

Evaluation of penicillin G residues by kidney inhibition swab tests in sow body fluids and tissues following intramuscular injection

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Abstract

In 2011, the USDA-Food Safety and Inspection Service (FSIS) changed the method used for screening swine tissues for antimicrobial residues from the Fast Antimicrobial Screen Test to the Kidney Inhibition Swab (KIS™). Here, we describe the use of KIS™ test for the detection of penicillin G residues in kidney, liver, plasma, urine, and skeletal muscle of heavy sows following the administration of a 5x label dose of penicillin G procaine. Such off-label use is legal in the United States under the Animal Medicinal Drug Use Clarification Act (AMDUCA) when label routes or doses are ineffective at treating disease and is commonly used to treat bacterial infections in heavy sows. Heavy sows (n=126; 228 ± 30.1 kg) were administered intramuscular (IM) doses of penicillin G (33,000 U/kg bw) for 3 consecutive days using 3 different administration patterns. Within treatment, six sows each were slaughtered after 5, 10, 15, 20, 25, 32, or 39 withdrawal days. The IM administration pattern had no discernible effect on penicillin G depletion from kidney, skeletal muscle, serum, urine or liver. Residues were depleted most rapidly from liver and skeletal muscle and more slowly from kidney and urine. While kidney residues were a poor predictor of penicillin G residues in skeletal muscle, kidney was the most sensitive target tissue for detecting penicillin G residues, with two positive results even after a 39-day withdrawal period. The most suitable ante-mortem matrix to replace FSIS on-site tests using kidney was urine. Serum, another ante-mortem matrix predicted muscle residue well albeit showing more positives than muscle. These data support a 15-day withdrawal period suggested by Food Animal Residue Avoidance Databank for extra-label penicillin G treated heavy sows with the caveat that kidney tissues be excluded from human consumption.

Introduction

Penicillin G is active against a variety of Gram-positive pathogens affecting livestock production and is indicated for treatment of a number of bacterial diseases in a variety of animal species including erysipelas in swine (NADA 065-010). For most food animals, the typical route of penicillin G administration is by IM injection at daily dose of 6,600 IU/kg with treatment not to exceed 4 consecutive days. Under label conditions, approved pre-slaughter withdrawal periods are 7 days for swine with zero tolerance for penicillin G residues in tissues. However, sows are commonly treated with higher doses which is allowed under a veterinarian's supervision when labeled doses are ineffective. Under AMDUCA, the veterinarian prescribing the off-label use must recommend an appropriate pre-slaughter withdrawal period to ensure that drug residues remaining in edible tissues deplete to safe levels (21 CFR Part 530).

There are very few data which describe the depletion of penicillin G residues under off-label conditions. The studies available indicate increased doses increase the elimination time. Apley et al. (2009) conducted a residue depletion study in sows using a single 5x label dose of 33,000 IU/kg administered by intramuscular injection or with a needle free device. Intramuscular injection in the "hip" produced flip-flop kinetics (KuKanich et al., 2005; Riviere 2011) in which the terminal elimination rate is controlled by the rate of absorption rather than by the rate of elimination. Between-animal plasma half-lives were highly variable. After an 8-day withdrawal period two of five hogs had a quantifiable residue. From these data, they recommended of a 28 day withdrawal period based on a 95% confidence interval that 99% of treated animals would have no detectable residue in the kidney.

The US-FSIS changed the method for screening swine tissues for antimicrobial residues from the Fast Antimicrobial Screen Test to the KIS™ test in September of 2011 (FSIS notice 45-11). An increase in the detection of penicillin residues in sow tissues subsequent to the adoption of the new screening assay may be attributed to the survey method change. This paper describes the detection of penicillin G procaine residues with the CHARM-KIS test in kidney, liver, plasma, urine, and skeletal muscles of heavy sows after an extra-label penicillin-G procaine administration. Sows were treated IM with 33,000

IU/kg bw for 3 consecutive days and were slaughtered with withdrawal periods extending to 39 days post-treatment.

