Impacts of Dairy Calf Housing on Antimicrobial Resistance

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Antimicrobial use and resistant bacteria in animal agricultural settings likely contribute to resistance in food-borne pathogens.

Minimizing transmission of resistant bacteria in the animal reservoir should help minimize transmission of resistant pathogens to humans either through the food chain or by direct contact. To improve our understanding of the forces driving antimicrobial resistance transmission, amplification, and persistence in bacteria in dairy settings, we focused on preweaned calves as a model system. Resistant *Escherichia coli* can cause disease in humans and animals and are also potential reservoirs of antimicrobial resistance genes.

Objective

Evaluate the impact of management practices on antimicrobial resistance dynamics in *E. coli* in dairy calves and develop strategies to minimize it. Here we present results from our first objective from studies conducted at WSU and Cornell University.

Hypotheses

Over-arching hypothesis: Pre-weaned dairy calves' housing configurations have an impact on emergence and transmission of multi-drug resistant *E. coli* on dairies.

WSU working hypothesis: Antimicrobial use and the proportion of antimicrobial resistant *E. coli* is higher for the youngest calves than for the oldest calves in conventional hutch rows, regardless of absolute age.

Cornell working hypothesis: Lower prevalence of antimicrobial resistance and higher multidrug resistance diversity in *E. coli* occurs in dairy calves housed in group pens compared to calves housed in individually pens.

WSU- Materials & Methods

E. coli were isolated from fecal samples collected from pre-weaned calves from four conventional large (>2000 cows) Washington dairies. Calves were housed in individual pens of various construction, in rows of variable length, and loaded sequentially. Calves were either fed pasteurized waste milk or non-medicated milk replacer. The youngest and oldest calves (4 – 5 calves in each group) in a row were sampled from each farm five times each at two week intervals.

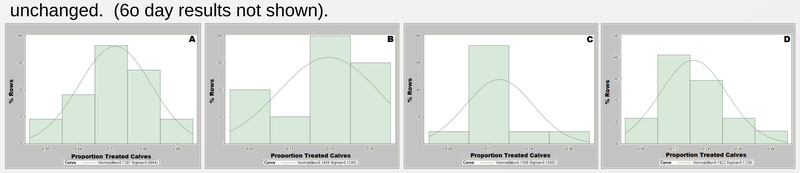
Fecal samples were serially diluted and spread plated on MacConkey agar for enumeration of total *E. coli*, on MacConkey media amended with ceftiofur sodium (2 µg/ml), tetracycline base (4 µg/ml), and trimethoprim-sulfamethoxazole (2/32 µg/ml) in order to estimate proportion of commensal *E. coli* with reduced susceptibility to these common antibiotics.

Treatment records and calf hutch and row locations of all pre-weaned sampled and non-sampled calves were obtained for each farm beginning two weeks prior to the onset of the bacteriologic sampling period through the final sampling date for each farm.

USDA National Institute of Food and Agriculture

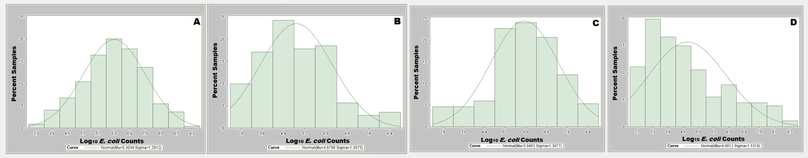
WSU Results

Treatment Histograms: The proportion of treated calves varies across farms within a row up to 30 days of age. Variation in proportion treated exists for up to 60 days of age as well; however, proportion treated up to 60 days on farms B and C increase while A and D remain relatively unchanged. (6o day results not shown).



Wilcoxon Rank Sums of Variation in treatment within row, up to 30 days of age (and 60 days of age- result not shown) is statistically significant (Kruskal-Wallis Test: Chi-Sq = 26.64, DF 3, Pr > Chi-Sq <.0001).

Resistance Histograms: Log_{10} Mean *E. coli* counts with reduced susceptibility to ceftiofur from calves on farms A-D



Results of multivariate logistic regression analyses:

I. Odds of **any antibiotic treatment** by **row group** (youngest v. oldest in row), controlling for farm (A-D) and row (1-11): Separate analyses were done because age was an effect modifier. The analysis was restricted to the first occurrence of each calf in the data. For calves younger 1-21 days of age, there was no significant association between being treated and being the youngest in a row (**odds ratio = 1.0, 95% Wald Confidence Limits = 0.5 – 1.6**). For calves 22 days and older, there was a significant negative association (**odds ratio = 0.2, 95% Wald CL = 0.1 – 0.7**).

