concentrations (PNEC) (KÜMMERER, 2001a).

In Germany as well as in other countries, regulations concerning drinking water quality demand to hold down the amount of chemical contaminants polluting or altering the quality of drinking water as low as possible. To implement these regulations, the knowledge of the distributions of antibiotics in the environment is essential. The objective of this study was to verify, if animal husbandry can be a source of antibiotic residues showing up in the aquatic environment in Northern Germany. Furthermore, the occurrence of these residues and potential differences in conventional and organic farms were evaluated.

METHODS

Liquid manure samples were collected during two seasons of leaching (October until February) in 2004/05 and 2005/06 every four weeks on two conventional and two organic dairy farms in Schleswig-Holstein, Germany. Manure was stirred mechanically in the slurry tank before sampling to guarantee a specimen as homogeneous as possible. All samples were filled into brown glass bottles and placed in the refrigerator (4°C) until further processing (<4 days).

At the same time, leachate samples were taken with help of suction cups on two fields per farm, one of grassland and one of maize. During the test period, the sampling interval of four weeks could not be maintained totally due to weather conditions, as a minimum of two litres of leachate is needed for analysis. In 2004/05, a total of eight samples, containing five manure samples, was analysed. These examinations served as preliminary tests to establish the analysis conditions for manure samples and to adjust the sensitive laboratory methods. In 2005/06, 34 samples were examined, consisting of ten manure samples, eleven leachate samples from grassland and 13 leachate samples from maize fields.

Chemical analysis of antibiotics from different matrices, especially manure, is complicated by the need for extraction. In this study, sample clean-up was performed by a 1:50 dilution with following centrifugation and via solid phase extraction. All samples were analysed with high-performance liquid chromatography in combination with tandem mass-spectrometry (HPLC-MS/MS). High recovery rates of about 70% for oxytetracycline, chlortetracycline, and tylosin enable this method for the analysis of antibiotics in difficult matrices like manure even in the low microgram per kilogram range. Samples were analysed for more than 20 substances listed in table 1. Additionally, to discriminate residues' origins, detailed information of the applied antibiotics on these farms were recorded accurately with questionnaires.

Table 1.

Antibiotics	**Limit of quantification (LOQ) in ng/l**	**Antibiotics**	**Limit of quantification (LOQ) in ng/l**
Amoxicillin	10	Dehydrato-Erythromycin	5
Ampicillin	10		
Benzylpenicillin	10	Roxithromycin	5
Cloxacillin	10	Spiramycin	5
Dicloxacillin	10	Tylosin	5
Flucloxacillin	10	Trimethoprim	5
Methicillin	10	Sulphadimidine	5
Mezlocillin	10	Sulphamethoxazole	5
Nafcillin	10	Ciprofloxacin	10
Oxacillin	10	Ofloxacin	5
Piperacillin	10	Chlortetracycline	25
Phenoxymethylpenicillin	10	Doxycycline	20
Azithromycin	5	Oxytetracycline	20
Clarithromycin	2	Tetracycline	20
Clindamycin	5	Vancomycin	50

RESULTS

Regarding the application of antibiotics, no differences between conventional and organic farms were assessed neither in the range of applied antibiotics nor in the amount of administered treatments. In general, most treatment took place after mastitis and diarrhoea with ß-lactam-antibiotics and tetracyclines. Out of the list of examined pharmaceuticals, amoxicillin, ampicillin, benzylpenicillin, cloxacillin, sulphadimidine, chlortetracycline, doxycycline, oxytetracycline and tetracycline were administered during the examination period. Furthermore, animals were treated with enrofloxacin and cephalosporines, but these substances could not be analysed by the used methods.

Limits of quantification were not exceeded in any sample, regardless of sample origin. Neither in liquid manure nor in leachate were the analysed antibiotics detected on levels above the limits of quantification. In two manure samples from the sampling period 2004/05, traces of sulphadimidine were found, laying above the limit of detection (2 ng/l), but beneath the limits of quantification (5 ng/l) and therefore not further quantifiable.

CONCLUSIONS

Compared to pig and poultry production, the use of antibiotics in dairy farming is still on a relatively low level, at least at the examined farms. The results can be attributed to the fact that the administered antibiotics either degrade very fast in the environment like ß-lactam-antibiotics or are highly adsorbent to soil like tetracyclines. The structure of ß-lactams such as penicillin, benzylpenicillin or cloxacillin, consisting of the ß-lactam-ring, contribute to the poor stability of this group in the environment: the ring can be opened by ß-lactamase, a widespread enzyme in bacteria, or by chemical hydrolysis. Thus, intact penicillins are usually not found in the environment (MYLLYNIEMI et al., 2000). Neither tetracyclines nor tylosin were detected in any water sample by HAMSCHER et al. (2002). Further confirmation of these findings is supported by LINDSEY et al. (2001) and ZHU et al. (2001). However, these substances have been detected in low levels in U.S. surface water samples (KOLPIN et al., 2002) and in higher levels in overland flow water (KRAPAC et al., 2005). In Northwest Germany, a study was conducted sampling a series from surface waters, detecting a wide range of antibiotics in all samples (CHRISTIAN et al., 2003): sulphonamides, macrolides and lincosamides were analysed frequently, whereas ß-lactams were rarely found. Tetracyclines were not detected because of their strong adsorption to organic matter. The presence of tetracycline-resistant bacterial isolates in lagoons and groundwater underlying two swine production facilities was published by CHEE-SANFORD et al. (2001). MACKIE et al. (2006) detected both tetracycline residues and tetracycline resistance genes in groundwater impacted by swine production facilities. However, antibiotic input by agricultural use is the minor origin of antimicrobials in the aquatic environment. Most of the analysed substances originated from discharge or sewage into rivers, only for a couple of samples could an influence of animal husbandry on the occurrence of antibiotics in surface waters be assumed. The major part of antibiotic input is carried out by human administration via hospital effluents or municipal wastewater, as reviewed by KÜMMERER (2001b).

Even if the occurrence, effects and fate of antibiotics have been put in the perspective of the scientific interest, still little is known about the actual risk to both humans and the environment. Significant gaps still exist in the understanding of the interaction between residues, metabolites and resistance promotion after excretion. But the consequences of increasing resistance in bacteria and the diminishing impact of therapeutic drugs reach far beyond geographic origins of antimicrobial compounds and are therefore of global concern. Without a doubt, a promising approach for proper risk assessment and management is the reduction of the emission of antibiotics into the environment, whether of human or veterinary medical origin. Appropriate use of antimicrobials in livestock production will preserve the long-term efficacy of existing antibiotics, support animal health and welfare and limit the risk factors of transferring antibiotic resistance to animals and humans. Thus, further and deeper studies are implicitly required.

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