

- a.) Vaccination reduces *Salmonella* prevalence in carcasses at harvest.
- b.) Reduction occurs in all species of *Salmonella*, not just homologous/Group C1 *Salmonellae*.
- c.) Vaccination can be a valuable tool in reducing risk of foodborne disease due to *Salmonella* in pork. Vaccination will not interfere with serologic categorization of farms' *Salmonella* status.

Vaccination may be considered as another potential tool for improving safety of pork by means of reducing the level of salmonella contamination of pork carcasses, and potentially ground or other fresh pork products. Commercial serologic tests may be utilized in conjunction with vaccination to evaluate the success of *Salmonella* reduction programs in swine.

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## QUANTIFYING TETRACYCLINE RESISTANCE

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CANDACE GLENDENING\*, ANDREW MACK, ANDREW BOWMAN and JULIE FUNK

Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, 1900 Coffey Road, Columbus, OH 43210, Ph: 614-292-3412, Fax: 614-292-4142, Email: glendening.2@osu.edu

\*Presenting author

**Summary:** This study's goal was to evaluate the impact of sub-therapeutic feeding of chlortetracycline (CTC) on the fecal concentration of tet(C), a gene that confers tetracycline resistance via an efflux mechanism. We developed a real-time quantitative PCR assay to measure the quantity of tet(C) in whole fecal DNA samples. The vast proportion of variability in tet(C) (91%) was associated with differences in concentration between the individual pigs, and there was no significant difference in the copy number of tet(C)/mg of feces between the treatment and control pigs ( $p > 0.05$ , linear regression, SPSS 11.0.5)

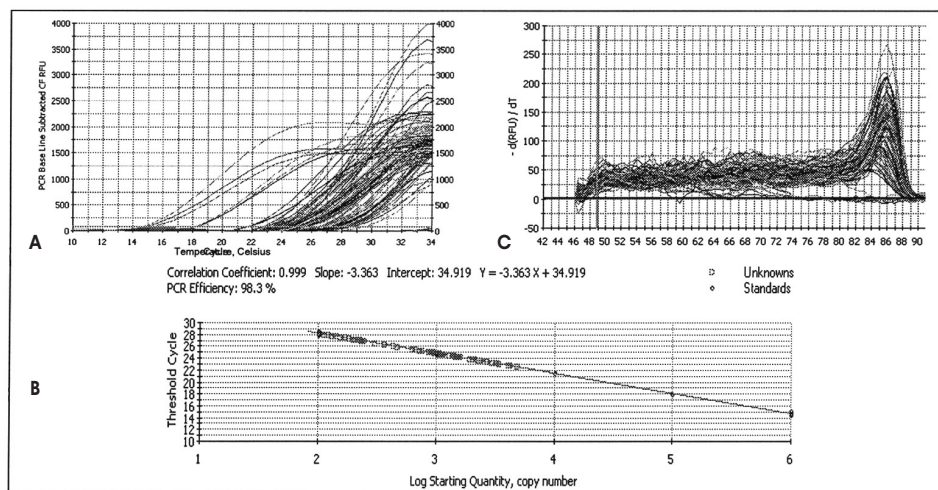
**Keywords:** real-time quantitative PCR, tet(C)

**Introduction:** Antimicrobial resistance (AR) research has been predominantly confined to the study

of cultivable bacteria. Although this approach provides pertinent information regarding AR, it is a very limited sample of the normal gastrointestinal flora, which could serve as a reservoir for AR genes. Therefore, tools for detection, quantification and tracking of AR genes are very attractive for understanding the ecology of AR. The goal of this study was to quantify the AR reservoir attributable to tet(C) in the feces of swine fed CTC in the diet as compared to pigs not fed CTC. This was accomplished by using real-time quantitative PCR (Q-PCR) to measure the amount of the tet(C) gene present in DNA extracted from fecal samples.

**Materials and Methods:** Treatments (50g CTC/ton of feed or no CTC) were assigned to 3 temporally matched barn pairs. Fecal samples (200 mg) were collected from 48 pigs per barn. Total fecal DNA was isolated using the QIAamp DNA Stool Mini Kit. From one barn pair, DNA was extracted from each individual pig fecal sample in triplicate to assay the variability of tet(C) concentration within a fecal sample. Quantification of tet(C) in the fecal DNA was done using the Qiagen QuantiTect SYBR Green PCR Kit on a BioRad iCycler iQ Real-Time Detection System with tet(C) primers (Aminov, 2002). A linear regression model was constructed to assess the effect of treatment and farm on tet(C) copy number. A multilevel linear model was constructed to assess the effect of treatment and hierarchical sources of variation in tet(C) concentration for the replicate fecal samples (MLwiN 2.1a, London).

