DTU Food
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The effect of antimicrobial usage on occurrence of resistance genes in fecal samples from slaughter pig batches

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Introduction & Objective: Genes coding for antimicrobial resistance (AMR) are regularly obtained from bacteria present in the intestinal flora of slaughter pigs. Variations in the occurrence of AMR relates predominantly to the antimicrobial (AM) usage (Aarestrup, 1999). Based on register data three proxy measurements of AM usage were estimated varying both for the time component and the population level. Each measurement was analysed for the quantitative effect on occurrence of AMR genes derived by Whole Community Sequencing (WCS) in fecal samples from slaughter pig batches.

Methods (Figure 1): Data from the Central Husbandry Register (CHR), VetStat and the Movement Database (MD) were applied in obtaining ten herds, five with prior low AM usage and five with prior high usage, and to estimate three Animal Daily Doses per kg (ADDkg) measurements; ADDkg *Lifetime exposure*, ADDkg *Herd usage* and ADDkg *Slaughter pigs*. Fecal samples were collected from the floor of 30 randomly selected slaughter pig pens and pooled into one, purposely to represent a batch of slaughter pigs. The ten batches were back traced through the production line. A daily use of aminoglycosides, lincosamides, macrolides, broad-spectrum penicillin, sulfonamides and tetracycline was estimated as an average daily use based on the interval between two prescriptions and summed according to each of the ADDkg measurements. Known resistance genes for aminoglycosides, lincosamides, macrolides, beta-lactams, sulfonamides and tetracycline were found using WCS, and the results were obtained as Reads Per Kilobase per Million (RPKM). The quantitative effect of each measurement on occurrence of AMR genes were assessed through uni-variable linear regression analyses.

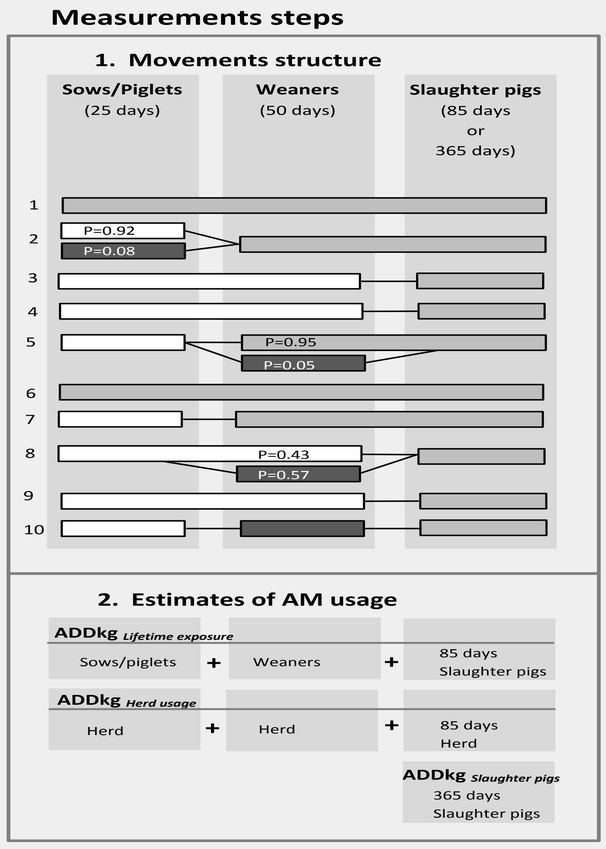

Figure 1. Illustrates the construction of the three ADDkg measurements. Step 1: The ten horizontal bars depicts the movements of the ten sampled slaughter pig batches prior to sampling. A colour shift in a bar stands for a different herd from the one where sampling took place and P the proportion of pigs being moved. The number 1 to 5 were the initial high users and 6 to 10 the initial low users. The three vertical bars represents the assumed days of exposure to AM in the; Sow-piglet unit, Weaning unit and Slaughter pig unit, from the onset of the latter. Step 2: The bars shows how the three measurements were estimated.

Results: Figure 2 shows the total estimated ADDkg for the measurements. The regression analyses displayed 1) significant effect of ADDkg *Lifetime exposure* on AMR genes for broad-spectrum penicillin, macrolides, tetracycline and sulfonamides (Figure 1) and 2) significant effect of ADDkg *Herd usage* on AMR genes for aminoglycosides, lincosamides and tetracycline. When extreme data points were removed from the regression models with significant result, the ADDkg *Lifetime exposure* still provided significant results for all but broad-spectrum penicillin, while the ADDkg *Herd usage* only remained significant for tetracyclines. The ADDkg *Slaughter pigs* provided no significant results.

Figure 2. ADDkg $_{Lifetime\ exposure}$, ADDkg $_{Herd\ usage}$ and ADDkg $_{Slaughter\ pigs}$ of the total aminoglycosides, lincosamides, macrolides, broad-spectrum penicillin, sulfonamides and tetracycline usage and the distribution within the ten herds (1 to 5 initial high users and 6 to10 initial low users) between sow/piglets, weaners and slaughter pigs.

