

Table S1. Panel of 12 antibiotics using breakpoint agar dilution for *E. coli* obtained in 2002 – 2003 (Berge AC, Hancock DD, Sicho WM, Besser TE. Geographic, farm, and animal factors associated with multiple antimicrobial resistance in fecal *Escherichia coli* isolates from cattle in the western United States. *J Am Vet Med Assoc.* 2010 Jun 15;236(12):1338-44.)

Antimicrobial	Breakpoint concentration ( $\mu\text{g/ml}$ )
Amikacin	32
Amoxicillin/clavulanic acid	32/16
Ampicillin	16
Cephalothin	32
Ceftiofur	4
Chloramphenicol	16
Gentamicin	8
Nalidixic acid	32
Streptomycin	12
Sulfisoxazole	100
Tetracycline	8
Sulfamethoxazole/ trimethoprim	76/4

Table S2. Panel of antibiotics tested in the 2008 study by agar diffusion.

Antimicrobial	( $\mu\text{g}/\text{disk}$ )	Zone size breakpoint for resistance (mm)
Amikacin	30	d14
Amoxicillin/clavulanic acid	20/10	d13
Ampicillin	10	d13
Cephalothin	30	d14
Ceftiofur	30	d17
Chloramphenicol	30	d12
Gentamicin	10	d12
Nalidixic acid	30	d13
Streptomycin	10	d11
Sulfisoxazole	250	d12
Tetracycline	30	d14
Trimethoprim	1.25/23.75	d10
/Sulfamethoxazole		

Table S3. Summary of the number of samples collected from 30 dairy farms.

Type of sample	Total	Average			
		per farm	Median	Min	Max
<u>Fecal</u>					
Calf <sup>a</sup>	1,173	39.1	43	11	46
Fresh cows <sup>b</sup>	31	1.0	1	0	2
Hospital pens <sup>b</sup>	28	0.9	1	0	2
Other adult cows <sup>b</sup>	60	2.0	2	0	6
<u>Environmental</u>					
Lagoon <sup>c</sup>	31	1.0	1	1	2
Milk filter <sup>c</sup>	28	0.9	1	0	1
Total	1351	45.0	48	15	52

Table S4. Number of samples per type of selective media and number of *E. coli* isolates confirmed by *iudA* PCR tested for *bla*<sub>CMY-2</sub> and/or *bla*<sub>CTX-M</sub> for each type of media.

	Cefepime (4 µg/ml)	Cefoxitin (4 µg/ml)	Plain MacConkey
Number of samples plated	1,347	1,347	796
Number of isolates from samples	2,785	3,913	1,847
Number of isolates positive/tested for <i>bla</i> <sub>CTX-M</sub>	2,508/2,785	8/318	26/585
Number of isolates positive/tested for <i>bla</i> <sub>CMY-2</sub>	380/2,785	3,080/3,912	189/591

Table S5. Number (percent) of 3,687 *E. coli* isolates that were tested for both *bla*<sub>CMY-2</sub> and *bla*<sub>CTX-M</sub> by PCR.

Media	<i>bla</i> <sub>CTX-M</sub> <sup>+</sup> ,	<i>bla</i> <sub>CTX-M</sub> <sup>-</sup> ,	<i>bla</i> <sub>CMY-2</sub> <sup>+</sup> ,	<i>bla</i> <sub>CMY-2</sub> <sup>-</sup> ,	Total
	<i>bla</i> <sub>CMY-2</sub> <sup>+</sup>	<i>bla</i> <sub>CMY-2</sub> <sup>+</sup>	<i>bla</i> <sub>CMY-2</sub> <sup>-</sup>	<i>bla</i> <sub>CMY-2</sub> <sup>-</sup>	
Cefepime (4 µg/ml) <sup>a</sup>	339 (12.2)	41 (1.5)	2,169 (77.9)	236 (8.5)	2,785
Cefoxitin (4 µg/ml) <sup>a</sup>	2 (0.05)	235 (74.1)	6 (1.9)	74 (23.3)	317
Plain MacConkey	3 (0.5)	185 (31.6)	23 (3.9)	374 (63.9)	585

<sup>a</sup> Antibiotic supplement to MacConkey after overnight growth in cefotaxime-supplemented LB broth (2 µg/ml).