Material and Methods

Chemicals and Supplies. Penicillin G procaine (300,000 U/mL; Norocillin; Norbrook Pharmaceuticals, Lenexa, KS) injection solution was purchased from Ivesco, LLC (Iowa Falls, Iowa). Penicillin G procaine monohydrate reference standard was purchased from U. S. Pharmacopeia, Rockville, MD. Kidney inhibition swabs, neutralization tablets, penicillin G controls, and heating blocks were obtained from Charm Laboratories (Lawrence, MA). Driploss containers were purchased from the Danish Meat Research Institute, Taastrup, Denmark.

Animal Housing and Treatment Assignment. A study protocol was approved by the North Dakota State University Institutional Animal Care and Use Committee prior to the initiation of the live-phase of the study. Heavy sows were purchased from the North Dakota Pig Cooperative (Larimore, ND) and delivered to the North Dakota State University Animal Research Center (Fargo, ND) and acclimatized for at least 14 days. Animals were randomly assigned to one of three treatments and were each provided unique identification numbers by ear tag. For treatment 1, sows received injections (10 mL on each side) in a single location for 3 days at the same location. For treatment 2, sows received consecutive injections (10 mL on each side) for 3 consecutive days at locations separated by approximately 2 inch intervals. For Treatment 3, sows received 20 mL in the one side with overflow injections occurring on the other side of the neck. Injections occurring on consecutive days were separated by approximately 2-3 inches.

Sows were slaughtered with 5, 10, 15, 20, 25, 32, or 39 day withdrawal periods relative to the last off-label dosing day. Positive control sows (n = 2 per withdrawal period) were treated with the label dose (6,600 U/kg bw) of penicillin G via IM administration for 3 consecutive days and were euthanized after a 7-day withdrawal period, consistent with the product label; or a 15-day withdrawal period. Negative control sows (n = 2) were dosed IM with sterile saline (1 mL per 45.5 kg) for 3 consecutive days and were slaughtered 5 days after the last saline injection.

On-Site Analysis of Kidneys. Kidney samples were screened for penicillin residues on the kill floor using the Charm-KIS microbial inhibition test according to FSIS procedures (FSIS CLG-ADD 3.01, 2011); the manufacturer's recommended incubation time, without the automatic shut-off option, was followed. All samples were determined in duplicate by separate operators and each result scored independently by both operators.

Sample Collection and Treatment. At collection, skeletal muscle and additional kidney were collected using a 3-cm diameter tissue coring device driven by a cordless drill and trimmed to approximately 3 x 3 cm. Samples were placed into Driploss containers and were frozen at -80 °C until analysis. Samples were thawed at room temperature for 1 hr and the tissue juices were collected from the drip tubes after centrifuging at 1,200 x g for 10 min. The collected tissue juices were used to saturate Charm-KIS swabs. Microbial inhibition tests for tissues were then conducted as described by the FSIS (2011) for determination of the presence of penicillin G. Liver required the addition of an equal volume of water followed by boiling (1 min) and centrifugation at 14,000 x g prior to swab. Urine or serum aliquots (500 µL) were combined with a single Charm-KIS neutralization tablet and vortexed; particulates were allowed to settle for 1 min. Serum/urine supernatant was adsorbed for 10 seconds with a cotton swab, after which, the CHARM-KIS microbial inhibition assay was performed.

Assay Sensitivity Determination. Control serum, urine, skeletal muscle juice, and kidney juice were prepared as previously described and tissue matrix aliquots were spiked with 0, 10, 20, 30, 40, and 50 ppb of penicillin G procaine. Liver juice was spiked with 0, 10, 25, 50, 75, and 100 ppb of penicillin G procaine. Fortification of each matrix was repeated on three separate days.

