RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: A study to correlate procaine penicillin G in tissues, plasma and oral fluids of sows and determine environmental transfer of penicillin to untreated sows - **NPB #13-236**

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Industry Summary:

Procaine penicillin G (PPG) residues are the most common violative residues detected in swine at slaughter (FSIS 2014). Recently, the United State Department of Agriculture Food Safety Inspection Service (FSIS) has replaced their PPG testing methods, which are in part responsible for widespread reports of PPG residue detection in cull sows. Direct measurement of the concentrations in tissue that result from typical administration routes and extra-label doses which are required to establish safe withdrawal periods are not well-described in the literature. This study describes the tissue depletion profile of PPG in sows after administration of either 3,000 IU/lb or 15,000 IU/lb once daily for 3 days using liquid chromatography mass spectrometry (LCMS) methodology. LCMS is able to isolate and quantify individual molecules based on their molecular structure and electronic properties, making it a very accurate assay for residue detection. This study also examined urine, plasma, and environmental samples as a method to detecting PPG residues prior to slaughter.

Forty-seven (47) sows were divided into three different treatment groups. Fifteen (15) sows were allocated to Treatment Group 1, and sixteen (16) sows were allocated to each of the remaining treatment groups. Treatment Group 1 (TG1) was administered 3,000 IU/lb PPG IM according to the labeled dose, Treatment Group 2 (TG2) was administered a dose of sterile saline IM once a day for three days and a volume equivalent to the average volume of PPG given to the PPG treated groups, and Treatment Group 3 (TG3) was administered an extra-label dose of 15,000 IU/lb PPG IM once daily for three consecutive days. Sows were housed in pens of three with one sow from each treatment in a pen and assigned to one of four necropsy time points, which corresponded to 1, 6, 14, and 28 days following the last administration of PPG. Blood samples and environmental samples were taken on all sows immediately before first administration of PPG, on day 3 post-administration of PPG, and immediately before euthanasia on sows to be necropsied. Urine and tissue samples (liver, kidney, injection site, semimembranosus/semitendinosus muscle) were collected at necropsy.

This study demonstrated that at extra-label dosing regiments tissue residues of PPG in the kidney can be detected in the kidney up to 6 days after final administration of PPG. Residues in the skeletal muscle were depleted more slowly than the kidney and detected out to at least 14 days. Injection site residues were the most marked and depleted the most slowly with PPG detected at high levels up to the conclusion of the trial at 28 days. Statistical modeling is needed to extrapolate the depletion profile of these tissues. From the raw data presented, the proposed 15 day withdrawal would not be sufficient for full tissue depletion. Further analysis of data will clarify whether the proposed 51 day withdrawal would be sufficient for full tissue residue depletion due to persistence at the injection site. Plasma concentrations very closely

correlate with tissue residue depletion of PPG in kidney and skeletal muscle. For extra-label doses and on-label doses, plasma concentrations of PPG were consistently higher than those found in kidney and skeletal muscle. With this information, plasma appears to be a good antemortem sample for PPG residue detection. However, the only method used for plasma residue detection was LCMS analysis. Because of the complexity of LCMS analysis, use of plasma as a practical, quick antemortem PPG residue sample is limited. Therefore, urine and environmental samples were also assessed for use in antemortem residue detection. Urine residues of PPG were found as consistently as plasma and tissue residues. TG1 sows had urine residues at Day 6 post-administration of PPG and TG3 sows had urine residues at Day 14 post-administration of PPG when analyzed by LCMS. Rapid testing methodology using both Charm MRL™ and SNAP™ tests demonstrated consistent results when detecting urine residues compared to the LCMS analysis. With this evidence, antemortem urine testing using these tests would provide accurate information about PPG residues in the sow of interest. However, PPG residues in environmental samples were not correlated to tissue residues. There was no consistency between the Charm MRL™ and SNAP™ tests to any of the other sample types. Residues were consistently detected in the environment with the SNAP™ test, which indicates presence of PPG residues in the environment surrounding sows treated with PPG within at least 28 days. The LCMS testing methodology was consistent with the KIS testing of kidneys used by the FSIS and is a reliable analytical tool to assess PPG residues.

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Keywords: swine, procaine penicillin G, tissue residues, environmental sampling, urine sampling

Scientific Abstract:

Procaine penicillin G (PPG) residues are the most common violative residues detected in swine at slaughter. The United State Department of Agriculture Food Safety Inspection Service (FSIS) has replaced their Fast Antimicrobial Test (FAST) with the Kidney Inhibition Swab (KIS) test, which is in part responsible for widespread reports of PPG residue detection in cull sows. Direct measurement of the concentrations in tissue that result from typical administration routes and extra-label doses are needed to establish safe withdrawal periods.

Forty-seven (47) sows were divided into three different treatment groups. Fifteen (15) sows were allocated to Treatment Group 1(TG1), and sixteen (16) sows were allocated to each of the remaining treatment groups. TG1 was administered 3,000 IU/lb PPG IM according to the labeled dose, Treatment Group 2 (TG2) was administered a dose of sterile saline IM once a day for three days and a volume equivalent to the average volume of PPG given to the PPG-treated groups, and Treatment Group 3 (TG3) was administered an extra-label dose of 15,000 IU/lb PPG IM once daily for three consecutive days. Sows were housed in pens of three with one sow from each treatment in a pen and assigned to one of four necropsy time points, which corresponded to 1, 6, 14, and 28 days following the last administration of PPG. Blood samples and environmental samples were taken on all sows immediately before first administration PPG, on Day 3 post-administration of PPG, and immediately before euthanasia on sows to be necropsied. Urine and tissue samples (liver, kidney, injection site, semimembranosus/semitendinosus muscle) were collected at necropsy.

This study demonstrated that at extra-label dosing regiments tissue residues of PPG in the kidney can be detected in the kidney up to 6 days after final administration of PPG. Residues in the skeletal muscle were depleted more slowly than the kidney and detected out to at least 14 days. Injection site residues were the most marked and depleted the most slowly with PPG detected at high levels up to the conclusion of the trial at 28 days. Statistical modeling is needed to extrapolate the depletion profile of these tissues. From the raw data presented, the proposed 15 day withdrawal would not be sufficient for full tissue depletion. Further analysis of data will clarify whether the proposed 51 day withdrawal would be sufficient for full tissue residue depletion due to persistence at the injection site. Plasma concentrations very closely correlate with tissue residue depletion of PPG in kidney and skeletal muscle. For extra-label doses and on-label doses, plasma concentrations of PPG were consistently higher than those found in kidney and skeletal muscle. With this information, plasma appears to be a good antemortem sample for PPG residue detection. However, the only method used for plasma residue detection was LCMS analysis. Because of the complexity of LCMS analysis, use of plasma as a practical, quick antemortem PPG residue sample is limited. Therefore, urine and environmental samples were also assessed for use in antemortem residue detection. Urine residues of PPG were found as consistently as plasma and tissue

residues. TG1 sows had urine residues at Day 6 post-administration of PPG and TG3 sows had urine residues at Day 14 post-administration of PPG when analyzed by LCMS. Rapid testing methodology using both Charm MRL™ and SNAP™ tests demonstrated consistent results when detecting urine residues compared to the LCMS analysis. With this evidence, antemortem urine testing using these tests would provide accurate information about PPG residues in the sow of interest. However, PPG residues in environmental samples were not correlated to tissue residues. There was no consistency between the Charm MRL™ and SNAP™ tests to any of the other sample types. Residues were consistently detected in the environment with the SNAP™ test, which indicates presence of PPG residues in the environment surrounding sows treated with PPG within at least 28 days. The LCMS testing methodology was consistent with the KIS testing of kidneys used by the FSIS and is a reliable analytical tool to assess PPG residues.

