

## Supporting information

### Impact of treatment strategies on cephalosporin and tetracycline resistance gene quantities in the bovine fecal metagenome

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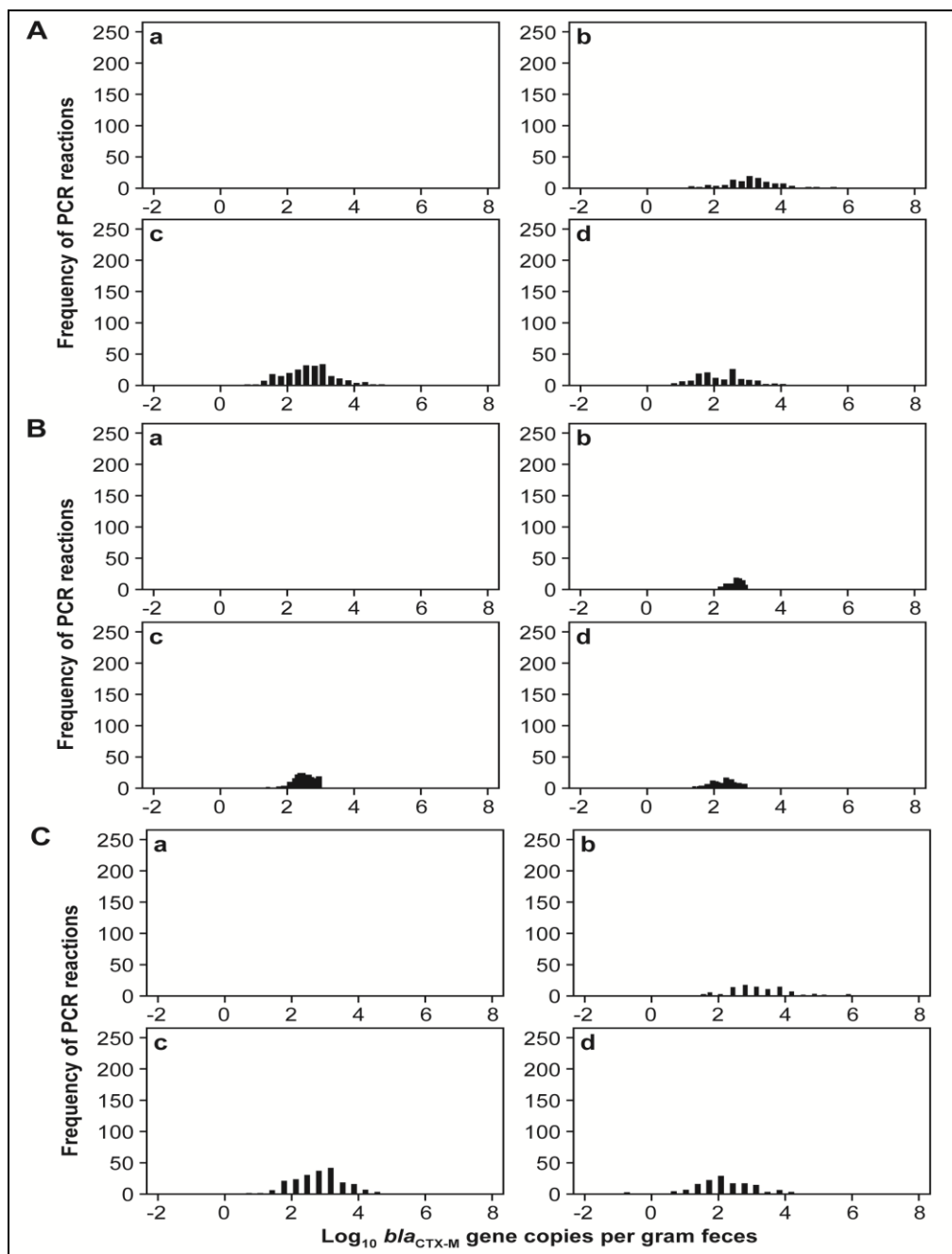
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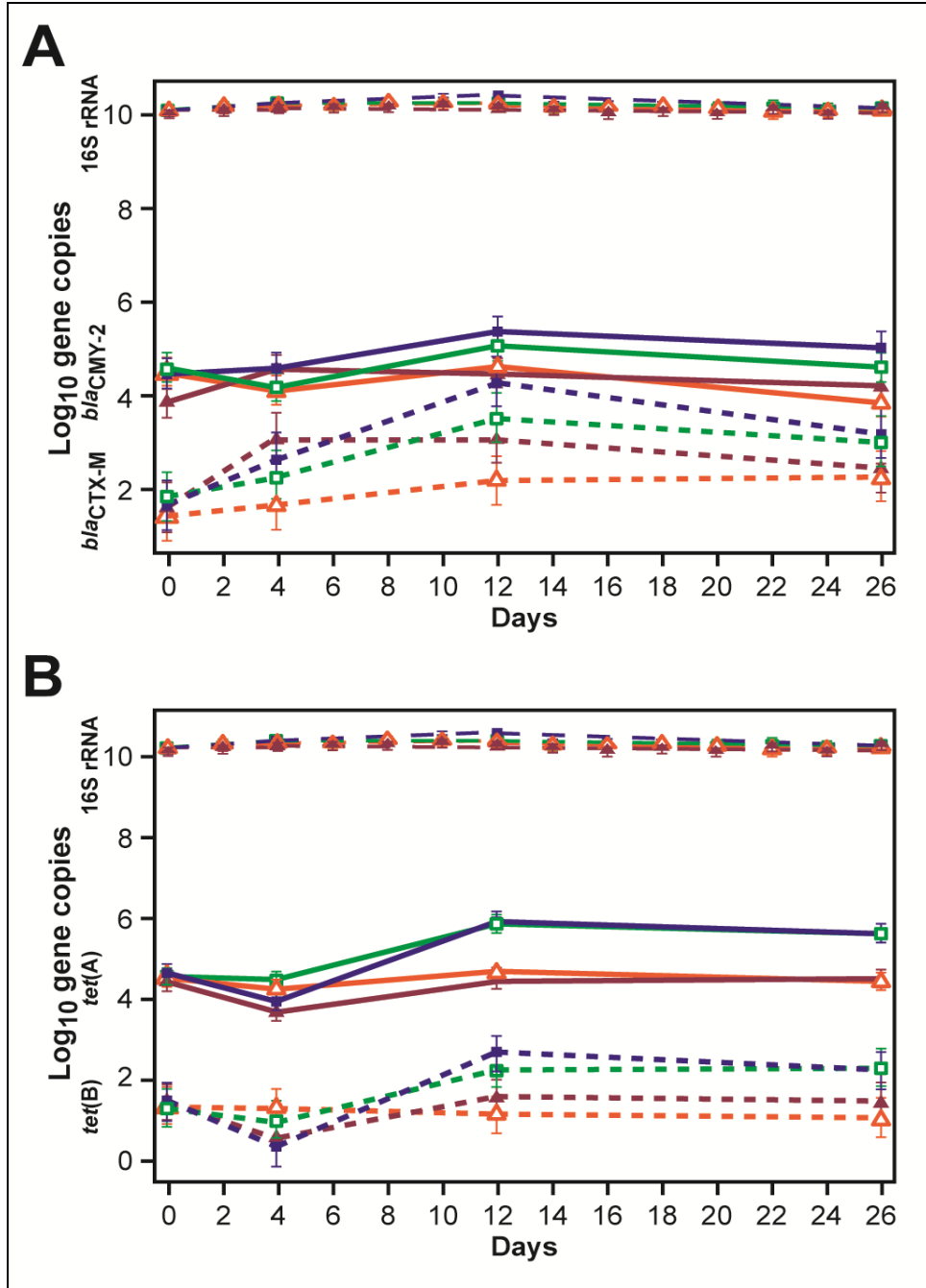
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Supplementary Figure S1: Histograms illustrating the distributions of the imputed estimates of missing observations of  $\log_{10} bla_{CTX-M}$  gene copies per gram feces obtained from a single imputation by three different imputation techniques: (A) linear regression, (B) truncated regression, and (C) interval censored, subgraphed by the number of missing observations (a=0, b=1, c=2, d=3) among the PCR triplicate assays.