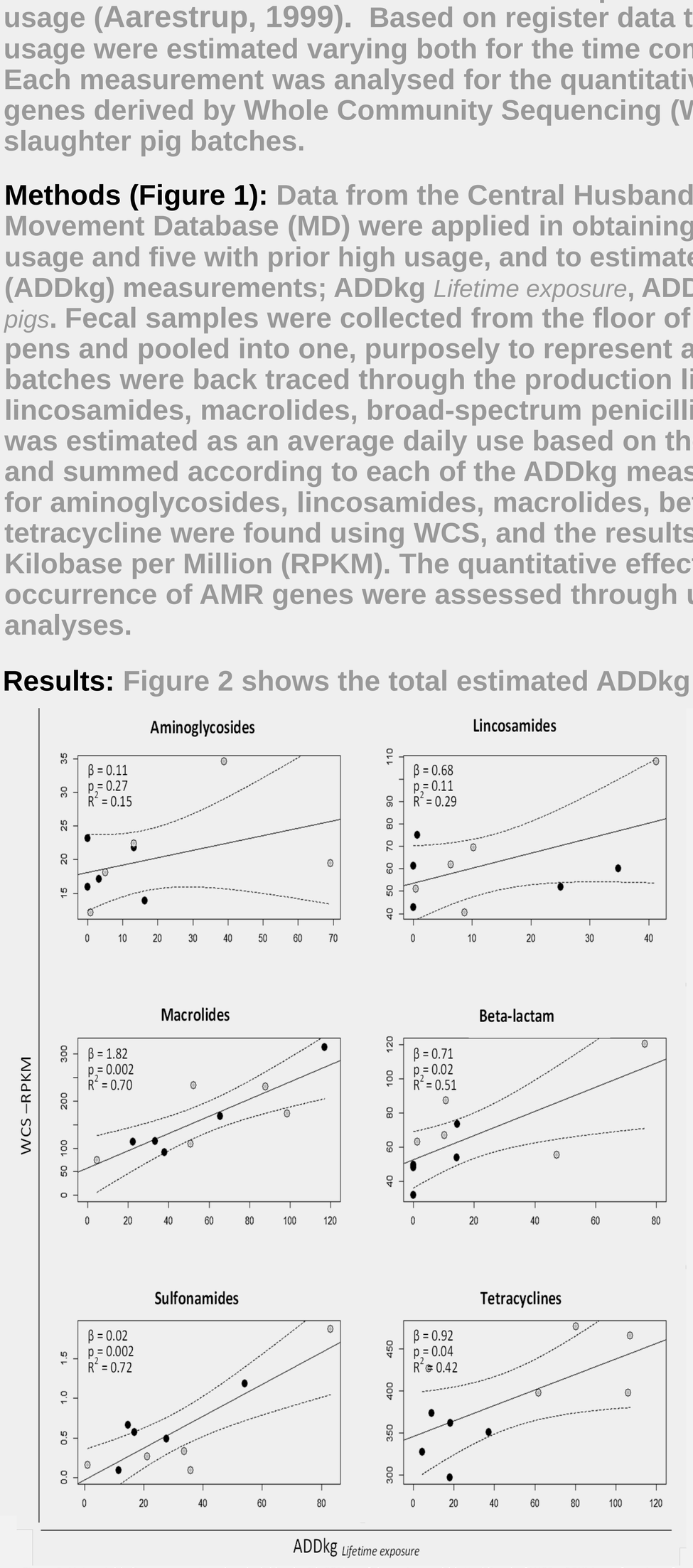

Figure 3. Uni-variable linear regression plots (solid line) with 95% confidence interval (dotted lines) of WCS – RPKM as a function of ADDkg $_{Lifetime\ exposure}$, for a period of 160 days for aminoglycosides, lincosamides, macrolides, penicillin, sulfonamides and tetracyclines. Grey points indicate initial high users and black points initial low users. The effect (β), the p-value (p) and the R-squared (R^2) are shown in the top left corner of each model.

Conclusion: Our results indicate that the ADDkg *Lifetime exposure* provides a useful proxy to describe the quantitative effect of AM usage on the amount of AMR genes in fecal samples from slaughter pigs. Furthermore, it underlines the importance of estimating exposure to AM not on single unit level, but rather as a lifetime exposure for a batch of pigs. These results supports the hypothesis that a large amount of the variation of AMR genes in the intestinal flora of a pig is a result of the AM exposure during their entire lifetime of approximately 5½ months and not merely the usage in the slaughter pig unit.

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Reference: Aarestrup, F. M., & Wegener, H. C. (1999). The effects of antibiotic usage in food animals on the development of antimicrobial resistance of importance for humans in campylobacter and escherichia coli. Microbes and Infection, 1(8), 639-644.