**Results:** Figure 1A is a representative amplification plot of the samples from real-time Q-PCR assays for tet(C) conducted using the BioRad iCycler iQ Real-Time Detection System. The plot displays the relative fluorescence of double stranded DNA for representative wells at every amplification cycle. Figure 1B is the standard curve, spanning  $10^6$  to  $10^2$  copies of tet(C), all samples run to date fall within this range, allowing accurate calculation of copies of tet(C)/mg of feces.

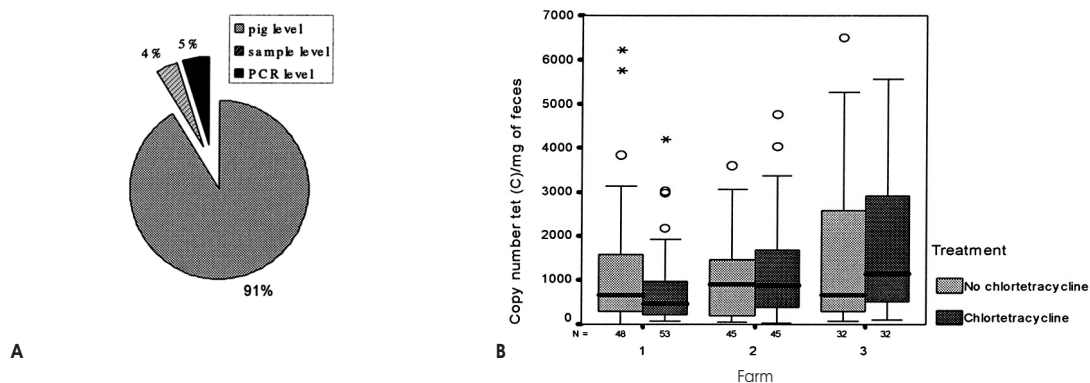


**Figure 1.** A. PCR Quantification screen output from the BioRad iCycler iQ Real-Time Detection System for representative samples for quantification of tet(C) in pig fecal samples. B. Standard curve of serial 10-fold dilutions of pBR322 from  $10^6$  to  $10^2$  copies. Boxes indicate threshold cycles of the fecal samples. The correlation coefficient was 0.999. C. Melt-curve profiles representing products generated from the amplification seen in Figure 1A.

The single prominent peak of the melting curve plot demonstrated in Figure 1C confirms product specificity of the tet(C) primers (expected product  $T_m$  of 84-86°C) and also shows no evidence of primer-dimers. The sequence of the tet(C) PCR product has been confirmed by sequence analysis. The greatest proportion of the variability in tet(C) copy number (91%) was associated with differences in concentration in the individual pig, while 4% and 5% of the variation in the copy number estimate was associated with fecal sub-samples and PCR replicates respectively (Fig. 2A). These data are

important as they demonstrate our ability to conduct the real-time quantitative PCR assay, and confirm that the variability in copy number estimates is associated with pig to pig differences in copy number and not variability in the assay technique.

The median copy number was 781 (range 18-5556) for the treatment group and 665 (range 11-6483) for the control group (Fig. 3B). There was no significant difference in the copy number of tet(C)/mg of feces between the treatment and control pigs ( $p>0.05$ ) despite the phenotypic differences previously identified in the Gram-negative fecal flora from these farms (Funk, 2003). Perhaps even more interesting is that Farm 2, which received therapeutic doses of tetracycline during the study and had the highest proportion of Gram-negative fecal isolates resistant to tetracycline overall, does not demonstrate an increase in the copy number of tet(C)/mg of feces relative to farms not receiving therapeutic CTC.



**Figure 2.** A. Proportion of variability in tet(C) copy number at pig, sample and PCR levels. B. Copy number of tet(C)/mg of feces in pigs receiving CTC in the feed vs. pigs not receiving CTC in the feed.

**Discussion:** Beyond qualitatively identifying the genes conferring AR present in commensals, there is an important question regarding what measurement of AR reflects the risk of transfer from animals to humans. Although qualitative assays can describe the number of animals that harbor a particular AR gene, it is unknown whether the relative quantity of this gene in an animal is important. In this study there was no detectable effect of short term changes in sub-therapeutic antimicrobial use on tet(C) copy number. Further studies to discern the contribution of other genes that confer tetracycline resistance are on-going.

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