(Note: The cut off set for the upper limit for the truncated regression imputation procedure was  $3 \log_{10} bla_{CTX-M}$  gene copies per gram. The cut off lower and upper limits for the interval censored regression imputation procedure were  $-\infty$  to  $7 \log_{10} bla_{CTX-M}$  gene copies per gram, respectively).

Supplementary Figure S2: Line graphs from non-imputed dataset illustrating: (A) non-standardized  $\log_{10}$  *bla*<sub>CTX-M</sub> (short dashed lines), non-standardized  $\log_{10}$  *bla*<sub>CMY-2</sub> (solid lines), and  $\log_{10}$  16S rRNA (long dashed lines) gene copies per gram wet feces, (B) non-standardized  $\log_{10}$  *tet*(B) (short dashed lines), non-standardized  $\log_{10}$  *tet*(A) (solid lines), and  $\log_{10}$  16S rRNA (long dashed lines) gene copies per gram wet feces, by treatment groups and each of the four sampling days. See Figure 3 for treatment group key. Data are marginal predicted means and error bars represent 95% confidence intervals.



Supplementary Table S1: Descriptive statistics (minimum, maximum, percentiles, mean and standard error) of  $\log_{10}$  gene copies observed both for raw and imputed data (first 5 imputations, as well as of all 20 imputations) cross-tabulated by the number of missing observations (0, 1, 2, 3) among each PCR triplicate assay.

Gene	Imputation <sup>a</sup>	Observations missing among qPCR triplicate	N <sup>b</sup>	Min <sup>c</sup>	Percentiles					Max <sup>d</sup>	Mean $\pm$ SE
					5	25	50	75	95		
<i>blactX-M</i>	No imputation (Raw data)	0	1347	1.96	2.53	3.02	3.49	4.09	5.17	6.99	3.61 $\pm$ 0.80
		1	192	1.98	2.18	2.55	2.85	3.22	3.89	4.96	2.92 $\pm$ 0.54
		2	108	1.82	2.15	2.38	2.77	3.08	4.16	4.99	2.86 $\pm$ 0.62
	1 <sup>st</sup>	1	96	1.19	1.77	2.63	3.11	3.54	4.31	5.69	3.10 $\pm$ 0.77
		2	216	0.85	1.46	2.12	2.63	3.06	3.93	4.87	2.63 $\pm$ 0.73
		3	123	0.66	1.15	1.67	2.09	2.62	3.31	4.18	2.19 $\pm$ 0.69
	2 <sup>nd</sup>	1	96	1.56	2.04	2.83	3.33	3.63	4.56	4.99	3.28 $\pm$ 0.69
		2	216	0.56	1.61	2.29	2.78	3.32	4.03	5.09	2.81 $\pm$ 0.74
		3	123	0.65	0.99	1.65	2.19	2.79	3.59	4.71	2.24 $\pm$ 0.84
	3 <sup>rd</sup>	1	96	1.46	2.06	2.77	3.19	3.61	4.59	5.24	3.24 $\pm$ 0.72