Figure S1. Dairy herd size by region of Washington State.

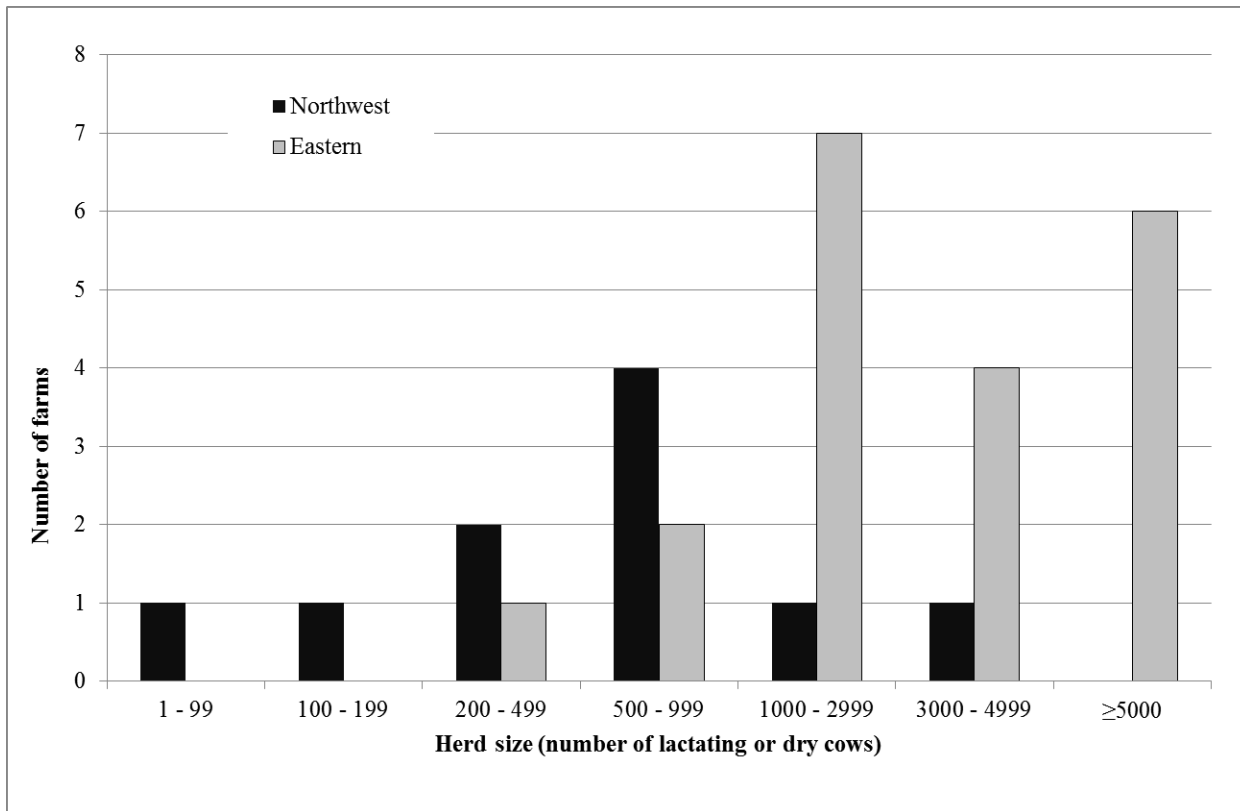
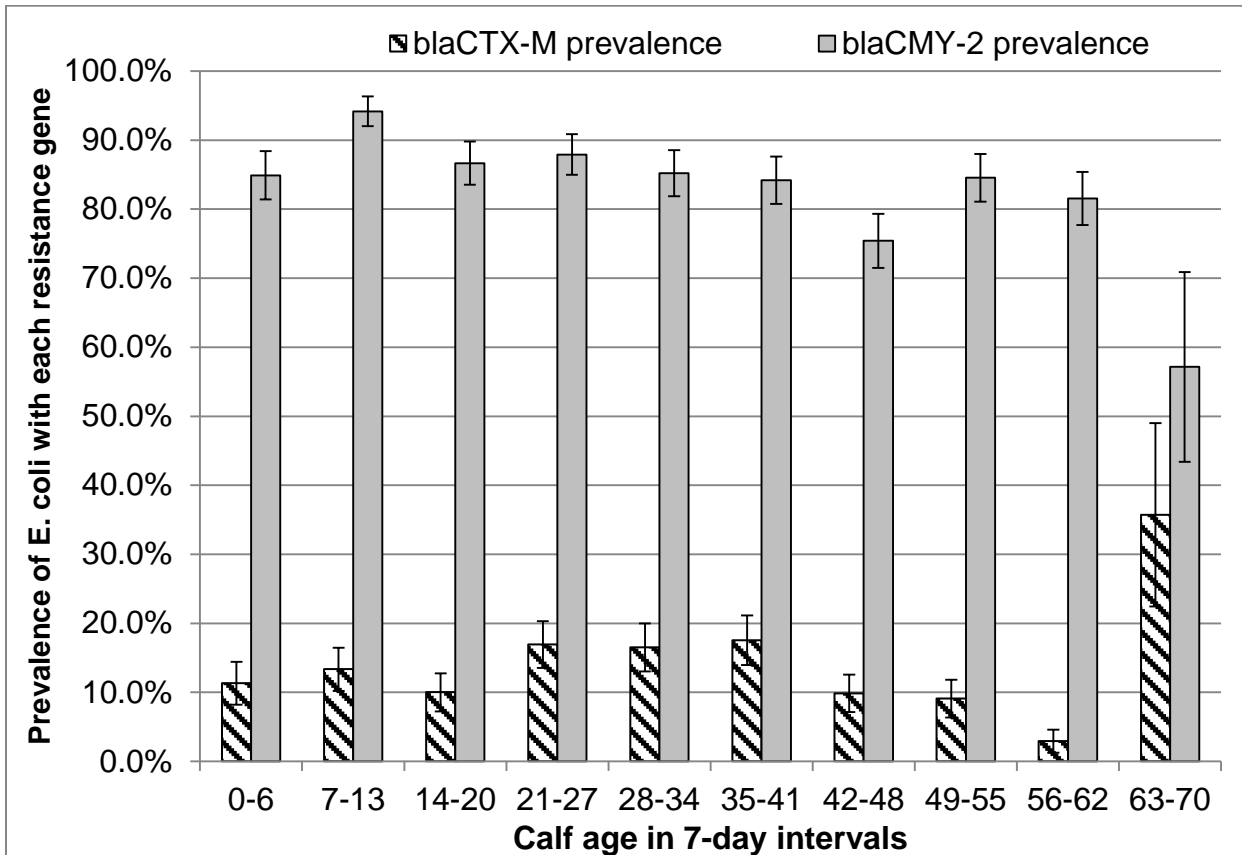