Introduction:

Procaine Penicillin G (PPG is approved for use in swine only for the treatment of *Erysipelothrix rhusiopathiae*. The Animal Medicinal Drug Use and Clarification Act (AMDUCA) provides a mechanism for veterinarians to use PPG at higher dosages and for additional target organisms beyond the original label. Veterinarians frequently find this use beneficial in sows for the treatment of a variety of pathogens. Iowa State University antimicrobial susceptibility testing during the 2014 calendar year revealed that greater than 89% *of Erysipelothrix rhusiopathiae*, 92% of *Pasteurella multocida* type A, 93% of *Pasteurella multocida* type D, and 78% of *Streptococcus suis* isolates were susceptible to PPG. Additionally, the known mechanism of action for PPG suggests that it would be effective for a number of common pathogens for which routine testing is not summarized (ISU VDL 2014). There is limited research which, in total, fails to establish with certainty the appropriate withdrawal time to ensure that extra-label doses, typically applied to sows, result in no residue in cull sow products (Apley et al. 2009). A Canadian study (Korsrud et al. 1998) found PPG residues in kidneys after extra-label injection persisted beyond the sampling timeframe of 7 days. From this data, researchers calculated, but did not directly measure, a withdrawal requirement of 15 days for the extra-label dose. This particular study used liquid chromatography detection technology which has been widely augmented by mass spectrometry to increase sensitivity of residue detection since the time of the study. A more recent study extrapolated a withdrawal of 51 days for extra-label administration of PPG. This extended time is needed to reduce kidney levels of PPG to below 25 ppb, the FSIS action level for PPG residues (Shelver et al. 2013).

The United State Department of Agriculture Food Safety Inspection Service (FSIS) has replaced the Fast Antimicrobial Test (FAST) with the Kidney Inhibition Swab (KIS) test. Additionally, the FSIS has updated confirmatory testing protocols that effectively switch from a biological test to a chemical test which allows for determination of the specific residue rather than recognition of general bacterial inhibition only. These steps have are believed to be in part responsible for widespread reports of PPG residue detection in cull sows. United States producers are prolific exporters of pork products and many destination countries have lower acceptable residue limits than those accepted by the USDA FSIS. Direct measurement of the concentrations in tissue that result from typical administration routes and extra-label doses was not found in an extensive literature search. This data is required to establish safe withdrawal periods. With increased scrutiny from domestic and export markets and evidence of PPG tissue residues in pigs, the swine industry must take a proactive stance towards prevention of carcass drug residue.

This study describes the tissue depletion profile of PPG in sows after administration of either 3,000 IU/lb or 15,000 IU/lb once daily for 3 days using a sensitive LCMS methodology. This study also examined a variety of antemortem methods including environmental sampling, plasma sampling, and urine sampling to assess drug tissue residues in sows prior to slaughter.

Objectives:

- Determine specific withdrawal times for typical extra-label penicillin regimens in adult sows by directly measuring drug concentrations in muscle (semimembranosus/semitendinosus), liver, kidney, and the injection site from the final day administration (right hip).

- Compare the results of the most sensitive current international standard for detection (LS/MS) with results obtained by new FSIS testing methods.

- Evaluate the environmental transfer of common penicillin G treatment protocols by validating oral fluids and environmental sampling for this purpose.

- Correlate the concentration of penicillin G procaine found in environmental samples, plasma, and urine with those found in tissues to develop an antemortem residue detection and prevention protocol.

Materials and Methods

Before the initiation of this experiment, all animal use, handling, and sampling techniques described were approved by the Iowa State University Animal Care and Use Committee (IACUC #4-14-7785-S).

Animals

Forty-seven (47) healthy cull sows were obtained from a commercial sow herd. None of the cull sows had previous PPG treatment for the 52 days immediately prior to the start of the study. Sows were placed in study pens according to their allotment upon arrival, and were acclimatized in their assigned pens and rooms for 72 hours. Each sow was identified by the use of a plastic livestock ear tag placed in the left ear of the sow at arrival. Three one inch diameter, circular tattoos were applied on the skin using a commercial tattoo applicator with a slap tattoo: one each on the right and left post-auricular areas and on the right hip. The post-auricular tattoos were approximately 2 inches ventral to the dorsal midline and 2 inches caudal to the ear. The hip tattoo was place approximately 5 inches ventral to the tuber ischii. A second circular tattoo was pressed into the skin 4 inches ventral to the each of the first three tattoos to serve as an injection site for injection volumes that exceeded the limit able to be administered in one site.

Pigs were housed at the Livestock Infectious Disease Isolation Facility (LIDIF) of Laboratory Animal Resources Research Facilities at the Iowa State University College of Veterinary Medicine. The entry weights were used to randomly allocate the sows into housing group based on anticipated necropsy date and treatment option. Sows were housed in four (4) rooms according to their scheduled necropsy time. Each room was divided into four (4) pens of three (3) sows. Each pen contained a sow from each of three treatment groups (TG1, TG2, and TG3). Housing conditions were in accordance with the recommendations outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Use and Research and Teaching 3rd Edition.

Pigs were fed an age-appropriate diet ad libitum that meets or exceeds NRC nutrient requirements and had free access to water. The feed was an age appropriate non-mediated Nature's Match Land O' Lakes ration.

Animal phase study design

Fifteen sows were allocated to TG1, sixteen (16) sows were allocated to TG2, and sixteen (16) sows were allocated toTG3. All sows in TG1 received 3,000 IU/lb PPG, sows in TG2received a sterile saline volume equivalent of 9,000 IU/lb PPG (an average of TG1 and TG3), and sows in TG3 received 15,000 IU/lb PPG (Agricillin, 300,000 IU PPG /mL, AgriLabs, St. Joseph, MO). Sows were weighed upon arrival and ranked by weight from heaviest to lightest. The heaviest one-third (16 pigs), middle one-third (16 pigs), and lightest one-third (15 pigs) were blocked by weight. They were given a random number and assigned to a treatment group (TG1, TG2, or TG3), necropsy group (G1-G4), and pen number demonstrated in **Table 1.**

Each sow was restrained with a hog snare and individual injections were administered at a specified time for each individual sow. These injections were administered IM with a 16 gauge, 1 inch needle inside the circular tattoo placed on arrival to ensure accurate recovery of the injection site. Up to 10 mL for TG1 and up to 20 mL for TG2 and TG3 was administered in the dorsal site and the remaining volume was given at the ventral site. Injections were administered at the same specified time each day for three consecutive days. Day 0 injections were given in the left post-auricular area, Day 1 injections were given on the right post-auricular area, and Day 2 injections were given on the right hip area.