		2	216	0.53	1.17	2.17	2.69	3.25	3.95	4.68	2.68 $\pm$ 0.78
		3	123	0.03	1.16	1.84	2.32	2.97	3.59	4.33	2.38 $\pm$ 0.80
	4 <sup>th</sup>	1	96	0.95	1.78	2.43	3.04	3.59	4.32	5.17	3.01 $\pm$ 0.82
		2	216	0.53	1.13	2.26	2.66	3.18	4.03	4.89	2.67 $\pm$ 0.79
		3	123	-0.48	0.87	1.82	2.28	2.82	3.97	4.45	2.32 $\pm$ 0.85
	5 <sup>th</sup>	1	96	0.99	2.01	2.86	3.24	3.70	4.47	5.23	3.25 $\pm$ 0.69
		2	216	1.01	1.65	2.25	2.74	3.21	4.03	5.05	2.76 $\pm$ 0.73
		3	123	0.37	1.30	1.89	2.43	2.89	3.59	4.22	2.39 $\pm$ 0.69
	Average of 20 imputations	1	1920	0.23	1.85	2.66	3.17	3.66	4.44	5.69	3.16 $\pm$ 0.77
		2	4320	0.02	1.42	2.20	2.69	3.23	4.01	5.69	2.71 $\pm$ 0.78
3		2460	-0.55	1.05	1.77	2.26	2.79	3.58	5.23	2.29 $\pm$ 0.77	
<i>tet(B)</i>	No imputation (Raw data)	0	1404	-0.41	0.63	1.52	2.29	3.11	4.15	5.39	2.33 $\pm$ 1.07
		1	164	-0.69	-0.29	0.41	1.06	1.76	2.46	4.29	1.08 $\pm$ 0.88
		2	83	-0.68	-0.30	0.34	0.97	1.78	2.69	4.47	1.07 $\pm$ 1.01
	1 <sup>st</sup>	1	82	-1.09	0.00	0.71	1.37	1.93	2.87	4.11	1.40 $\pm$ 0.95
		2	166	-1.14	-0.79	0.03	0.70	1.13	2.11	3.32	0.65 $\pm$ 0.88
		3	183	-2.76	-2.03	-1.02	-0.24	0.47	1.76	3.23	-0.22 $\pm$ 1.12
	2 <sup>nd</sup>	1	82	-0.33	-0.14	1.02	1.53	2.20	3.09	3.61	1.61 $\pm$ 0.94
		2	166	-2.39	-1.02	0.04	0.79	1.38	2.74	3.90	0.74 $\pm$ 1.13
		3	183	-2.95	-1.53	-0.68	-0.01	0.66	1.68	2.66	-0.02 $\pm$ 0.98
	3 <sup>rd</sup>	1	82	-1.61	-0.39	0.68	1.47	2.12	3.07	5.67	1.39 $\pm$ 1.13
		2	166	-3.29	-1.19	-0.24	0.45	1.17	2.29	2.93	0.47 $\pm$ 1.10
		3	183	-2.56	-2.04	-1.24	-0.51	0.42	1.16	1.98	-0.42 $\pm$ 1.02
	4 <sup>th</sup>	1	82	-0.64	-0.18	0.83	1.62	2.36	3.19	4.28	1.58 $\pm$ 1.08
		2	166	-2.29	-1.5	0.05	0.72	1.39	2.41	3.89	0.67 $\pm$ 1.12
		3	183	-3.66	-1.84	-0.54	0.22	0.99	2.13	3.17	0.18 $\pm$ 1.18
	5 <sup>th</sup>	1	82	-1.29	-0.32	0.73	1.53	2.03	2.89	3.89	1.43 $\pm$ 1.03
		2	166	-1.39	-0.85	-0.09	0.78	1.63	2.42	3.89	0.79 $\pm$ 1.09
		3	183	-3.01	-1.73	-0.76	-0.12	0.59	2.01	3.83	0.00 $\pm$ 1.12
	Average of 20 imputations	1	1640	-2.29	-0.32	0.75	1.48	2.15	3.16	5.70	1.45 $\pm$ 1.07
		2	3320	-3.55	-1.10	-0.04	0.69	1.42	2.43	4.51	0.68 $\pm$ 1.09
3		3660	-4.10	-1.86	-0.84	-0.13	0.60	1.69	3.83	-0.12 $\pm$ 1.07	

