Final Report Review

Project Title: **Development of a rapid, animal-side assay to screen biological fluid samples for the presence of violative drug residues to confirm residue clearance in tissues at slaughter**

Principal Investigator: Dr. Patrick Gordon, DVM, PhD

Co-investigators: Dr. Kristen Hayman, DVM
Dr. Troy Brick, DVM, MS
Dr. Rochelle Warner, DVM, MS
Dr. Larry Wulf, PhD

Primary Institution: Iowa State University

Award Amount: $40,845

Reviewer: Dr. James Russell

Date: November 15, 2020

Project Objective:

- The objective of this proposal is to develop a residue detection platform that can be used to perform rapid, animal-side screening of drug residues in biological fluids of cattle prior to harvest. To achieve this objective, the specific goals included:
    - Validation of laboratory procedures for drug detection on various biological fluids (urine, oral fluids, serum).
    - Conduct a live animal study in which calves are treated with CEF followed by the collection and analysis of biological fluids and tissue samples using rapid assays to detect the presence of violative residues.
    - Quantify drug residue concentrations in kidney tissues and biological fluids using LC/MS and correlate residue concentrations in the matrices.
    - Assessment and validation of the BetaStar® Advanced assay as a rapid, animal-side test to detect violative versus non-violative residues in bovine biological fluids.

Project Results:

- Ceftiofur (CEF) concentrated in urine after administration to calves resulting in positive BetaStar® tests across all time points through 5 days post-dosing.
    - Urine samples collected one day after the labelled withdrawal period for CEF administration tested positive to CEF residues with the BetaStar® even though kidney tissues had CEF residues below the legal tolerance levels (400 ppb).
- Bovine oral fluid samples had very low levels of CEF when quantified by LC/MS across all sampling points correlating to negative results on the BetaStar® test one day after CEF administration.

Final Report Summary

Nontechnical Summary:

Title: Evaluation of a rapid, animal-side assay (BetaStar®S) to screen bovine urine and oral fluid samples for the presence of violative ceftiofur residues prior to slaughter

Project Director: Patrick Gorden, DVM, PhD, D-ABVP-Dairy, D-ACVCP, 2416 Lloyd Veterinary Medical Center, College of Veterinary Medicine, Iowa State University (Email: pgorden@iastate.edu; Office #: 515-294-3096).

Project initiated: May 1, 2019

Project completed: May 1, 2020; November 5, 2020 (final report submission)

Total funds budgeted: $40,845

Total funds spent: $40,845

Nontechnical summary:

In total, fifteen (N=15) approximately 6-month-old Holstein heifers were randomly selected and enrolled. All heifers received an on-label daily dose of ceftiofur hydrochloride (Excenel RTU EZ, Zoetis Inc., Kalamzoo, MI) for five total doses. Immediately prior to the first ceftiofur (CEF) administration, baseline plasma, urine and oral fluids were collected. Upon completion of the CEF administration, daily plasma, urine, and oral fluid samples were collected from all study heifers for a total of five days – one day beyond the labelled meat withdrawal for the selected CEF product. Additionally, starting one day after the final CEF administration, three study animals were randomly selected for sacrificial necropsy and kidney tissue collection on a daily basis until the end of the study. CEF levels were quantified in all samples using liquid chromatography coupled with mass spectrometry (LC/MS) at the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA). Urine and oral fluid samples were screened for CEF residues with the commercially available BetaStar® S test kit (Neogen Corporation, Lansing, MI) and analyzed according the manufacturer suggested protocol utilizing the Raptor® Integrated Analysis Platform (Neogen Corporation, Lansing, MI). In bovine urine, CEF residues were found to concentrate after administration, resulting in positive BetaStar® S test kit results across all time points. Urine samples collected from animals one day after the labelled withdrawal period for the CEF product administered were found to be positive for CEF residues by the BetaStar® S test kit even though kidney tissues had quantifiable residues below the legally established tolerance (400 ppb). Bovine oral fluids samples were found to contain very low levels of CEF when quantified by LC/MS across all sampling time points, correlating to negative results on the BetaStar® S test kit one day after CEF administration - when CEF residues were at their highest in plasma. Results from this study demonstrate that use of the BetaStar® S test kit may have potential for residue screening in cattle treated with CEF when utilizing urine samples, but not oral fluids. Due to the observed excretion and concentration of CEF in bovine urine, finding a negative result on the BetaStar® S assay would indicate that tissue CEF residues would be below established tissue tolerance levels. However, the sensitivity of the assay will result in false positive test results when analyzing urine. Further work is currently being conducted by the investigators in collaboration with the BetaStar® S test kit manufacturers to correlate BetaStar® S test kit results from urine with CEF residues found in corresponding kidney tissues – the US regulatory target screening tissue.

Impacts

- As the majority of violative residues reported in beef in the United States are the β-lactam antimicrobial ceftiofur (CEF) found in culled dairy cows, successful correlation urine CEF residues detected with the BetaStar® S test kit to levels within kidney tissue (the regulatory screening test tissue) has the potential to provide producers with an on-farm, ante-mortem tool to prevent shipment of cattle containing violative CEF residues.

Conclusions

- Use of the BetaStar® S test kit to analyze urine may have the potential for rapid animal-side residue screening in cattle treated with CEF.
    - But the BetaStar® S test is inaccurate in oral fluids.
- The observed excretion and concentration of CEF in bovine urine imply that a negative BetaStar® S assay demonstrates that kidney tissue CEF residues would be below established tissue tolerance levels.
    - But the current assay will also yield in false positive results when analyzing urine.
- Further work needs to be conducted by the investigators in collaboration with the BetaStar® S test kit manufacturers to better correlate BetaStar® S test kit measurements in urine with the quantity of CEF residues found in corresponding kidney tissues, the U.S. regulatory target screening tissue.

Technology transfer

Refereed journal publications:

Hayman, K.P, T.A. Brick, R.M. Warner, L.W. Wulf, and P.J. Gorden. 2021. Evaluation of a rapid, animal-side assay (BetaStar®S) to screen bovine urine and oral fluid samples for the presence of violative ceftiofur residues prior to slaughter. *Journal of Food Safety*. (Planned Manuscript; Obtain reference June 2021).

Reviewed abstracts:

None reported

Extension/Outreach publications:

None reported

Scientific presentations:

None reported

Extension/Outreach presentations:

None reported

Podcasts:

None reported

Other:

None reported

Personnel support:

Faculty:

None

Post-doctoral associates:

One (Kristen P. Hayman; $10,465 for salary and benefits)

Farm labor:

None

Graduate students:

None

Undergraduate students:

None

Other:

None

Leveraged funds:

None reported, but BetaStar®S test kits were provided by manufacturer