Blood samples were obtained immediately prior to the first administration of injections, two days after completion of dosing regimen, and immediately prior to euthanasia (two time points total for G1 and three time points total for G2-G4). Sows had blood collected from the left or right jugular vein using a 4 inch 16 gauge hypodermic needle and 12 mL Luer lock syringe. They were physically restrained with a hog snare and at each sample point 8 mL of blood was obtained. All blood samples for use in analysis were collected in glass 10 mL heparin tubes. Blood samples were mixed by inverting

the tube, labeled with a unique identifier, and immediately placed on ice. The blood sample was centrifuged at 1000g g for 15 minutes, and the separated plasma was stored in cryovials at -80 ° C.

Environmental sampling was performed in each group to assess the presence of PPG in the environment. An unscented swiffer pad was placed in a 50mL vial containing 25 mL of physiologic saline until all liquid was absorbed by the swiffer pad. The wet swiffer was used to scrub a selected sampling area. Any excess fluid was mopped up by the pad and the entire swiffer pad was placed in a plastic bag and the fluids were extracted by digital manipulation of the pad. The fluid was removed from the plastic bag and poured back into the 50mL vial and stored at -80° C until analysis. Environmental samples were collected on during the acclimation period before PPG administration, on Day 2 post- PPG administration, and each day of necropsy.

Urine samples were collected at necropsy. Free catch urine was collected from sows prior to euthanasia in a 50 mL Falcon tube. If the free catch sample was unable to be obtained antemortem, the bladder was expressed post-mortem by applying pressure to the flank and collecting the manually expressed urine in a 50 mL Falcon tube. One sow was unable to be collected by either method so five mL of urine was aspirated from the urinary bladder using a 6 mL Luer Lock syringe with an attached 22ga x3/4" needle. All samples were transferred to a non-additive red top tube and stored at -80 degrees Celsius prior to analysis.

Necropsies were performed on each pig assigned to the necropsy group with at the assigned days post-administration of injections. These necropsies occurred at 1, 6, 14, and 28 days after final administration of PPG and sterile saline. Any gross pathological abnormalities were noted. Kidney, liver, semitendinosus/ semimembranosus muscle, and the right hip injection sites were collected and submitted for analysis. For injection sites, a two inch circumference around the final injection sites (right hip area) was dissected out for sampling. These tissue samples were stored in Whirl-Pak bags and placed on ice until permanent storage at -80 ° C prior to analysis.

Sample Collection, Processing, and Analysis

All drug concentrations in collected samples were analyzed at Iowa State University Veterinary Diagnostic Lab and the Iowa State University-Pharmacology Analytical Support Team (ISU-PhAST).

Porcine Tissue Extraction for Liquid Chromatography-Mass Spectrometry (LC-MS/MS) (Liver, Kidney, Muscle, Injection Site)

Calibration standards for tissue (liver, kidney, muscle, injection site) were prepared using standard additions of procaine penicillin G with 2 grams of ground/processed blank tissue. Blank tissue refers to tissue with no known exposure to penicillin G. Final concentrations of penicillin G were 1, 10, 50, 100, 250, 500, 1000 ng/mL. Standards were mixed using a vortex mixer and allowed to sit for 5 minutes. Internal standard, penicillin G –d7 ethylperidinium salt (Sigma, St. Louis, MO), was added to the standards/samples to give a final concentration of 500 ng/mL.

Ten mL of acetonitrile:water (4:1) were added and standards/samples were mixed using a multi-tube vortexer for 5 minutes. Samples/standards were then centrifuged at 2500 rpm for 5 minutes. Supernatent was transferred to a 50 mL centrifuge tube containing 0.5 g of C18 sorbent. Ten mL of hexane saturated with acetonitrile was added. Samples/standards were vortexed for 1 minute and centrifuged at 3500 rpm for 5 minutes. Hexane was then aspirated to waste. Two mL of samples/standards were evaporated to dryness, resuspended in 50 µL of 25% (v/v) acetonitrile: water and 150 µL water and then transferred to an autosampler vial with glass insert. Samples were centrifuged for 20 minutes at 2400 rpm prior to LC-MS/MS analysis.

Porcine Plasma Extraction for LC-MS/MS

Plasma samples were thawed and centrifuged. Aliquots of 500 µL were transferred to test tubes. Standards were prepared by adding penicillin G to 500 µL of blank plasma to obtain final concentrations of 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 ng/mL. Quality control (QC) samples were prepared by adding penicillin G to 500 µL of blank plasma to obtain final concentrations of 15, 150, 750 ng/mL. Internal standard, penicillin G-d7 ethylperidinium salt (Sigma), to give a final concentration of 40 ng/mL was added to each sample/standard. A volume of 2.5 mL acetonitrile was added to each standard/sample, followed by mixing with a vortex mixer, and then centrifugation for 20 min at 2400 rpm. Supernatant was transferred to a test tube and evaporated to dryness using a stream of nitrogen. Standards/samples were reconstituted in 50 µL of 25% (v/v) acetonitrile in water and mixed with a vortex mixer. Then 150 µL of water was added and mixed

with a vortex mixer. Standards/samples were transferred to an autosampler vial with glass insert, centrifuged for 20 min at 2400 rpm and analyzed via LC-MS/MS.

Porcine Urine Extraction for LC-MS/MS
Frozen standards/samples were thawed at room temperature. Standards for urine were prepared by spiking 150 µL of blank urine to concentrations of 10, 20, 50, 100, 200, 500, 1000, 2000, 5000, 10000 ng/mL penicillin G. QC samples were prepared at concentrations of 30, 300, 3000, and 30,000 ng/mL penicillin G by spiking 150 µL of urine. Internal standard, penicillin G-d7 ethylperidinium salt (Sigma), giving a final concentration of 250 ng/mL was added to each standard/QC/sample and then diluted 1:8 with water. Standards/QCs/samples were transferred to an autosampler vial with glass insert, centrifuged for 20 minutes at 2400 rpm and analyzed via LC-MS/MS. Samples that had a concentration higher than 10,000 ng/mL were diluted accordingly with water until their concentrations decreased enough to be in the range of the standard curve.

LC-MS/MS analysis of Porcine Tissue (Liver, Kidney, Muscle, Injection Site) Plasma, and Urine
Concentrations of penicillin G in tissue samples were measured using LC-MS/MS. Separation was achieved via high-performance liquid chromatography (HPLC) using a Surveyor pump and autosampler from Thermo Scientific (San Jose, CA, USA). Data collection was achieved using a Thermo TSQ Quantum Discover Max triple quadrupole mass spectrometer. The HPLC system utilized a Kinetex C18 column (100mm x 2.1 mm, 2.6 µm particle size) from Phenomenex (Torrance, CA, USA) maintained at 40 °C. The mobile phase consisted of A: 0.1% (v/v) formic acid in water and B: 0.1% (v/v) formic acid in acetonitrile. The flow rate was 0.25 mL/min. The mobile phase began at 20% B with a linear gradient to 95% B which was maintained for 2 minutes before re-equilibration to 20% B.