Figure S2. Prevalence of *E.coli* carrying *bla*<sub>CMY-2</sub> compared to prevalence of CTX-M EC in dairy calves by calf age. Wilcoxon signed rank test for the difference between calf age prevalence *P*-value = 0.002. Error bars represent +/- the standard error of the mean.



**APPENDIX:**

**2012 SURVEY OF  
WASHINGTON DAIRY FARM OPERATIONS:  
MOVEMENT OF ANTIBIOTIC RESISTANT  
BACTERIA BETWEEN ANIMALS AND FARMS**



This is a collaborative effort among practicing veterinarians, animal agricultural industries, the School for Global Animal Health and the Department of Veterinary Clinical Sciences Washington State University



## DAIRY BACKGROUND AND ANIMAL MOVEMENT

1. **Which one category best describes the current dairy herd size for this operation? Please include both milking and dry cows.**

*(Please check the ONE box that matches your answer)*

- <sub>1</sub> None
- <sub>2</sub> 1 - 99 cows
- <sub>3</sub> 100 - 199 cows
- <sub>4</sub> 200 - 499 cows
- <sub>5</sub> 500 - 999 cows
- <sub>6</sub> 1000 -3000 cows
- <sub>7</sub> 3000 -5000 cows
- <sub>8</sub> Over 5000 cows

2. **Which one category best describes the current maximum number of pre-weaned calves on your operation in the last 12 months?**

