# The Effect of Metaphylactic Antimicrobial Use on the Development of Antimicrobial Resistance in Fecal *E. coli* Isolates of Feedlot Cattle

Sylvia Checkley, *DVM*<sup>1</sup>; Manuel Chirino-Trejo, *DVM*, *MSc*, *PhD*<sup>2</sup>; Eugene Janzen, *DVM*, *MVS*<sup>1</sup>; Cheryl Waldner, *DVM*, *PhD*<sup>1</sup>; John Campbell, *DVM DVSc*<sup>1</sup>

<sup>1</sup>Department of Large Animal Clinical Sciences

<sup>2</sup>Department of Veterinary Microbiology

Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5B4

#### Introduction

Much of the long-term economic and environmental stability of beef production seems to be driven by marketplace issues such as food safety and quality assurance that would include the potential presence of antimicrobial resistant bacteria. Long-acting injectable antimicrobials are widely used for metaphylaxis in calves on arrival at the feedlot. Previous feedlot trials by other researchers have associated metaphylaxis with lowered rates of morbidity and mortality from respiratory disease. Average daily gain, feed efficiency, case fatality rates, treatment rates, and relapse rates for bovine respiratory disease have also improved in animals where metaphylaxis is used. Metaphylaxis, therefore, has great industry significance from both an economic and animal welfare perspective. The objectives of this study were: 1) to determine the prevalence of fecal E. coli resistant to antimicrobials in auction-mart derived weaned calves, 2) to determine the effect of metaphylactic antimicrobial usage on the development of fecal E. coli resistant to various antimicrobials, and 3) to determine the persistence of antimicrobial resistant fecal E. coli and to evaluate the potential risk of their presence preslaughter.

#### **Materials and Methods**

A randomized controlled clinical trial was performed at the research feedlot at the University of Saskatchewan. This feedlot has open-air pens with dirt floors, 20% porosity fencing, and a central alley representative of larger feedlots in Western Canada. Two hundred and eighty eight auction-mart-derived Charolais-cross steers with an average weight on arrival of 636 lb (289 kg) (range 563-777 lb; 256-353 kg) were procured and underwent routine processing on arrival, including vaccination, topical parasiticidal treatment,

hormonal implants and eartags. Treatment groups were also assigned at this time. Feedlot staff was blinded as to the allocation of the treatment groups. Twelve steers were randomly blocked by weight into each of the 24 pens. Adjoining pens (sharing waterers) were also randomly assigned to one of three treatments. The treatment groups were: 1) control: no antimicrobials given on arrival, 2) medicated feed: 2g/head/day of oxytetracycline in the starter ration beginning on Day 0 for 14 days, and 3) injectable antimicrobial treatment: 9.1 mg/ lb (20 mg/kg) body weight of long-acting oxytetracycline administered subcutaneously on Day 0 of the trial period. All sick animals were treated according to predefined protocols. Fecal samples were collected prior to treatment allocation on arrival, and also on Days 7, 15, 35, 70, 100, 150, and preslaughter (208 day average).

### Laboratory Analysis

Fresh feces were cultured overnight on MacConkey's agar; three individual strains of *E. coli* were randomly chosen for subculture in litmus milk. No further passage occurred *in vitro*. Subcultures were stored at -70°C. To generate resistance profiles, subcultures were thawed when all had been collected, and cultured immediately on blood agar. Minimal inhibitory concentrations of seven antimicrobials were determined using the Mueller-Hinton agar dilution method. The antimicrobials tested were ampicillin, tetracycline, florfenicol, gentamicin, enrofloxacin, sulfamethoxazole, and trimethoprim/sulfadoxine. NCCLS standards were followed for all laboratory analyses.

#### Results

Data has been collected and is currently being analyzed using statistical modelling techniques to describe the effect of antimicrobial usage on the development of antimicrobial resistance and to identify levels of clustering within the data. Preliminary results show that the use of long-acting oxytetracycline for metaphylaxis on arrival does not result in significant increases in the proportion of antimicrobial resistant fecal *E. coli* isolates. There is no treatment effect of antimicrobial use at the onset of the feeding period leading to antimicrobial resistance evident in the *E. coli* isolates in preslaughter fecal samples.

#### Conclusions

Preliminary conclusions are that metaphylaxis does not significantly contribute to antimicrobial resistance of commensal E. *coli* isolates from cattle feces at the time of slaughter. The use of antimicrobials late in the feeding period may be of some importance in the development of antimicrobial resistance and its persistence at slaughter.

## Diagnostic Testing for Johne's Disease in the Cow-Calf Herd

Sylvia Checkley, *DVM*<sup>1</sup>; Cheryl Waldner, *DVM PhD*<sup>1</sup>; John Campbell, *DVM DVSc*1; Greg Appleyard, *BSc MSc PhD*<sup>2</sup>; Edward Clark, *DVM MSc PhD*<sup>3</sup>; Eugene Janzen, *DVM MVS*<sup>1</sup>

<sup>1</sup>Department of Large Animal Clinical Sciences

<sup>2</sup>Department of Veterinary Microbiology

<sup>3</sup>Prairie Diagnostic Services

Western College of Veterinary Medicine, University of Saskatchewan, Saskatchewan, S7N 5B4

#### Abstract

Johne's disease is caused by Mycobacterium avium subspecies paratuberculosis (MAP) infection. Public health concerns about the possible link between Johne's disease and Crohn's Disease (and the resulting media attention), along with changes in international trade regulations have elevated the importance of control and management of Johne's disease to a new level. However, very little research on Johne's disease has been done with beef cattle. The objectives of this study were to describe several cow-calf herds with a definitive diagnosis of Johne's disease (histopathology) in at least one animal, and describe the prevalence of MAP within the herd at the time of diagnosis. Furthermore, the longitudinal nature of this study allows comparison of how the prevalence within the herd begins to change when certain control measures are put into effect. Comparisons of agreement will be possible for five different live animal diagnostic tests. Finally, this study also provided the unique opportunity to create a blind comparison of necropsy findings in a batch of cull beef cows in a highprevalence Johne's herd for determination of the best tissues to examine on necropsy. A herd with a ten-year history of mature cow chronic diarrhea had two cows with MAP infection confirmed on necropsy. This was the inciting incident for the Special Field Investigation

Unit at WCVM to become involved in a longitudinal study involving various diagnostic testing issues important in management of this disease. Two other herds were identified through similar means and have been added to the study. Samples were collected during routine fall processing, using Vacutainers for tail vein blood collection. Fecal samples were collected with individual sleeves for each animal. A visual assessment of the animal's general health was also recorded. Prevalence of disease within the herds, age distribution of cases, and changes in the prevalence over time with or without control strategies will be reported. Measures of agreement will be examined for five diagnostic tests including the ELISA, AGID, fecal culture, direct fecal PCR, and PCR on MAP culture slants incubated for six weeks. Procedures for all tests are standardized and performed by blinded experienced laboratory personnel.

Investigators collected fecal and tissue samples from 40 animals culled from a high prevalence Johne's herd. All of these samples were collected at the slaughterhouse and processed in the laboratory by a standardized technique. Culture of feces and histology of ileum, cecum, colon and mesenteric lymph nodes was performed by an experienced blinded pathologist. The prevalence of MAP for all tissues and agreement between tissue findings and culture of feces will be reported.