Title: Special Call for Review of the Critical Literature: Fate and Transport of Antibiotic Residues and Antibiotic Resistance Genetic Determinants during Manure Storage, Treatment, and Land Application with emphasis on the Environmental Persistence and Transferability of these Determinants - **NPB# 04-161**

Investigator: Roderick I. Mackie[1,2]

Co-Investigators: Satoshi Koike[1], Ivan Krapac[3], Joanne Chee-Sanford[4], Scott Maxwell[5], Yu-Feng Lin[6] and Rustam I. Aminov[7]

Institutions:

[1] Department of Animal Sciences, and Division of Nutritional Sciences, University of Illinois
[2] Institute for Genomic Biology, University of Illinois
[3] Illinois State Geological Survey, Natural Resources Building, University of Illinois
[4] USDA-ARS, and Department of Crop Sciences, University of Illinois
[5] Department of Natural Resources and Environmental Sciences, University of Illinois
[6] Illinois State Water Survey, Department of Natural Resources
[7] Rowett Research Institute, Bucksburn, Aberdeen

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For more information contact:
National Pork Board, P.O. Box 9114, Des Moines, Iowa USA
800-456-7675, **Fax:** 515-223-2646, **E-Mail:** porkboard@porkboard.org, **Web:** http://www.porkboard.org/

EXECUTIVE SUMMARY

Antibiotics are used at therapeutic levels to treat disease, at slightly lower levels as prophylactics, and at low, sub-therapeutic levels for growth promotion and improvement of feed efficiency. Over 88% of swine producers in the United States gave antimicrobials to grower /finisher pigs in feed as a growth promoter in 2000. It is estimated that *ca*.75% of antibiotics are not absorbed by animals and are excreted in urine and feces. The extensive use of antibiotics in swine production has resulted in antibiotic resistance in many intestinal bacteria, which are also excreted in swine feces, resulting in dissemination of resistance genes into waste holding environments. These animal waste products are generally stored before disposal into the environment. The most common method to dispose of swine effluent in the United States is through land application.

Antibiotics used in animal agriculture can enter the environment via a number of routes including the drug manufacturing process, disposal of unused drugs and containers, and through the use and application of waste material containing the drug. The excretion of waste products by grazing animals, atmospheric dispersal of feed and manure dust containing antibiotics, and the incidental release of products from spills or discharge are also potential pathways into the environment. Laboratory investigations have documented the physico-chemical interactions of most veterinary antibiotics with soil and soil components. Determining what concentrations of antibiotics are environmentally relevant is important in making inferences about the actual impacts of antibiotics detected in the environment but there is limited field information on the biological activity of low concentrations of antibiotics and antibiotic residues, and the fate and transport of antibiotics. Much of the information is simply occurrence or frequency data. In general, the available field data suggests that antibiotics are relatively immobile in the environment and when detected are at the lower µg/L range. Antibiotics are generally not very persistent in manure with half-lives generally less than 100 days. Thus, the application of manure to agricultural fields will likely introduce antibiotic breakdown products to the environment. However, data are very scarce concerning the occurrence, fate and transport of antibiotic breakdown products in the environment. In addition, little information is available concerning the biological activity of breakdown products or for parent antibiotics that are sorbed to soil components. More sensitive techniques are required to assess the persistence of antibiotics and their residues in the environment.

The differences of manure storage systems and land application methods can affect the dissemination of antibiotic resistance genes into the environment. Intestinal bacteria in manure that are introduced to the environment, can survive in soil for as long as 8 weeks to 6 months, but vary depending on species and temperature. DNA from viable, as well as dead, bacteria could be a source of antibiotic resistance genes. More research is needed on how bacterial populations are partitioned in pit/lagoon environments; what proportion and types are associated with the solid and liquid phases This has practical implications for waste management from both an antibiotic resistance perspective as well as pathogen reduction, and will become of much greater importance as the industry inevitably moves toward separation of liquids and solids in order to comply with phosphorus-based limits on land application of manure. In addition, more research is needed to obtain a thorough understanding of the effect of soil heterogeneity on spatial and temporal patterns of microbial communities and the potential for gene transfer. Quantitative measurements of antibiotic resistance gene levels are needed, in addition to measurements of diversity and frequency, in order to address questions of accumulation and persistence of these determinants. Bacteria and

viruses have great potential to move deep into the subsurface environment. Several studies concluded that application of animal manure to soil can readily lead to groundwater contamination with fecal bacteria, especially under moist soil conditions, and that macropores are important in the transport of bacteria through soil.

Phylogenetic analysis indicates that antibiotic resistance genes have evolved and been maintained in bacteria prior to the modern antibiotic era, even though the origin and purpose of these genes is not yet clear. These resistance genes are exchanged among a broad range of bacteria. There is evidence that increased occurrence of lateral gene transfer has occurred recently, most likely accelerated by indiscriminate use of antibiotics. Trace amounts of antibiotics, chemicals or other substances such as heavy metals could act as a selective pressure for the maintenance and transfer of antibiotic resistance genes. Very little is known about gene transfer between bacteria in the environment and the maintenance or reduction of antibiotic resistance in manure storage environments. In order to obtain key data on gene flow, higher throughput and more sequencing of antibiotic resistance genes both from known organisms and bulk genomic DNA from different environments is essential.
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