*(Please check the ONE box that matches your answer)*

- <sub>2</sub> 1 - 19 calves
- <sub>3</sub> 20 - 39 calves
- <sub>4</sub> 40 - 64 calves
- <sub>5</sub> 65 - 199 calves
- <sub>6</sub> 200 - 499 calves
- <sub>7</sub> 500 - 999 calves
- <sub>8</sub> More than 1000 calves

3. **Do you raise bull calves on premises?**


- <sub>1</sub> No
- <sub>2</sub> Yes

4. **Which one category best describes the maximum number of heifers (4 to 24 months of age) on your operation in the last 12 months?**

*(Please check the ONE box that matches your answer)*

- <sub>1</sub> None
- <sub>2</sub> 1 - 99 heifers
- <sub>3</sub> 100 - 199 heifers
- <sub>4</sub> 200 - 499 heifers
- <sub>5</sub> 500 - 999 heifers
- <sub>6</sub> 1000 - 3000 heifers
- <sub>7</sub> 3000 - 5000 heifers
- <sub>8</sub> Over 5000 heifers


5. Does this dairy operation have more than one physical location?

- <sub>1</sub> No
- <sub>2</sub> Yes 

6. How many physical locations does this operation have for accommodating this number of dairy cows or herd size?

\_\_\_\_\_ Number of physical locations


7. Have you sent any heifer calves off premises within the last 12 months?

- <sub>1</sub> No
- <sub>2</sub> Yes 


8. If yes, where do/did you send your heifer calves?

- <sub>1</sub> Commercial heifer raiser
- <sub>2</sub> Other dairy location
- <sub>3</sub> Other, explain \_\_\_\_\_


9. Have you purchased lactating cows in the past 6 months?

- <sub>1</sub> No
- <sub>2</sub> Yes  How many \_\_\_\_\_


10. Have you purchased replacement heifers (age 60 days to about 2 years) in the past 6 months?

- <sub>1</sub> No
- <sub>2</sub> Yes  How many \_\_\_\_\_

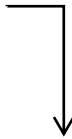
11. Have you purchased calves (age 0 to 60 days) in the past 6 months?

- <sub>1</sub> No
- <sub>2</sub> Yes  How many \_\_\_\_\_

12. Have you purchased bulls in the past 6 months?

- <sub>1</sub> No
- <sub>2</sub> Yes  How many \_\_\_\_\_

13. Do you send your heifer's off-farm to be bred?

- <sub>1</sub> Yes
  - <sub>2</sub> No
- 

14. If yes, do other dairies use the same heifer breeder/premises?

- <sub>1</sub> Yes
- <sub>2</sub> No

15. What percentages of feeds does this dairy grow that are fed to cows on this dairy?

(check the best one for each feed category)

- <sub>1</sub> Less than 25 %
- <sub>2</sub> 26 - 50%
- <sub>3</sub> 51 - 75%
- <sub>4</sub> Greater than 75%
- <sub>5</sub> None

	Corn	Hay	Other
Less than 25 %			
26 - 50%			
51 - 75%			
Greater than 75%			
None			

Please list 'Other' feed types: \_\_\_\_\_

## CALVING PEN PRACTICES

16. Where are close-ups cows kept prior to calving?

- <sub>1</sub> Dry lot
- <sub>2</sub> Bedded pack
- <sub>3</sub> Individual calving pen/stall
- <sub>4</sub> Free stalls
- <sub>5</sub> Tie stall
- <sub>6</sub> Pasture
- <sub>7</sub> Other, explain \_\_\_\_\_

**17. Where do the cows calve?**

- <sub>1</sub> Dry lot
- <sub>2</sub> Bedding pack
- <sub>3</sub> Individual calving pen/stall
- <sub>4</sub> Free stalls
- <sub>5</sub> Tie stall
- <sub>6</sub> Pasture
- <sub>7</sub> Calving cows are housed in close up pen
- <sub>8</sub> Other, explain\_\_\_\_\_

**18. Are sick cows ever housed with cows that are calving?**

- <sub>1</sub> Yes
- <sub>2</sub> No

**19. When cleaning the pen where cows calve, what procedures do you perform?**

(Check all that apply)

- <sub>1</sub> Scraped
- <sub>2</sub> Add lime
- <sub>3</sub> Add new straw
- <sub>4</sub> Add new shaving
- <sub>5</sub> Add new composted manure
- <sub>6</sub> Add new sand
- <sub>7</sub> Vacuum
- <sub>8</sub> Other, explain\_\_\_\_\_