Results and Discussion

Typical color indication for positive, negative, and "caution" KIS results are shown in Figure 1. Sensitivity for penicillin residues in kidney juice, muscle juice, urine, and serum was 20, 30, 20, and 30 ppb respectively and can be lot dependent. When dilution factor is accounted for, liver juice sensitivity was 100 ppb.

Negative control sows that received normal saline and which were slaughtered on withdraw day 5 had negative readings for all matrices tested. The positive control sows, which received the label dose and were slaughtered at withdrawal day 7 showed positive Charm-KIS results for all tissues. For withdrawal day 15, one out of two positive control animals tested positive using Charm-KIS in kidney and urine samples but the

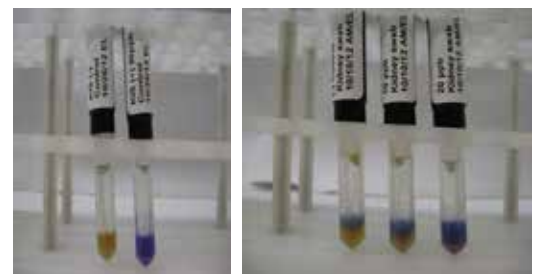


Figure 1. Typical results returned from the Charm-KIS rapid screening assay, from left to right, KIS negative control, KIS positive control, negative, caution, and positive results from kidney swabs. A caution results is considered to be negative by FSIS.

rest of matrices returned negative results. As seen in Figure 2 there is no difference in the various treatments indicating the differences from the various injection patterns are not discernibly different. The variability is probably due to the variability in absorption since penicillin G procaine would demonstrate flip-flop kinetics. Apley's (2009) estimate of a 28 day withdrawal period is at least reasonable and our data suggest it may be longer before kidney levels become non-detectable as we observed positives even at day 39. Slow penicillin depletion could be attributed to absorption from the injection sites where residues remain and the fact that penicillin concentrated in the kidney where it is excreted by an active process. From sow penicillin results reported by Korsrud et al (1998), penicillin concentrations in kidney were approximately 40-70 times greater than residues in corresponding muscle samples. The Charm-KIS assay returned smaller numbers of muscle positives than in kidneys for the same animal and time point, in agreement with Korsrud et al's findings. Charm-KIS assays of liver returned fewer positive results than the assays of other tissues, even at day 5 (Figure 3) possibly caused by the much poorer assay sensitivity with liver compared to other matrices.