Both penicillin G and penicillin G d-7, had a retention time of 4.6 minutes. The transitions used for penicillin G identification were (m/z) 335 → 114/160/176. The transitions used for the internal standard, penicillin G-d7, were 342 → 114/160/183. All data was collected in positive ion mode.

All standard curves for tissues, plasma, and urine had a coefficient of determination that exceeded 0.98. QC samples were deemed to have passed when calculated concentration values were with 20% of expected levels.

Urine analyzed using Charm MRL™ beta-lactam test for urine (Charm Sciences Inc.)
Standards were prepared by diluting a 1 ppt (part per thousand or 1 µg/µl) stock solution of Penicillin G, potassium salt, in 60:40 acetonitrile:water with water purified using a Millipore (Billerica,MA) Milli-Q water purification system. Standards had final concentrations of 1, 5, 10, 20, 30, 40, 50, 60, 100 and 200 ppb. Purified water was also used as the negative control. The positive control was prepared by dissolving one tablet of penicillin G, supplied in the test kit, in 1.0 ml of water purified using a Millipore (Billerica, MA) Milli-Q water purification system.
50 µl of sample were diluted with 450 µl of dilution buffer supplied in the test kit. 300 µl of this mixture was applied to the sample pad on the test strip. The strip was incubated for 8 min at 55°C, visually examined and then inserted into a ROSA™ Reader from Charm Sciences Inc. (Lawrence, MA). Prior to analysis, positive and negative calibration strips, supplied by the manufacturer, were read as a daily performance check. Samples were reported as positive or negative, according to the ROSA™ Reader result, as long as the positive control gave a reading greater than +400 and the negative control gave a reading less than -400, according to test kit instructions.

Urine analyzed using SNAP™ beta-lactam test kit (IDEXX)
Urine samples were centrifuged at 1200 g for 3 min prior to analysis. The New SNAP™ Beta-Lactam Test from IDEXX (Westbrook, ME) was used according to instructions. A disposable pipette, provided with each SNAP™ device, was used to draw up 450 µL (±50 µL) of sample. The sample was placed in the sample tube provided which contained a reagent pellet. After mixing thoroughly, the sample tube was incubated for 5 min at 45°C (±5°C). Following incubation, the sample was poured into the sample well on the SNAP™ device. As soon as the edge of the activation circle began to disappear, the activator was pushed down and the test device was left on the heater block for another 4 min. SNAP™ devices were verified visually and read using a SNAPshot™ Reader.
Standards, with concentrations of 1, 5, 10, 20, 30, 40, 50 and 60 ppb were prepared in purified water by diluting a 1 ppt (part per thousand or 1 µg/µl) stock solution of penicillin G, potassium salt, in 60:40 acetonitrile:water. Purified water was used as negative control and to reconstitute the positive control, penicillinG, supplied in the test kit. Samples were

reported as negative when the reading on the IDEXX SNAPshot™ Reader was 1.05 or lower, according to test kit instructions.

Porcine kidneys analyzed using KIS test (Charm Sciences Inc.)
The Kidney Inhibition Swab (KIS) test was performed on porcine kidneys, according to test instructions. The juice from porcine kidney, with no known exposure to antibiotics, was used as the negative control. Positive control was prepared by mixing 0.8 ml of juice from kidney with no known exposure to antibiotics with 0.5 ml of 50 ppb penicillin G. This solution of 50 ppb penicillin G, as well as other standards, with concentrations of 10, 20, 30 and 40 ppb were prepared in purified water by diluting a 1 ppt (part per thousand or 1 µg/µl) stock solution of penicillin G, potassium salt, in 60:40 acetonitrile:water. In each case, 0.5 ml of standard was mixed with 0.8 ml of kidney juice, not known to contain antibiotics. Following incubation, the agar color was compared to the colors shown on the interpretation card included with the KIS test supplies. Results were interpreted as positive or negative.

Environmental samples analyzed using Charm MRL™ beta-lactam test for urine (Charm Sciences Inc.)
Environmental samples were also analyzed according to test kit instructions: 50 µl of sample were diluted with 450 µl of dilution buffer supplied in the test kit. 300 µl of this mixture was applied to the sample pad on the test strip. The strip was incubated for 8 min at 55°C, visually examined and then inserted into a ROSA™ Reader from Charm Sciences Inc. (Lawrence, MA). Purified water was used as the negative control. The positive control was prepared by dissolving one tablet of penicillin G, supplied in the test kit, in 1.0 ml of water purified using a Millipore (Billerica, MA) Milli-Q water purification system. Prior to analysis, positive and negative calibration strips, supplied by the manufacturer, were read as a daily performance check. Samples were reported as positive or negative, according to the ROSA™ Reader result, as long as the positive control gave a reading greater than +400 and the negative control gave a reading less than -400, according to test kit instructions.

Environmental samples analyzed using SNAP™ beta-lactam test kit (IDEXX)
Environmental samples were mixed using a vortex mixer then centrifuged at 15000 rpm for 20 min prior to analysis. The New SNAP™ Beta-Lactam Test from IDEXX (Westbrook, ME) was used according to instructions. A disposable pipette, provided with each SNAP™ device, was used to draw up 450 µL (±50 µL) of sample. The sample was placed in the sample tube provided which contained a reagent pellet. After mixing thoroughly, the sample tube was incubated for 5 min at 45°C (±5°C). Following incubation, the sample was poured into the sample well on the SNAP™ device. As soon as the edge of the activation circle began to disappear, the activator was pushed down and the test device was left on the heater block for another 4 min. SNAP™ devices were verified visually and read using a SNAPshot™ Reader.
Purified water was used as negative control and to reconstitute the positive control, penicillin G, supplied in the test kit. Samples were reported as negative when the reading on the IDEXX SNAPshot™ Reader was 1.05 or lower, according to test kit instructions.

Results and Discussion

- **Determine specific withdrawal times for typical extra-label penicillin regimens in adult sows by directly measuring drug concentrations in muscle (semimembranosus/semitendinosus), liver, kidney, and the injection site from the final day administration (right hip).**

Residues in TG1 sows administered the labelled dose of PPG were discovered in kidney muscle, and injection sites by LCMS. Kidney residues were detected in 33% (1/3) of sows at Day 1, 25% (1/4) of sows at Day 6 and in 25% (1/4) of sows at Day 28 post-administration at a level of 48.4 ng/g. PPG residues in muscle were detected in 100% (4/4) of sow tissues sampled at Day 1 post-administration, but in no other groups. Injection site residues were detectable in at least one sow in all time points, with a 207.7 ng/g residue detected at Day 28. For a complete set of tissue residue data see **Table 3.**

TG2 sows were administered no PPG and were instead injected with sterile saline to serve as a negative control in this study. No detectable residues were found in kidney, muscle, or liver tissues in any sow at any time point. Injection site residues were detected in five sows with at least one sow at each sampling time point. Many of the detectable injection site residues in TG1 and TG3 were found at very high levels, some over 1 million ng/g of tissue. This high concentration possibly could remain in the column after analysis of treated animals and produce a false positive result.