**20. How frequently is new bedding added to the calving/maternity area?**

(Check all that apply)

- <sub>1</sub> Twice a week
- <sub>2</sub> Bi-weekly
- <sub>3</sub> Weekly
- <sub>4</sub> Monthly
- <sub>5</sub> Other, explain\_\_\_\_\_


21. How soon are calves removed from calving area or removed from cow?

- <sub>1</sub> As soon as they are born
- <sub>2</sub> Within 30 minutes
- <sub>3</sub> Within 2 hours
- <sub>4</sub> Within 6 hours
- <sub>5</sub> Within 12 hours
- <sub>6</sub> Within 24 hours
- <sub>7</sub> Greater than 24 hours
- <sub>8</sub> Other, explain \_\_\_\_\_

## FEEDING PRACTICES

22. Do you feed colostrum to your calves during the first 24 hours?

(Please check the ONE box that best matches your regular practices)

- <sub>1</sub> No
- <sub>2</sub> Yes 

23. If yes, how much colostrum do your calves receive during the first 24 hours?

(Please check the ONE box that matches your answer)

- <sub>1</sub> Less than 2 quarts
- <sub>2</sub> 2 quarts to 4 quarts
- <sub>3</sub> More than 4 quarts
- <sub>4</sub> Do not receive colostrum
- <sub>5</sub> Don't Know

24. Do you feed milk from buckets or bottles?

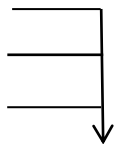
- <sub>1</sub> Buckets
- <sub>2</sub> Bottles

25. **When feeding your calves what direction do you most commonly feed the calves?**

- <sub>1</sub> Youngest to oldest
- <sub>2</sub> Oldest to youngest
- <sub>3</sub> Both directions
- <sub>4</sub> Other, explain \_\_\_\_\_

26. **What do you feed your pre-weaned calves after the first 24 hours?**

(Please check all that apply)

- <sub>1</sub> Non- medicated milk replacer
  - <sub>2</sub> Medicated milk replacer
  - <sub>3</sub> Fresh cow milk
  - <sub>4</sub> Hospital milk
  - <sub>5</sub> Bulk tank milk
- 

27. **Do you pasteurize the milk before feeding?**

- <sub>1</sub> No
- <sub>2</sub> Yes

28. **If you use medicated milk replacer or add ingredients to the milk or milk replacer, what products do you use most often?**

(Please check all that apply)

- <sub>1</sub> Decoquate (Deccox) - coccidiosis
- <sub>2</sub> Lasalocid (Bovatec) - coccidiosis
- <sub>6</sub> Other, explain \_\_\_\_\_

29. **What type of grain do you feed pre-weaned calves after the first 24 hours?**

(Please check all that apply)

- <sub>1</sub> Grain made on farm without antibiotic
- <sub>2</sub> Grain made on farm with antibiotic
- <sub>3</sub> Grain purchased without antibiotic
- <sub>4</sub> Grain purchased with antibiotic

30. **If antibiotics/antimicrobials (ionophores/coccidiosis) are in the grain, what is the name of the antibiotic/antimicrobials you use most often?**

- <sub>1</sub> Rumensin \_coccidiosis
- <sub>2</sub> Elanco -coccidiosis
- <sub>3</sub> Oxytetracycline
- <sub>4</sub> Other, explain \_\_\_\_\_

31. **How frequently do you clean the buckets/bottles that you feed milk in?**

- <sub>1</sub> Daily or more frequently
- <sub>2</sub> Every other day to weekly
- <sub>3</sub> Monthly
- <sub>4</sub> Between calves
- <sub>5</sub> Other, explain \_\_\_\_\_

32. **How are the buckets/bottles cleaned or disinfected?**

(Check all that apply)

- <sub>1</sub> Water
- <sub>2</sub> Soap and water
- <sub>3</sub> Disinfectant
- <sub>4</sub> Other, explain \_\_\_\_\_

33. **How frequently do you clean the buckets that you supply water in?**

- <sub>1</sub> Daily or more frequently
- <sub>2</sub> Every other day to weekly
- <sub>3</sub> Monthly
- <sub>4</sub> Between calves

