Results of anti-mortem screening methodology to predict prescribed drug withholding periods for flunixin and ceftiofur in heifers

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**Introduction**

A simple, cow-side test for the presence of drug residues in live animals would be useful for drug residue avoidance programs. Simple inhibition tests used at slaughter do not detect some drug tolerance concentrations such as those for flunixin and ceftiofur metabolites. This experiment evaluated an adaptation of a beta-lactam and flunixin lateral flow (LF-modification) test for use with urine and serum samples from treated heifers and determined the ability of the test to predict the labeled slaughter withhold of ceftiofur and flunixin.

**Materials and Methods**

Heifers were treated with Naxcel (1 mg/lb, IM) or Banamine (50 mg/100 lb, IV). Initially three heifers were dosed. Urine was collected daily for five days. Blood and saliva were collected immediately before and at one and four days after treatment. For the second dosing, urine and blood samples were collected daily for six days from 12 treated heifers. All samples were tested by liquid chromatography with LF-modification, and by kidney inhibition swab (KIS) test.

**Results**

The LF-modification limit of detection (LOD) for flunixin was 0.03 ppm and the LOD for ceftiofur was 0.6 ppm. After the first dosing of ceftiofur, all urine and serum samples yielded negative results via KIS and LF-modification. Results for saliva samples were inconsistent, therefore, testing was discontinued. After the second dosing of ceftiofur, seven of 12 urine samples collected one day after treatment yielded positive results via KIS; whereas, 10 of 12 urine samples had ceftiofur concentrations $\geq 0.6$ ppm (the LOD) via LF-modification, with high performance liquid chromatography (HPLC) results for the 12 samples ranging from 0.2 to 3.6 ppm ceftiofur. For urine samples collected two days after the second dosing, one of 12 samples yielded positive results via KIS, three of 12 samples had ceftiofur concentrations $\geq 0.6$ ppm via LF-modification, with HPLC results for the 12 samples ranging from 0.04 to 0.55 ppm ceftiofur. For urine samples collected three days after the second dosing, only one of 12 samples yielded positive results via KIS and HPLC results for the samples ranged from 0.2 to 0.4 ppm. Results for serum samples obtained after the second dosing of ceftiofur were as follows: two of 12, 0 of 11, and 0 of 11 samples were positive via KIS; six of 12, one of 11, and 0 of 11 samples were positive via LF-modification; and the HPLC results for the samples ranged from 0.2 to 1.2 ppm, 0.1 to 0.3 ppm, and 0.01 to 1.01 ppm at one, two, and three days after treatment, respectively. For HPLC, ceftiofur was converted to its metabolite, desfuroyl ceftiofur acetate (DCA), for quantitation.

After the first dosing of flunixin, all serum and urine samples collected yielded negative results via KIS. However, all the urine samples collected one day after the first dosing had LF-modification results $> 0.15$ ppm (1:5 dilution). The HPLC performed on urine samples revealed an absence of the flunixin parent compound but the presence of the $\beta$-glucuronide-flunixin metabolite. Serum samples collected immediately before and four days after the first dosing yielded negative results via LF-modification. After the second dosing of flunixin, all serum and urine samples collected yielded negative results via KIS. All urine samples collected one day after the second dosing had LF-modification results $> 0.75$ ppm (1:25 dilution); the HPLC results ranged from 0.29 to 1.94 ppm flunixin, with three samples $< 0.75$ ppm flunixin. For urine samples collected two days after the second dosing, nine of 12 undiluted samples had LF-modification results $> 0.03$ ppm and four of 12 samples at the 1:10 dilution were $> 0.3$ ppm; the HPLC results ranged from 0.03 to 0.24 ppm flunixin. For urine samples collected at three and four days after the second dosing, six of 12 undiluted samples had a flunixin concentration $> 0.03$ ppm via LF-modification. The HPLC results for urine samples collected three days after the second dosing ranged from 0.02 to 0.26 ppm flunixin, with two samples $> 0.15$ ppm. The HPLC results for urine samples collected four days after the second dosing ranged from 0.02 to 0.07 ppm flunixin, with only one sample $> 0.05$ ppm. For serum samples collected one day after the second dosing, all undiluted
samples had LF-modification results > 0.03 ppm, and the flunixin concentration in seven samples was > 0.15 ppm at the 1:5 dilution; the HPLC ranged from -0.02 to 0.83 ppm flunixin, with only three samples > 0.1 ppm. For serum samples collected two days after the second dosing, two samples yielded positive results via LF-modification and had flunixin concentrations of 0.1 and 0.6 ppm as determined via HPLC. All serum samples collected > 2 days after the second dosing had negative results via LF-modification and HPLC. For untreated control samples, the LF-modification method had an approximately 4% false positive rate.

**Significance**

The slaughter withdrawal time for both ceftiofur and flunixin is four days. In ceftiofur-treated heifers, the results for urine and serum tests were similar and neither showed detectable DCA residues > 2 days after ceftiofur administration. For flunixin, urine was more predictive than serum because the LF-modification method was detecting the excreted glucuronide metabolite. Understanding incurred-drug-residue relationships between tissue, urine, and serum samples is important for interpretation of the results of residue screening tests. Additional work is ongoing to evaluate residues in antemortem tissue biopsy samples taken from the treated animals.