II. Odds of **any isolation of *E. coli* with reduced susceptibility to ceftiofur** by **row group**, controlling for farm (A-D), sample visit (1-5), row (1-11), and distance in feet to the most recently ceftiofur-treated calf (0 ft or is a treated animal, 1-28 ft, 29-99 ft, 100-299 ft, 300-999 ft and ≥1000 ft or none treated in row). There was no significant association between being the youngest calf in a row (**odds ratio = 0.8, 95% Wald CL = 0.5 - 1.4**).

Conclusions:

The distributions of both treatment intensity and *E. coli* with reduced susceptibility to ceftiofur varied markedly between farms. Although there was no support for the hypothesis that the youngest group in a row was more likely to be treated, there was a significant negative association between being youngest in a row and treatment with calves older than 21 days. There was no association between row group and prevalence of *E. coli* with reduced susceptibility to ceftiofur.

The variation between four farms with regard to resistance and treatment protocols suggests that summary analyses should be viewed cautiously. An analysis comparing row length and age ranges within rows with regard to treatment frequency is underway.

Cornell- Materials & Methods

Escherichia coli were isolated from fecal swabs collected from pre-weaned calves five to ten weeks of age. Calves were either housed in individual pens (IP) and fed pasteurized milk twice a day, or housed in group pens (GP) and fed acidified milk free-choice. No antibiotic was added to the milk of either IP or GP calves. All samples were collected from one farm.

Fecal swabs were cultured on MacConkey agar at 37ºC and three typical *Escherichia coli* colonies were picked and streaked on Trypticase Soy Agar (TSA) plates with 5% Sheep Blood for isolation. Confirmed *E. coli* isolates were tested for susceptibility to 12 antimicrobials using a Kirby-Bauer disk diffusion assay in accordance with guidelines published by the CLSI (Clinical Laboratory Standards Institute).

Cornell Results

Table 1. Descriptive data for Individual Pen (IP) and Group Pen (GP) calves.

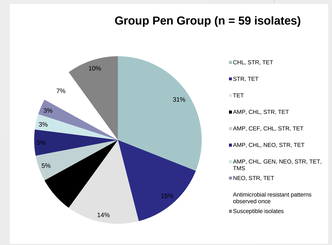
Description	IP* (n=71)	GP** (n=59)
Average age of calf at sampling (in days)	40.4	59.44
Received antibiotic treatment prior to sampling	24%	13.5%
Distribution of antibiotic treatments:		
Tulathromycin (Draxxin)	14%	0%
Enrofloxacin (Baytril)	10%	0%
Oxytetracycline (Bio-Mycin)	0%	13.5%

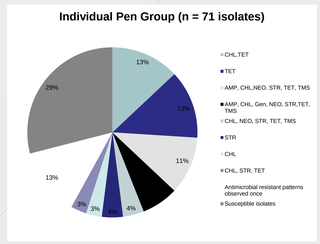
Table 2. Outcome of logistic regression evaluating the effect of antimicrobial resistance of Escherichia coli isolates from the IP and GP groups.

Antibiotic	Status	*IP	**GP	Odds (**GP vs *IP)	P-value
Streptomycin	Susceptible	56.34	23.73		
	Intermediate	2.82	1.69		
	Resistant	40.85	74.58	4.211	**0.0002**
Tetracycline	Susceptible	26.76	10.17		
	Intermediate	11.27	1.69		
	Resistant	61.97	88.14	4.329	**0.0017**
Trim/Sulfa	Susceptible	73.24	91.53		
	Intermediate	1.41	0		
	Resistant	25.35	8.47	0.256	**0.0113**

Note: Only odds with P-value <.05 are shown. Ceftiofur, ceftriaxone, ciprofloxacin and nalidixic acid were not included in the analysis because resistant isolates where not present in both IP and GP. *IP: calves housed in individual pens; **GP: calves housed in group pens.

Antimicrobial resistance profiles for *E. coli* isolates recovered from fecal samples of calves





* Abbreviations: TET, tetracycline; AMP, ampicillin; TMS, trimethoprim-sulfamethoxazole; GEN, gentamycin; CEF, cefoxitin; CHL, chloramphenicol; NEO, neomycin; STR, streptomycin; XNL, ceftiofur.

Conclusions:

: Counter to the hypothesis, a greater number of *E. coli* isolates from GP were resistant to the antimicrobials tested, and resistant isolates were distributed in fewer antimicrobial resistant patterns compared to resistant isolates in IP. This preliminary data suggest that the direct contact between calves in GP can be a factor responsible for dissemination of antimicrobial resistance even in a calf housing system where less calves are treated with parenteral antibiotics.

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