34. **How do you clean or disinfect water buckets?**

(Check all that apply)

- <sub>1</sub> Water
- <sub>2</sub> Soap and water
- <sub>3</sub> Disinfectant
- <sub>4</sub> Other, explain \_\_\_\_\_

## CALF HOUSING PRACTICES

### 35. What type of calf hutch do you currently have on the dairy?

(Check all that apply)

- <sub>1</sub> Double individual wood style
- <sub>2</sub> Single individual wood style
- <sub>3</sub> Plastic hutch with run
- <sub>4</sub> Plastic enclosed
- <sub>4</sub> Plastic with tether
- <sub>4</sub> Triplet hutch wood style (California style hutch)
- <sub>4</sub> Other, explain \_\_\_\_\_

### 36. What type of calf hutch bedding do you currently have on the dairy?

(Check all that apply)

- <sub>1</sub> Shavings
- <sub>2</sub> Straw
- <sub>3</sub> Sand
- <sub>3</sub> None
- <sub>4</sub> Other, explain \_\_\_\_\_

### 37. How often is bedding added to the hutches?

- <sub>1</sub> Twice a week
- <sub>2</sub> Weekly
- <sub>3</sub> Monthly
- <sub>4</sub> Between calves
- <sub>5</sub> Other, explain \_\_\_\_\_

### 38. How often are the hutches cleaned?

- <sub>1</sub> Twice a week
- <sub>2</sub> Weekly
- <sub>3</sub> Monthly
- <sub>4</sub> Between calves
- <sub>5</sub> Other, explain \_\_\_\_\_



**39. When cleaning the hutches, what method do you use most often?**

(Check all that apply)

- <sub>1</sub> Scraping
- <sub>2</sub> Water
- <sub>3</sub> Soap and water
- <sub>4</sub> Disinfectant
- <sub>5</sub> Brush-scrubbing
- <sub>6</sub> Power washer
- <sub>7</sub> Other, explain \_\_\_\_\_

**40. After a hutch row is emptied, are those hutches moved to a new area?**

- <sub>1</sub> No
- <sub>2</sub> Yes


**41. What is the minimum length of time that empty hutches remain empty between calves?**

- <sub>1</sub> Less than one week
- <sub>2</sub> One week
- <sub>3</sub> Two weeks
- <sub>4</sub> Three weeks
- <sub>5</sub> Four weeks or greater
- <sub>6</sub> Other, explain \_\_\_\_\_

**42. When pre-weaned calves get sick, do you put them in a separated housing area?**

- <sub>1</sub> No
- <sub>2</sub> Yes

43. Do you use any type of fly control during the year?

- <sub>1</sub> No  
<sub>2</sub> Yes 

44. If yes, what type of fly control do you use?

- <sub>1</sub> Fly traps  
<sub>2</sub> Larvicides  
<sub>3</sub> Fly parasite release programs  
<sub>4</sub> Residual fly sprays  
<sub>5</sub> Insecticide-impregnated ear tags  
<sub>6</sub> Other, explain \_\_\_\_\_

## PERSONNEL TREATMENT PRACTICES

45. Do the same personnel care for the sick calves (feeding or treatments) as care for the healthy calves?

- <sub>1</sub> No  
<sub>2</sub> Yes

46. Do calf treaters change boots or foot covers between sick calf hutches?

- <sub>1</sub> No  
<sub>2</sub> Yes

47. When treating animals do the people treating the animals wear gloves?

- <sub>1</sub> No  
<sub>2</sub> Yes

48. How do the people treating sick calves keep their hands clean while treating?

- <sub>1</sub> Change gloves in-between each calf
- <sub>2</sub> Clean hands/gloved hands with sanitizer between each calf
- <sub>3</sub> Clean hands/gloved hands with sanitizer every \_\_\_\_ number of calves  
(ie: 5 calves then wash hands/gloves)
- <sub>4</sub> Do not wash/sanitize hands/gloved hands during treatment
- <sub>5</sub> Other, explain \_\_\_\_\_