Sows administered the extra-label dose of PPG in TG3 had detectable residues in all tissue sample types. Kidney PPG residues were detected in 100% (4/4) of sows at Day 1 post-administration and 50% (2/4) of sows at six days post-administration. One sow at Day 1 had measurable residues in the liver. Muscle residues were detected out to 14 days post-administration and were found in 50% (2/4) of sows sampled. Very high levels of residue were detected in the injection sites of the TG3 sows. 100% (4/4) sows in Day 1, Day 6, and Day 14 necropsy time points had injection site residues. These values ranged from 31,689 ng/g to 12,518,210 ng/g on Day 1 and from 53.0 ng/g to 3,645.9 ng/g on Day 6. Injection site residues at Day 14 were lower, ranging from 51.7 ng/g to 599.8 ng/g. Only 25% (1/4) of sows had injection site residues at Day 28 post injection. High levels of PPG in the injection sites at all time points could be due to the large injection volume and slow distribution of PPG through the tissues. Sows have a thick layer of fat overlaying the hip region where injections were given. A one inch needle may not have penetrated the muscle in more well-conditioned sows and some of the high levels of residues could be due to PPG that is bound in the fat layer.

PPG tissue residue depletions appears to be concentration dependent. As concentration increased, a higher percentage of sows had tissue residues in all four tissues sampled. Additionally, the concentration of PPG residue found in TG3 was on average higher than sows in TG1. Based on the preliminary data, it appears that tissue residues can be found in sows administered extra-label doses at least 28 days post-administration. This is much greater than the estimated 15 day withdrawal extrapolated from the Korsrud et al. study. Further statistical analysis and modeling is needed to further the understanding of a complete tissue residue depletion profile.

- **Compare the results of the most sensitive current international standard for detection (LC/MS) with results obtained by new FSIS testing methods.**

Methods used by the FSIS to detect residues using LCMS were similar to the testing methods used in this study. The FSIS reported lower limits of quantitation for kidney, liver, and muscle to be at 15, 30, and 5 ng/g of tissue, respectively. Initially this study used lower LOQ's of 5 ng/g tissue, but a large amount of variability was seen at this level, so the data was finalized using the FSIS standards.

The FSIS also uses the kidney inhibition swab (KIS) test to screen for residues. A comparison of the international standard for detection (LCMS) values with the results of the KIS test on the same samples is listed in **Table 4**. In TG1 the LCMS detected three more positive residue tests of kidneys than the KIS test. The KIS test only reported one positive residue test at Day 1. The two assays agreed completely with the TG2 controls. They correlated well in the extra-label TG3 sows, with both the LCMS and KIS test detecting 100% of samples with residues at Day 1 and 50% of samples with residues at Day 6. However, the samples that tested positive at Day 6 were not the same sows for both assays. The LCMS had a more accurate level of detection than the KIS test on these samples.

- **Evaluate the environmental transfer of common penicillin G treatment protocols by validating oral fluids and environmental sampling for this purpose.**

The initial study proposal included oral fluids sampling for PPG. Oral fluids were proposed to provide a practical sample of potential PPG contamination of the environment from treated sows. A 2015 study of oral fluid collection in individually housed sows revealed an oral fluid collection rate of only 23.2% of sows that were sampled on their first day of rope exposure. Exposure on a second increased the rate to only 47.8% of sows that chewed on oral fluids ropes. The study also noticed an association with younger sows and a willingness to chew on ropes (Pepin et al. 2015). In this study, ropes were hung in each pen during the 72 hour acclimatization period once a day for 30 minutes on each day of acclimatization. During this period only one sow in one pen (in a total of 16 pens) chewed on ropes. Due to the findings in the recent study and failure of sows in this study to provide oral fluid samples, environmental sampling was selected to be used as a more reliable assay for sampling the environment than oral fluids.

Environmental samples analyzed with the Charm MRL™ and SNAP™ rapid tests results are reported in **Table 6**. Widely varying results were seen between the two assays. All environmental samples tested with Charm MRL™ were negative for PPG residues. This is in contrast to the SNAP™ tests run on the same samples. At

time 0, before any administration of PPG product, two positive tests were detected by SNAP™ testing in the room containing the G3 sows. On Day 1 post-administration of PPG 66% (2/3) out of samples analyzed were positive on the SNAP™ test. All other samples were positive with the test except for one pen of sows on Day 3. Based on these results SNAP™ appears to be a more sensitive assay for the detection of PPG residues. However, it is possible that some of the positives may be false positives. It would be more useful than Charm MRL™ to use as a screening test for presence of PPG residues in the environment. Based on these two assays , the accuracy of using environmental sample testing to detect PPG residues in a group of sows may not be an accurate antemortem assessment. However, it does provide some evidence to support further research for PPG excretion into the environment by treated sows.

- **Correlate the concentration of penicillin G procaine found in plasma, urine, and environmental samples with those found in tissues to develop an antemortem residue detection and prevention protocol.**

Plasma samples of TG1 revealed PPG residues in 100% (4/4) sows at Day1 and Day 6 post-administration. 92% (11/12) sows sampled on Day 3 had detectable plasma residues. No residues were found on plasma samples in sows necropsied on Day 14 or Day 28. Residue concentration decreased as time increased from last administration. Specific concentrations are listed in **Table 2a**. In the negative control group (**Table 2b**), there were no plasma samples that had PPG residues. TG3 had 100% (4/4) residue detection in plasma samples at Day 1 and Day 6 (**Table 2c**). 100% (12/12) of samples tested at Day 3 were also positive for PPG residues by LCMS testing. 75% (3/4) of sows had residues detected in plasma samples at Day 14, but no residues were detected at Day 28. Similar to the tissue residue pattern, extended residue detection is seen with the TG3 sows versus the TG1 sows. Plasma detection of PPG residues by LCMS was more sensitive than detection of residues in kidney tissue by LCMS. Plasma PPG residues were detected in 100% of TG3 sows at Day 14 and kidney residues were only detected in 50% of sows at Day 6 for the same treatment group. However, plasma detection of PPG residues was similar to the detection level seen in the skeletal muscle. Plasma concentrations were no indication of injection site concentrations.

