

PUBLIC HEALTHWORKER SAFETY

Title: Tetracycline Concentrations and Circulation of *tet* Genes in Swine Feeding Operations and Adjacent Environments – NPB #07-027

Investigator: Andrei L. Barkovskii, Associate Professor of Microbiology

Institution: Georgia College & State University

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Scientific Abstract

Three farms varying in antibiotic (AB) usage from permanent to no usage were surveyed for the presence of sixteen tetracycline resistance (TCR) genes including *tet*(A), A(P), (B), (C), (D), (E), (G), (K), (L), (M), (O), (Q), (S), (T), (W), and (X) to evaluate the impact of AB usage in farms on the TCR gene profiles, TCR gene persistence, and their environmental occurrence. Animal feces and feed, manure treatment lagoons, irrigated and non-irrigated soils, ponds and creeks were sampled throughout 2007. PCR was used for detecting the above genes with plasmid-based positive controls as references and a ladder for the amplicon size control. TCR genes were observed in all three farms with no apparent difference in their profiles where *tet*(A), A(P), (M), (O), (Q), (W), and (X) dominated. No *tet*(K) and *tet*(T) were observed in any farm. *Tet*(B) was found related to chlorotetracycline usage and animal life phase, and was transient being detected either only in animal feces or in the feces and the lagoons directly accepting these feces, and disappearing below the detection limit in consequent lagoons. *Tet*(D) and (L) were also transient in the farms. *Tet*A(P), (C), (D), (E), (G), (M), and (O) were detected in animal feed, but their profiles in the feed varied between the farms and even between the houses within the same farm.

Tet(A), (C), (E), (G),(M), (O), (Q), and (X) were persistent in the feces and lagoons, and were found around the farms. The identities of the corresponding genes detected in the lagoons and in adjacent environments were confirmed with sequence of gene-specific amplicons. Their numbers were determined with SYBR Green-based qPCR, normalized per gram or milliliter of the matrix, and to the numbers of the "housekeeping" 16S rRNA genes. The recovery efficiencies of TCR genes from environmental matrices were determined, and the detection limits for these genes established. Their environmental occurrence around farms was found proportional to the degree of AB usage, and was most prominent in the constantly using AB farm. In the lagoon system of that farm, concentrations of the "persistent" *tet*(M), (O) and (X) genes increased 2-3 orders of magnitude during manure passage through the lagoons, though the concentrations of the "housekeeping" 16S rRNA genes remained stable and, in some cases, decreased. While individual concentrations of these genes varied between 10^5 - 10^9 /g(ml), their concentrations in the proximal to the farm soil and water bodies varied between 10^1 - 10^4 /g(ml) with *tet*(A) and (O) being the highest. No environmental occurrence of TCR genes was observed around the AB-free "finisher" branch farm.

It was concluded that (i) except for *tet*(B), there is no apparent correlation between AB usage and TCR gene profiles, (ii) the presence of *tet*(B) is associated with farrowing animals and piglets, and indicates antibiotic usage, (iii) TCR gene profiles are composed of "persistent" and "transient" genes, some of the former originate from animal feed, while the others are likely promoted by farm environment, (iv) persistent genes propagate in manure treatment lagoons, and their environmental occurrence and concentrations correlate to the frequency of AB usage at the farms, number of manure treatment lagoons, and the amount of precipitation in the region.

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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>