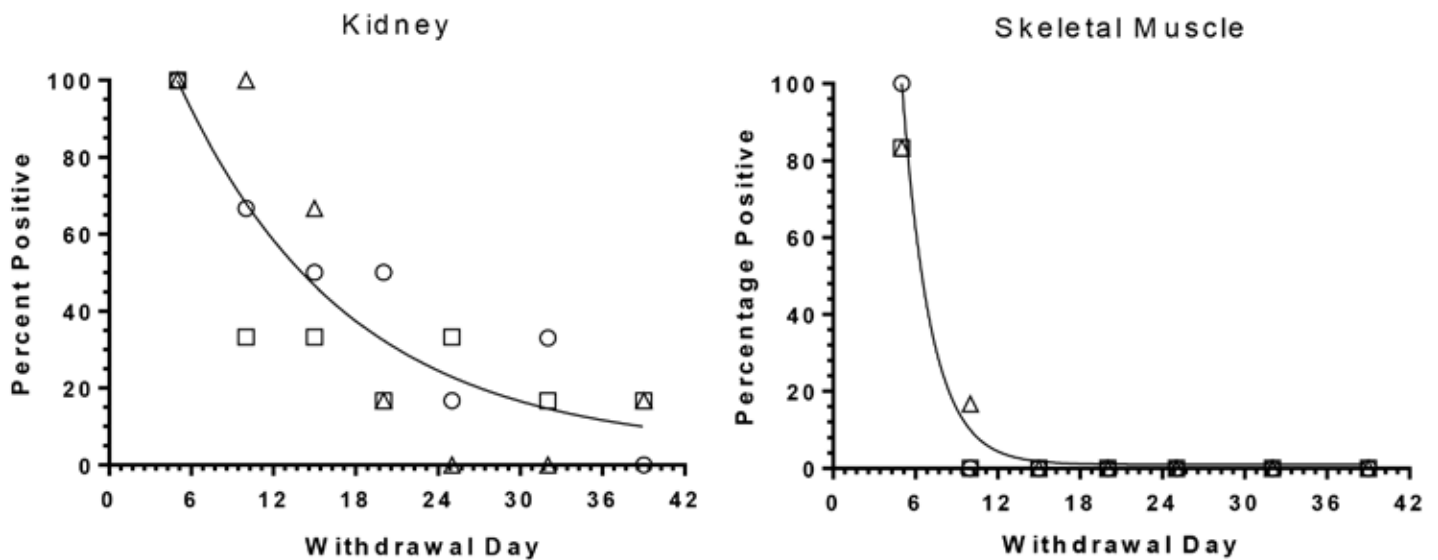


Figure 2. Depletion of Penicillin G residues in kidneys (left panel) and muscle (right panel) of heavy sows as detected by the Charm-KIS rapid screening assay. Line is a first order depletion fit of combined data from Treatments; false positive results (where there is no measureable residues from LC-MS/MS, data not shown) were removed from the skeletal muscle data prior to plotting.

Applicability of using the Charm-KIS for pre-slaughter screening of treated animals was tested with assays of serum and urine. No false-positive Charm-KIS assay results were returned for either matrices (LC-MSMS data not shown). The urine was a much better surrogate matrix for detecting potential violative penicillin G residues in kidney (Figure 3) than was serum. Serum results clearly demonstrate that serum is an inadequate surrogate for kidney because serum is more rapidly cleared of penicillin than kidney.

Conclusion

Based on kidney results a prolonged withdrawal period is needed prior to culling sows that have been previously treated with high doses of penicillin G procaine. The labeled dose can also return positive kidney penicillin tests beyond the prescribed withdrawal period. Urine can serve as an ante-mortem test reflecting the levels which will be observed in the kidney.

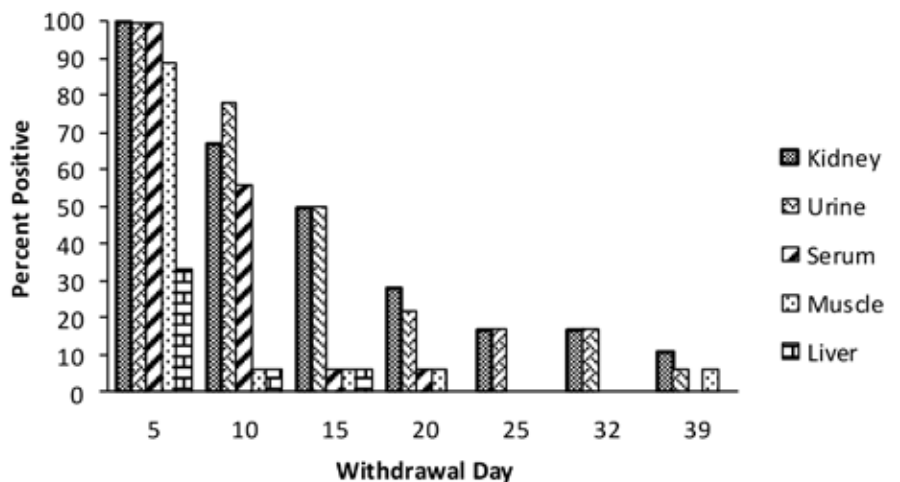


Figure 3. Comparison of Charm-KIS test results in kidneys, urine, serum, muscle, and liver of off-label treated sows as a function of withdrawal day.

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