Urine samples were analyzed by LCMS, SNAP™and Charm MRL™ rapid tests. Analysis with LCMS found PPG residues in 100% of samples at Day 1 (4/4) and Day 6 (3/3) for TG1. All other time points for TG1 tested negative. The TG2 control group had no residues in urine samples in any sow at any time point. PPG residues in TG3 sows were detected out to Day 14. 100% of sows at Day 1 (4/4) and Day 6 (4/4) had very concentrated residues, ranging from a high of over 1 million ng/mL at day 1 to around 16,000 ng/mL on Day 6. 50% (2/4) sows necropsied on Day 14 post-administration of PPG had PPG residues present in the urine, and no sows had PPG residues present at Day 28. A complete list of urine analysis is found in **Table 5.** The Charm MRL™ tests that reported a positive urine sample correlated exactly with the positive LCMS samples in TG1 with residues present at least 6 days post-administration of PPG. All but one positive on the Charm MRL™ test agreed with the positive LCMS samples of TG3, indicating residues present at at least 14 days post-administration. TG2 Charm MRL™ tests had two samples test positive, which did not agree with the LCMS and should not be present in a truly negative animal. These samples that tested positive were present in one sow sampled at Day 1 and one sampled a Day 14. The SNAP™ tests performed on these two particular samples was also positive. The SNAP™ test for TG2 also had two more positive tests, another at Day 1 and one on Day 6. SNAP™ tests performed on TG1 sows agreed with the Charm MRL™ and LCMS except for two sows (2/4) on Day 14 and one sow (1/2) on Day 28. SNAP™ test results for TG3 also correlated well with the Charm MRL™ and LCMS results. There were two more positive tests on the SNAP™ than the Charm MRL™, one sow at Day 14 and another at Day 28. Mild discrepancies in positive test results among the three assays were mostly seen as time increased from cessation of PPG administration. This could be due to inaccuracies in the assays at low levels of quantitation. The SNAP™ and Charm MRL™ tests also are less specific than the LCMS and may be more likely to report false positive results.

Very little correlation can be made between environmental samples and the tissue residue depletion seen with the LCMS analysis. Environmental sampling may be useful to detect presence of residues in an area, but not for individual sow assessment of PPG residues.

Conclusion

At extra-label doses, tissue residues of PPG in the kidney can be detected at up to at least 6 days after final administration of PPG. Residues in the skeletal muscle were depleted more slowly than the kidney and detected out to at least 14 days. Injection site residues were the most prominent and depleted the most slowly with PPG detected at high levels up to the conclusion of the trial at 28 days. Statistical modeling is needed to extrapolate the depletion profile of these tissues. From the raw data presented, the proposed 15 day withdrawal would not be sufficient for full tissue depletion. Further analysis of data will clarify whether the proposed 51 day withdrawal would be sufficient for full tissue residue depletion due to persistence at the injection site. Plasma concentrations very closely correlate with tissue residue depletion of PPG in kidney and skeletal muscle. For extra-label doses and on-label doses, plasma concentrations of PPG were consistently higher than those found in kidney and skeletal muscle. With this information, plasma appears to be a good antemortem sample for PPG residue detection. However, the only method used for plasma residue detection was LCMS analysis. Because a lab with proper equipment and established residue analysis protocols is required for LCMS analysis, use of plasma as a practical, quick antemortem PPG residue sample is limited. Urine and environmental samples were also assessed for use in antemortem residue detection. As a sample type, urine residues of PPG were found correlated to plasma and tissue residues. TG1 sows had urine residues at Day 6 post-administration of PPG and TG3 sows had urine residues at Day 14 post-administration of PPG when analyzed by LCMS. Both rapid tests, Charm MRL™ and Snap, had consistent results when detecting urine residues compared to the LCMS analysis. With this evidence, antemortem urine testing with either Charm MRL™ or SNAP™ tests would provide accurate information about PPG residues in the sow of interest. Environmental samples were not an accurate representation of the tissue residues. There was no consistency between the Charm MRL™ nd SNAP™ tests or when correlated back to any of the other sample types. Residues were consistently detected in the environment with the SNAP™ test, which indicates presence of PPG residues in the environment surrounding sows treated with PPG within at least 28 days. Comparison of the LCMS methods with current FSIS methods of testing revealed that lower LOQ's than what are being used by FSIS testing are less accurate and produce more variability at low levels. The LCMS testing methodology was consistent with the KIS testing of kidneys used by the FSIS and is a reliable analytical tool to assess PPG residues.

Table 1. Study animal weights and necropsy group allocation information. Sows in Treatment Group 1 (TG1) and Treatment Group 3(TG3) received a dose of 3,000 IU/lb and 15,000 IU/lb procaine penicillin G IM for three consecutive days. Sows in Treatment Group 2 (TG2) received a dose of sterile saline equal to the average volume of procaine penicillin G administered to TG1 and TG3, but not exceeding 20 mL.

		Treatment Group 1		**Treatment Group 2**		**Treatment Group 3**	
Necropsy Group	**Pen Number**	**Sow ID**	**Weight (lb)**	**Sow ID**	**Weight (lb)**	**Sow ID**	**Weight (lb)**
G1 (day 1)	**1**	N/A	N/A	468	525.5	447	554.0
	2	342	536.5	463	452.0	441	384.0
	3	473	508.5	462	536.5	444	512.5
	4	339	554.5	471	496.0	453	516.0
G2 (day 6)	**1**	470	586.5	456	456.0	450	489.5
	2	446	564.5	466	564.5	467	567.5
	3	345	582.5	452	575.5	455	590.5
	4	474	592.5	350	588.5	346	640.5
G3 (day 14)	**1**	472	499.0	458	593.5	443	571.5
	2	349	587.5	347	494.5	457	441.0
	3	344	471.5	451	469.5	454	539.0
	4	440	461.5	445	524.5	461	446.0
G4 (day 28)	**1**	340	509.0	459	594.5	341	515.0
	2	348	492.5	460	608.5	442	590.0
	3	448	542.0	343	334.0	464	489.0
	4	465	506.5	469	525.5	449	505.0

Table 2a. Plasma procaine penicillin G concentrations (ng/mL) in Treatment Group 1 (TG1) after IM administration at 3,000 IU/mL. Concentrations that were below the level of quantification (LOQ) (5 ng/mL) was designated "<LOQ".

Necropsy Group	Pen Number	Sow ID	Days Post-Administration					
			0	1	3	6	14	28
G1 (day 1)	1							
	2	342	<LOQ	169.0				
	3	473	<LOQ	247.5				
	4	339	<LOQ	124.0				
G2 (day 6)	1	470	<LOQ		23.0	15.8		
	2	446	<LOQ		85.3	40.2		
	3	345	<LOQ		24.5	7.4		
	4	474	<LOQ		100.3	43.8		
G3 (day 14)	1	472	<LOQ		22.7		<LOQ	
	2	349	<LOQ		136.6		<LOQ	
	3	344	<LOQ		20.1		<LOQ	
	4	440	<LOQ		71.8		<LOQ	
G4 (day 28)	1	340	<LOQ		42.2			<LOQ
	2	348	<LOQ		<LOQ			<LOQ
	3	448	<LOQ		18.2			<LOQ
	4	465	7.5		70.1			<LOQ

Table 2b. Plasma procaine penicillin G concentrations (ng/mL) in Treatment Group 2 (TG2) after IM administration of sterile saline at the volume equivalent of 9,000 IU/mL procaine penicillin G and not exceeding 20 mL. Concentrations that were below the level of quantification (LOQ) (5 ng/mL) was designated "<LOQ".