49. When feeding calves electrolytes and or colostrum, do you use esophageal feeders?

- <sub>1</sub> No
- <sub>2</sub> Yes



50. If yes, when and how do you clean the esophageal feeders?  
(Check all that apply)

- <sub>1</sub> Clean with soap and water
- <sub>2</sub> Clean with disinfectant
- <sub>3</sub> Clean with water only
- <sub>4</sub> After each calf
- <sub>5</sub> After more than one calf
- <sub>6</sub> Daily
- <sub>7</sub> Weekly
- <sub>8</sub> Other, explain \_\_\_\_\_

51. When treating calves, are syringes re-used between calves?

- <sub>1</sub> No
- <sub>2</sub> Changed after a certain number of calves \_\_\_\_\_
- <sub>3</sub> Changed daily
- <sub>4</sub> Changed weekly
- <sub>5</sub> Other, explain \_\_\_\_\_

**52. When treating calves, are needles re-used between calves?**

- <sub>1</sub> No
- <sub>2</sub> Changed after a certain number of calves \_\_\_\_\_
- <sub>3</sub> Changed daily
- <sub>4</sub> Changed weekly
- <sub>5</sub> Other, explain \_\_\_\_\_

**DAIRY PERSONNEL PRACTICES**

**53. On average how many people are on the dairy each day?**

- <sub>1</sub> Less than 10
- <sub>2</sub> 11 - 25
- <sub>3</sub> 26 - 50
- <sub>4</sub> 51 - 75
- <sub>5</sub> Greater than 75


**54. On average, how many new employees do you hire on a monthly basis?**

- <sub>1</sub> None
- <sub>2</sub> 1 - 2
- <sub>3</sub> 3 - 5
- <sub>4</sub> 6 - 7
- <sub>5</sub> Greater than 8

55. For each group of people listed below please indicated how frequently they visit the farm:

	<b>Frequency</b>					
	Daily	2X weekly	Monthly	2X Monthly	Six Months	Yearly
	▼	▼	▼	▼	▼	▼
56. Veterinarian .....	<input type="checkbox"/> <sub>1</sub>	<input type="checkbox"/> <sub>2</sub>	<input type="checkbox"/> <sub>3</sub>	<input type="checkbox"/> <sub>4</sub>	<input type="checkbox"/> <sub>5</sub>	<input type="checkbox"/> <sub>6</sub>
57. Feed Delivery Trucks .....	<input type="checkbox"/> <sub>1</sub>	<input type="checkbox"/> <sub>2</sub>	<input type="checkbox"/> <sub>3</sub>	<input type="checkbox"/> <sub>4</sub>	<input type="checkbox"/> <sub>5</sub>	<input type="checkbox"/> <sub>6</sub>
58. Nutritionist .....	<input type="checkbox"/> <sub>1</sub>	<input type="checkbox"/> <sub>2</sub>	<input type="checkbox"/> <sub>3</sub>	<input type="checkbox"/> <sub>4</sub>	<input type="checkbox"/> <sub>5</sub>	<input type="checkbox"/> <sub>6</sub>
59. Hoof Trimmer .....	<input type="checkbox"/> <sub>1</sub>	<input type="checkbox"/> <sub>2</sub>	<input type="checkbox"/> <sub>3</sub>	<input type="checkbox"/> <sub>4</sub>	<input type="checkbox"/> <sub>5</sub>	<input type="checkbox"/> <sub>6</sub>
60. Breeders .....	<input type="checkbox"/> <sub>1</sub>	<input type="checkbox"/> <sub>2</sub>	<input type="checkbox"/> <sub>3</sub>	<input type="checkbox"/> <sub>4</sub>	<input type="checkbox"/> <sub>5</sub>	<input type="checkbox"/> <sub>6</sub>

61. Do any employees or farm family members work at least once every two weeks on other dairy farms?

<sub>1</sub> No  
<sub>2</sub> Yes 

62. If yes, how many employees work at/own locations with other dairy animals?

<sub>1</sub> None  
<sub>2</sub> 1 - 2  
<sub>3</sub> 3 - 5  
<sub>4</sub> Unknown

**63. If you have any opinions or comments about antibiotic-resistant bacteria on the dairy, please write them in the box below.**

Thank you very much for your time! If you would like to receive summary results from the study, please list the appropriate name and address below:

Name: \_\_\_\_\_

Address: \_\_\_\_\_

City, State, Zip: \_\_\_\_\_