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Correlation between Quinolone-Resistant Commensal *E. coli* in Dairy Calves and Enrofloxacin Use

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INTRODUCTION

The use of enrofloxacin in food-producing animals can result in rapid development of resistant bacterial populations, including bacterial pathogens[6]. Prior to 2008, the use of fluoroquinolones in cattle was limited to non-dairy animals, however in 2008 the use of enrofloxacin for respiratory disease was approved for dairy heifers younger than 20 months of age. Enrofloxacin is not approved in dairy heifers older than 20 months of age at this time. We hypothesized that retrospective testing could detect an increase in the prevalence of resistance to quinolone antibiotics (nalidixic acid (Nal) and ciprofloxacin (Cip)) among dairy calf *E. coli* populations after the approval of enrofloxacin use in dairy heifer calves.

METHODS

E. coli isolates for this study were drawn from previous studies in dairy cattle along with isolates from a 2012 project targeting enrofloxacin use. For all studies prior to 2012, fecal samples were collected from individual animals and plated onto various selective and non-selective plates. To estimate prevalence of ciprofloxacin resistance (Cip-R) from archived isolates, we focused on isolates grown on non-selective media. Isolates that were nalidixic acid-resistant (Nal-R) by a standard disk diffusion method[5] were further tested for Cip-R using the same method.
The 2012 isolates were obtained from 30 dairy farms in Washington State. Calf fecal samples were plated onto MacConkey agar supplemented with Cip (2ug/ml) to determine the prevalence of Cip-R *E. coli*. Questionnaire data were also collected to determine whether the dairies were currently using enrofloxacin. Isolates were stored at -80^{O}C in 10% glycerol.

RESULTS

On one large dairy farm sampled in 2006-2008 and in 2011, the prevalence of Nal-R *E. coli* from calves increased from 1.3 % in 2006 to 47.9 % in 2011 ($P < 0.01$) (Table 1). On the same farm, prevalence of Nal-R *E. coli* from adult dairy cows increased from 0 % in 2008 to 16.9 % in 2011 (Table 2). Among those Nal-R *E. coli* in 2011 the prevalence of Cip-R was 53.6%. Among these farms, five farms had data from previous years which is shown in Table 3.
In 2012, the prevalence of *E. coli* growth from samples plated directly to cip-supplemented media ranged from 0 to 86.0% among 30 farms (Table 5).
The median of this prevalence was 62.0% among farms that reported enrofloxacin use compared to 34.0% among those that did not (Wilcoxin one-way P=0.06) (Table 4). Among the dairies that used enrofloxacin, the most common application for the drug included diarrhea and pneumonia in dairy heifers under 20 months of age, while none of the farms reported use in the heifers older than 20 months of age.

Table 1. Percent of all *Escherichia coli* isolates[a] from calves on Farm 60 that were resistant to nalidixic acid in 2006-2008, and 2011.

Year	Percent resistant[bc] Nal	Number of isolates tested	95% CI
2006	1.3	610	(0.6-2.7)
2007	2.2	1,133	(1.5-3.3)
2008	17.4	2,462	(17.5-19)
2011	47.9	507	(43.5-52.4)

[a] Isolates obtained by plating fecal samples onto non-selective media (VRB-MUG or MacConkey).
[b] Zone of inhibition < 13 mm diameter.
[c] Bartholomew's test for trend: chi-sq = 679.13, 2-tailed P < 0.01

Table 2. Adult Cows on Farm 60

Year	% Resistant Nal	Total tested	95% CI
2008	0	1,057	(0-.05)
2011	16.9	142	(11.3-24.3)

Among all Nal-R isolates, the prevalence of Cip-R = 90/168 (53.6%)

Table 3. Percent of all *Escherichia coli* isolates from calves on various farms other than Farm 60 that were resistant to nalidixic acid in 2008 and 2011.

Farm	2008 % resistant Nal	2008 Number tested	95% CI	2011 % resistant Nal	2011 Number tested	95% CI
3	0.2	2,833	(0.1-0.5)	0	32	(0-13.3)
35	0.2	3,152	(0.1-0.4)	NA	NA	(NA)
41	NA	NA	(NA)	35.5	31	(19.8-54.6)
46	NA	NA	(NA)	0	13	(0-28.3)
120	NA	NA	(NA)	0	12	(0-40.7)
Total	0.2	5,985	(0.1-0.4)	12.5	88	(6.7-21.7)

[a]Isolates obtained by plating fecal samples onto non-selective media (VRB-MUG or MacConkey).

Table 4. Prevalence of Cip-R by reported enrofloxacin use in dairy calves, 2012

	Farms with any enrofloxacin use N=18	Farms with no enrofloxacin use N=12
Mean	57%	36%
Median	63%	34%

Prevalence of Cip-R was not normally distributed so a nonparametric test (Wilcoxon Two-Sample Test- normal approximations) was used. P=0.03

Table 5. Prevalence of Cip-R among the 2012 isolates.

Cip-R Prevalence (%)	Frequency	Percent	Mean (%)	Median (%)
0	4	13.33	0	0
1-40	7	23.3	23	29
41-70	12	40	51	64
71-86	7	23.3	79	79
Total	30	100	32	62

Isolates were collected off of MacConkey with 2ug/ml of Cip

CONCLUSIONS

These data support the hypothesis that quinolone resistance among *E. coli* from dairy animals increased after the approval of enrofloxacin use in dairy heifers. In table 3, two of the five farms reported that they did not use enrofloxacin in their calves less than 20 months of age and had never used the product even after it was approved.
While our observations were confined to commensal *E. coli* populations, the same selective process may be taking place in pathogenic bacterial populations present on dairies, including *Salmonella enterica* and *Campylobacter* spp. We would like to continue our work to see what effect the use of enrofloxacin has on pathogenic bacterial populations.

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