Necropsy Group	Pen Number	Sow ID	Days Post-Administration					
			0	1	3	6	14	28
G1 (day 1)	1	468	<LOQ	<LOQ				
	2	463	<LOQ	<LOQ				
	3	462	<LOQ	<LOQ				
	4	471	<LOQ	<LOQ				
G2 (day 6)	1	456	<LOQ		<LOQ	8.2		
	2	466	<LOQ		<LOQ	<LOQ		
	3	452	<LOQ		<LOQ	<LOQ		
	4	350	<LOQ		<LOQ	<LOQ		
G3 (day 14)	1	458	<LOQ		<LOQ		<LOQ	
	2	347	<LOQ		<LOQ		<LOQ	
	3	451	<LOQ		<LOQ		<LOQ	
	4	445	<LOQ		<LOQ		<LOQ	
G4 (day 28)	1	459	<LOQ		<LOQ			<LOQ
	2	460	<LOQ		<LOQ			<LOQ
	3	343	<LOQ		<LOQ			<LOQ
	4	469	24.9		<LOQ			<LOQ

Table 2c. Plasma procaine penicillin G concentrations (ng/mL) in Treatment Group 3 (TG3) after IM administration at 15,000 IU/mL. Concentrations that were below the level of quantification (LOQ) (5 ng/mL) was designated "<LOQ".

Necropsy Group	Pen Number	Sow ID	Days Post-Administration					
			0	1	3	6	14	28
G1 (day 1)	1	447	<LOQ	641.3				
	2	441	<LOQ	620.3				
	3	444	<LOQ	871.9				
	4	453	<LOQ	520.4				
G2 (day 6)	1	450	<LOQ		336.3	213.1		
	2	467	<LOQ		401.0	149.5		
	3	455	<LOQ		470.6	29.5		
	4	346	<LOQ		299.4	220.6		
G3 (day 14)	1	443	<LOQ		226.0		88.2	
	2	457	<LOQ		411.6		<LOQ	
	3	454	<LOQ		713.6		61.2	
	4	461	<LOQ		298.9		9.6	
G4 (day 28)	1	341	<LOQ		277.0			<LOQ
	2	442	<LOQ		552.4			<LOQ
	3	464	<LOQ		145.9			<LOQ
	4	449	<LOQ		136.2			<LOQ

Table 3. Tissue procaine penicillin G (ng/g) in kidney, liver, semitendinosus/semimembranosus muscle, and injection site (s). Concentrations that were below the level of quantification (LOQ) was designated "<LOQ". The following LOQ values were applied: kidney, 15 ng/g; liver, 30 ng/g; muscle 5 ng/g; injection site, 50 ng/g . Injections were given in two locations on the hip: "dorsal" and "ventral." For TG1, up to 10 mL of procaine penicillin G was injected in the dorsal location first, with remaining volume injected on the ventral location. For TG2 and TG3, up to 20 mL was injected in the dorsal location and remaining volume was injected ventrally.

	Treatment Group 1						
Necropsy Group	Pen Number	Sow ID	Kidney	Liver	Muscle	Injection Site (Dorsal)	Injection Site (Ventral)
G1 (day 1)	1	N/A	N/A	N/A	N/A	N/A	N/A
	2	342	<LOQ	<LOQ	13.7	535872	N/A
	3	473	31.7	<LOQ	15.5	351282	N/A
	4	339	16.4	<LOQ	15.6	1151.0	N/A
G2 (day 6)	1	470	<LOQ	<LOQ	<LOQ	105.5	N/A
	2	446	96.4	<LOQ	<LOQ	895.2	652.2
	3	345	<LOQ	<LOQ	<LOQ	<LOQ	N/A
	4	474	<LOQ	<LOQ	<LOQ	97.8	N/A
G3 (day 14)	1	472	<LOQ	<LOQ	<LOQ	105.1	N/A
	2	349	<LOQ	<LOQ	<LOQ	<LOQ	N/A
	3	344	<LOQ	<LOQ	<LOQ	<LOQ	N/A
	4	440	<LOQ	<LOQ	<LOQ	<LOQ	N/A
G4 (day 28)	1	340	<LOQ	<LOQ	<LOQ	<LOQ	N/A
	2	348	48.4	<LOQ	<LOQ	207.7	N/A
	3	448	<LOQ	<LOQ	<LOQ	<LOQ	N/A
	4	465	<LOQ	<LOQ	<LOQ	<LOQ	N/A
	Treatment Group 2						
G1	1	468	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

(day 1)	**2**	463	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	3	462	<LOQ	<LOQ	<LOQ	98.7	555.4
	4	471	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
G2 (day 6)	**1**	456	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	2	466	<LOQ	<LOQ	<LOQ	24.6	<LOQ
	3	452	<LOQ	<LOQ	<LOQ	<LOQ	185.0
	4	350	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
G3 (day 14)	**1**	458	<LOQ	<LOQ	<LOQ	945.5	79.1
	2	347	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	3	451	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	4	445	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
G4 (day 28)	**1**	459	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	2	460	<LOQ	<LOQ	<LOQ	<LOQ	114.4
	3	343	<LOQ	<LOQ	<LOQ	N/A	N/A
	4	469	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Treatment Group 3						
G1 (day 1)	**1**	447	19.4	<LOQ	98.2	177772	357204
	2	441	38.8	67.1	72.6	12518610	N/A
	3	444	44.7	<LOQ	115.7	1469383	2674478
	4	453	679.9	<LOQ	59.6	460021	31689
G2 (day 6)	**1**	450	64.9	<LOQ	21.7	436.5	3645.9
	2	467	16.3	<LOQ	19.8	227.9	31667
	3	455	<LOQ	<LOQ	<LOQ	53.1	527.6
	4	346	<LOQ	<LOQ	22	53.0	90121
G3 (day 14)	**1**	443	<LOQ	<LOQ	6.8	<LOQ	234.7
	2	457	<LOQ	<LOQ	<LOQ	85.7	<LOQ
	3	454	<LOQ	<LOQ	6.4	51.7	71.6
	4	461	<LOQ	<LOQ	<LOQ	599.8	<LOQ
G4 (day 28)	**1**	341	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	2	442	<LOQ	<LOQ	<LOQ	433.0	194.5
	3	464	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	4	449	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table 4. Kidney Inhibition Swab (KIS) Test on kidney tissues. Results were reported as positive (POS) or negative (NEG). A positive or negative result was determined by a color change. LCMS kidney values from **Table 3** are included for comparison and reported in ng/g.