<sup>a</sup>Data considered for descriptive statistics: Non-imputed versus imputed data (total, n=20 imputations).

<sup>b</sup>Number of reaction wells included in each category.

<sup>c</sup>Minimum estimated  $\log_{10}$  gene copies per gram feces.

<sup>d</sup>Maximum estimated  $\log_{10}$  gene copies per gram feces.

Supplementary Table S2: Descriptive statistics (minimum, maximum, percentiles, mean and standard error) of  $\log_{10}bla_{CTX-M}$  gene copies observed both for raw and imputed data (average of 20 imputations using 3 imputation procedures) cross-tabulated by number of missing observations (0, 1, 2, 3) among each PCR triplicate assay.

Imputation <sup>a</sup>		Observations missing among qPCR triplicate	N <sup>b</sup>	Min <sup>c</sup>	Percentiles					Max <sup>d</sup>	Mean $\pm$ SE
					5	25	50	75	95		
No imputation (Raw data)		0	1347	1.96	2.53	3.02	3.49	4.09	5.17	6.99	3.61 $\pm$ 0.80
		1	192	1.98	2.18	2.55	2.85	3.22	3.89	4.96	2.92 $\pm$ 0.54
		2	108	1.82	2.15	2.38	2.77	3.08	4.16	4.99	2.86 $\pm$ 0.62
Average of 20 imputations	Linear regression	1	1920	0.23	1.85	2.66	3.17	3.66	4.44	5.69	3.16 $\pm$ 0.77
		2	4320	0.02	1.42	2.20	2.69	3.23	4.01	5.69	2.71 $\pm$ 0.78
		3	2460	-0.55	1.05	1.77	2.26	2.79	3.58	5.23	2.29 $\pm$ 0.77
	Truncated regression	1	1920	1.35	2.13	2.46	2.66	2.83	2.96	2.99	2.61 $\pm$ 0.27
		2	4320	0.94	1.98	2.32	2.54	2.75	2.95	2.99	2.51 $\pm$ 0.29
		3	2460	0.97	1.79	2.14	2.38	2.62	2.90	2.99	2.37 $\pm$ 0.34
	Interval censored regression	1	1920	0.44	1.84	2.56	3.09	3.66	4.43	6.11	3.11 $\pm$ 0.79
		2	4320	-0.14	1.40	2.19	2.71	3.21	3.95	5.44	2.69 $\pm$ 0.77
		3	2460	-0.89	0.90	1.66	2.16	2.67	3.46	5.13	2.17 $\pm$ 0.77

Note: Table illustrates the results of a truncated regression imputation procedure with the cut off set for an upper limit of 3  $\log_{10}bla_{CTX-M}$  gene copies per gram. The cut off lower and upper limits for the interval censored regression imputation procedure were  $-\infty$  to 7  $\log_{10}bla_{CTX-M}$  gene copies per gram, respectively.

<sup>a</sup>Data considered for descriptive statistics: Non-imputed versus imputed data (total, n=20 imputations).

<sup>b</sup>Number of reaction wells included in each category.

<sup>c</sup>Minimum estimated  $\log_{10} bla_{CTX-M}$  gene copies per gram feces.

<sup>d</sup>Maximum estimated  $\log_{10}bla_{CTX-M}$  gene copies per gram feces.

Supplementary Table S3: Marginal predicted means for *bla*<sub>CTX-M</sub> and *tet*(B) log<sub>10</sub> gene copy numbers per gram wet feces across different factor combinations obtained from the main multi-level mixed linear regression analysis utilizing the non-imputed dataset with low non-zero values as well as datasets where missing observations were imputed utilizing three imputation techniques.