		Treatment Group 1			**Treatment Group 2**			**Treatment Group 3**		
Necropsy Group	**Pen Number**	**Sow ID**	**LCMS**	**KIS Result**	**Sow ID**	**LCMS**	**KIS Result**	**Sow ID**	**LCMS**	**KIS Result**
G1 (day 1)	**1**	N/A	N/A	N/A	468	<LOQ	NEG	447	19.4	POS
	2	342	<LOQ	NEG	463	<LOQ	NEG	441	38.8	POS
	3	473	31.7	NEG	462	<LOQ	NEG	444	44.7	POS
	4	339	16.4	POS	471	<LOQ	NEG	453	679.9	POS
G2 (day 6)	**1**	470	<LOQ	NEG	456	<LOQ	NEG	450	64.9	POS
	2	446	96.4	NEG	466	<LOQ	NEG	467	16.3	NEG
	3	345	<LOQ	NEG	452	<LOQ	NEG	455	<LOQ	NEG
	4	474	<LOQ	NEG	350	<LOQ	NEG	346	<LOQ	POS
G3 (day 14)	**1**	472	<LOQ	NEG	458	<LOQ	NEG	443	<LOQ	NEG
	2	349	<LOQ	NEG	347	<LOQ	NEG	457	<LOQ	NEG
	3	344	<LOQ	NEG	451	<LOQ	NEG	454	<LOQ	NEG
	4	440	<LOQ	NEG	445	<LOQ	NEG	461	<LOQ	NEG

G4 (day 28)	**1**	340	<LOQ	NEG	459	<LOQ	NEG	341	<LOQ	NEG
	2	348	48.4	NEG	460	<LOQ	NEG	442	<LOQ	NEG
	3	448	<LOQ	NEG	343	<LOQ	NEG	464	<LOQ	NEG
	4	465	<LOQ	NEG	469	<LOQ	NEG	449	<LOQ	NEG

Table 5. Urine procaine penicillin G concentrations (ng/mL) as determined by LCMS, Charm MRL™, and SNAP™ beta-lactam test kit. Concentrations that were below the level of quantification (LOQ) (50 ng/mL) with LCMS was designated "LOQ". Charm test values were assigned "positive" if greater than +400 and "negative" if lower than -400. Snap test values were assign "positive" with a reading of 1.06 or higher and "negative" with a reading of 1.05 or lower.

	Treatment Group 1				
Necropsy Group	**Pen Number**	**Sow ID**	**LCMS**	**Charm**	**SNAP**
G1 (day 1)	1	N/A	N/A	N/A	N/A
	2	342	12488.0	POS	POS
	3	473	31502.2	POS	POS
	4	339	37580.8	POS	POS
G2 (day 6)	1	470	11270.8	POS	POS
	2	446	869.2	POS	POS
	3	345	2335.2	POS	POS
	4	474	N/A	POS	POS
G3 (day 14)	1	472	<LOQ	NEG	NEG
	2	349	<LOQ	NEG	POS
	3	344	<LOQ	NEG	NEG
	4	440	<LOQ	NEG	POS
G4 (day 28)	1	340	<LOQ	NEG	POS
	2	348	N/A	NEG	NEG
	3	448	<LOQ	NEG	NEG
	4	465	<LOQ	NEG	NEG
	Treatment Group 2				
G1 (day 1)	1	468	<LOQ	NEG	NEG
	2	463	<LOQ	POS	POS
	3	462	<LOQ	NEG	POS
	4	471	<LOQ	NEG	NEG
G2 (day 6)	1	456	<LOQ	NEG	NEG
	2	466	<LOQ	NEG	NEG
	3	452	<LOQ	NEG	POS
	4	350	<LOQ	NEG	NEG
G3 (day 14)	1	458	<LOQ	POS	POS
	2	347	<LOQ	NEG	NEG
	3	451	<LOQ	NEG	POS
	4	445	<LOQ	NEG	NEG
G4 (day 28)	1	459	<LOQ	NEG	NEG
	2	460	<LOQ	NEG	NEG
	3	343	<LOQ	NEG	NEG
	4	469	<LOQ	NEG	NEG
	Treatment Group 3				
G1 (day 1)	1	447	1156912.8	POS	POS
	2	441	654840.0	POS	POS
	3	444	313796.6	POS	POS
	4	453	417324.0	POS	POS
G2 (day 6)	1	450	48974.6	POS	POS
	2	467	26788.7	POS	POS

	3	455	16663.2	POS	POS
	4	346	56246.8	POS	POS
G3 (day 14)	1	443	<LOQ	POS	POS
	2	457	<LOQ	NEG	POS
	3	454	5419.4	POS	POS
	4	461	1145.7	POS	POS
G4 (day 28)	1	341	<LOQ	NEG	NEG
	2	442	<LOQ	NEG	NEG
	3	464	N/A	NEG	POS
	4	449	<LOQ	NEG	NEG

Table 6. Environmental Sample Procaine Penicillin G residues as determined by Charm MRL™, and SNAP™ beta-lactam test kit. Charm test values were assigned "positive" if greater than +400 and "negative" if lower than -400. Snap test values were assign "positive" with a reading of 1.06 or higher and "negative" with a reading of 1.05 or lower.

Charm MRL™								
			Days Post-Administration					
Necropsy Group	**Pen Number**	**Sow ID**	**0**	**1**	**3**	**6**	**14**	**28**
G1 (day 1)	**1**	468, 447	NEG	NEG				
	2	342, 463, 441	NEG	NEG				
	3	473, 462, 444	NEG	NEG				
	4	339, 471, 453	NEG	NEG				
G2 (day 6)	**1**	470, 456, 450	NEG		NEG	NEG		
	2	446, 466, 467	NEG		NEG	NEG		
	3	345, 452, 455	NEG		NEG	NEG		
	4	474, 350, 346	NEG		NEG	NEG		
G3 (day14)	**1**	472, 458, 443	NEG		NEG		NEG	
	2	349, 347, 457	NEG		NEG		NEG	
	3	344, 451, 454	NEG		NEG		NEG	
	4	440, 445, 461	NEG		NEG		NEG	
G4 (day 28)	**1**	340, 459, 341	NEG		NEG			NEG
	2	348, 460, 442	NEG		NEG			NEG
	3	448, 343, 464	NEG		NEG			NEG
	4	465, 469, 449	NEG		NEG			NEG
SNAP™								
			Days Post-Administration					
Necropsy Group	**Pen Number**	**Sow ID**	**0**	**1**	**3**	**6**	**14**	**28**
G1 (day 1)	**1**	468, 447	NEG	N/A				
	2	342, 463, 441	NEG	NEG				
	3	473, 462, 444	NEG	POS				
	4	339, 471, 453	NEG	NEG				
G2 (day 6)	**1**	470, 456, 450	NEG		POS	POS		
	2	446, 466, 467	NEG		POS	POS		
	3	345, 452, 455	NEG		POS	POS		
	4	474, 350, 346	NEG		POS	POS		
G3	**1**	472, 458, 443	NEG		POS		POS	

(day14)	**2**	349, 347, 457	NEG		POS		POS	
	3	344, 451, 454	POS		POS		POS	
	4	440, 445, 461	POS		POS		POS	
G4 (day 28)	**1**	340, 459, 341	NEG		POS			POS
	2	348, 460, 442	NEG		POS			POS
	3	448, 343, 464	NEG		POS			POS
	4	465, 469, 449	NEG		NEG			POS

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