Factors <sup>a</sup>			<i>bla</i> <sub>CTX-M</sub>					<i>tet</i> (B)				
CTC <sup>b</sup>	Mix <sup>c</sup>	Day <sup>d</sup>	n <sup>e</sup>	Low non-zero <sup>f</sup>	Linear regression <sup>g</sup>	Truncated regression <sup>h</sup>	Interval-censored regression <sup>i</sup>	n <sup>e</sup>	Low non-zero <sup>f</sup>	Linear regression <sup>g</sup>	Truncated regression <sup>h</sup>	Interval-censored regression <sup>i</sup>
0	0	0	46	1.64 (1.11-2.17)	2.99 (2.73-3.26)	2.92 (2.67-3.16)	2.96 (2.69-3.24)	22	1.44 (0.98-1.89)	1.82 (1.42-2.21)	1.81 (1.43-2.18)	1.77 (1.38-2.16)
0	0	4	16	3.11 (2.58-3.64)	3.60 (3.35-3.84)	3.56 (3.32-3.80)	3.58 (3.33-3.82)	52	0.56 (0.11-1.02)	1.17 (0.77-1.57)	1.21 (0.81-1.61)	1.12 (0.72-1.52)
0	0	12	15	3.08 (2.55-3.61)	3.53 (3.28-3.77)	3.50 (3.26-3.75)	3.53 (3.28-3.78)	20	1.59 (1.13-2.05)	1.83 (1.43-2.22)	1.82 (1.45-2.20)	1.82 (1.42-2.21)
0	0	26	24	2.46 (1.92-2.99)	3.18 (2.93-3.43)	3.15 (2.91-3.40)	3.19 (2.94-3.44)	36	1.44 (0.98-1.90)	1.89 (1.49-2.28)	1.89 (1.50-2.28)	1.94 (1.54-2.35)
0	1	0	49	1.44 (0.91-1.97)	2.83 (2.58-3.09)	2.79 (2.54-3.03)	2.73 (2.46-3.00)	25	1.36 (0.90-1.81)	1.69 (1.30-2.08)	1.69 (1.31-2.08)	1.65 (1.26-2.05)
0	1	4	43	1.66 (1.13-2.19)	2.88 (2.63-3.14)	2.84 (2.60-3.09)	2.79 (2.54-3.05)	28	1.31 (0.85-1.77)	1.68 (1.28-2.07)	1.66 (1.28-2.04)	1.64 (1.24-2.03)
0	1	12	32	2.19 (1.66-2.72)	3.11 (2.87-3.36)	3.07 (2.83-3.31)	3.06 (2.80-3.31)	30	1.17 (0.72-1.63)	1.50 (1.10-1.89)	1.51 (1.13-1.90)	1.49 (1.10-1.89)
0	1	26	25	2.27 (1.74-2.81)	3.03 (2.79-3.28)	2.99 (2.75-3.24)	2.98 (2.72-3.23)	33	1.07 (0.61-1.53)	1.49 (1.09-1.88)	1.49 (1.10-1.88)	1.54 (1.13-1.96)
1	0	0	44	1.65 (1.12-2.18)	2.94 (2.69-3.20)	2.87 (2.62-3.11)	3.00 (2.73-3.26)	20	1.47 (1.01-1.93)	1.76 (1.38-2.15)	1.77 (1.39-2.14)	1.79 (1.40-2.18)
1	0	4	22	2.67 (2.14-3.20)	3.32 (3.07-3.57)	3.28 (3.04-3.52)	3.37 (3.12-3.62)	47	0.31 (-0.14-0.77)	0.93 (0.53-1.32)	0.96 (0.58-1.34)	1.00 (0.61-1.40)
1	0	12	3	4.31 (3.78-4.85)	4.40 (4.16-4.64)	4.40 (4.15-4.64)	4.41 (4.16-4.66)	8	2.68 (2.22-3.13)	2.77 (2.38-3.15)	2.77 (2.39-3.14)	2.78 (2.40-3.17)
1	0	26	12	3.19 (2.66-3.73)	3.55 (3.31-3.80)	3.53 (3.29-3.77)	3.59 (3.34-3.84)	10	2.24 (1.78-2.70)	2.40 (2.01-2.79)	2.39 (2.01-2.78)	2.43 (2.04-2.82)
1	1	0	45	1.84 (1.31-2.37)	3.12 (2.87-3.37)	3.07 (2.83-3.32)	3.14 (2.88-3.40)	31	1.31 (0.86-1.77)	1.66 (1.27-2.05)	1.67 (1.29-2.06)	1.67 (1.28-2.06)
1	1	4	29	2.30 (1.77-2.83)	3.16 (2.91-3.41)	3.10 (2.86-3.35)	3.17 (2.92-3.42)	42	1.02 (0.56-1.47)	1.49 (1.09-1.89)	1.53 (1.15-1.91)	1.55 (1.15-1.96)
1	1	12	16	3.54 (3.00-4.07)	4.01 (3.77-4.26)	3.99 (3.74-4.23)	4.02 (3.77-4.27)	17	2.28 (1.82-2.73)	2.45 (2.06-2.84)	2.46 (2.08-2.84)	2.49 (2.10-2.88)
1	1	26	14	3.02 (2.49-3.56)	3.42 (3.18-3.67)	3.41 (3.17-3.65)	3.45 (3.20-3.69)	10	2.30 (1.84-2.76)	2.44 (2.05-2.82)	2.43 (2.05-2.81)	2.47 (2.08-2.86)

<sup>a</sup>Independent variables in the regression models.

<sup>b</sup>Pen-level CTC treatment referred to whether all cattle in a pen received CTC subsequent to the CCFA treatment (coded 1).

<sup>c</sup>Differential pen-level CCFA treatment referred to whether CCFA was administered to either one (coded 1) or else all 11 animals within pens (coded 0).

<sup>d</sup>Day: Study day.

<sup>e</sup>Number of observations missing in each category.

<sup>f</sup>Results from dataset where missing observations were substituted with a low non-zero value.

<sup>g</sup>Results from dataset where missing observations were imputed utilizing a linear regression imputation approach.

<sup>h</sup>Results from dataset where missing observations were imputed utilizing a truncated regression imputation approach.

<sup>i</sup>Results from dataset where missing observations were imputed utilizing an interval censored regression imputation approach.