

Supplementary Material

Association between respiratory disease pathogens in calves near feedlot arrival with treatment for bovine respiratory disease and subsequent antimicrobial resistance status

Jennifer N. Abi Younes¹, John R. Campbell¹, Sheryl P. Gow², Amelia R. Woolums³, Cheryl L. Waldner^{1*}

¹Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada

²Canadian Integrated Program for Antimicrobial Resistance Surveillance, Public Health Agency of Canada, Saskatoon, SK, Canada

³Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, United States

*** Correspondence:**

Cheryl L. Waldner

cheryl.walder@usask.ca

1 Supplementary Tables

Table S0. Bovine respiratory disease clinical scoring system used to identify calves eligible for first treatment. Adapted from Step *et al.* (24).

BRD Clinical Scoring System – Reference List			
Score	BRD Code	Key Clinical Signs (*one or more signs are observed)	Occasional Clinical Signs (*may also be observed)
0	Normal	<ul style="list-style-type: none">▪ Clinically normal animal	
1	Mild BRD	<ul style="list-style-type: none">▪ Mildly ↑ respiratory rate▪ Mild – moderate gauntness▪ Quiet when observed from afar, but becomes alert when sees a human▪ Shallow or dry cough	<ul style="list-style-type: none">▪ Nasal discharge (clear, cloudy, white, or yellow)

BRD Clinical Scoring System – Reference List			
Score	BRD Code	Key Clinical Signs (*one or more signs are observed)	Occasional Clinical Signs (*may also be observed)
2	Moderate BRD	<ul style="list-style-type: none"> ▪ Mild or moderate depression <ul style="list-style-type: none"> • lethargic, but may become alert when approached • lower than normal head carriage • “hiding” behavior ▪ Mild – moderate muscle weakness <ul style="list-style-type: none"> • droopy ears • slow stepping or mild incoordination ▪ Coughing repeatedly ▪ Breathing with slightly ↑ abdominal effort ▪ Moderate gauntness 	<ul style="list-style-type: none"> ▪ ↑ Respiratory rate ▪ Nasal discharge (clear, cloudy, white, or yellow)
3	Severe BRD	<ul style="list-style-type: none"> ▪ Severe depression or weakness <ul style="list-style-type: none"> • when approached: does not become alert, low head carriage maintained, does not move away • cross stepping ▪ Repeated deep cough ▪ Marked respiratory effort <ul style="list-style-type: none"> • open mouth breathing or panting • moderate – marked ↑ abdominal effort 	<ul style="list-style-type: none"> ▪ ↑ Respiratory rate ▪ Nasal discharge (clear, cloudy, white, or yellow) ▪ Moderate – extreme gauntness
4	Moribund (near death)	<ul style="list-style-type: none"> ▪ Down, does not rise when approached or stimulated ▪ Standing, but does not move unless directly stimulated <ul style="list-style-type: none"> • very weak: drags feet, sways, stumbles, falls ▪ Eyes very sunken, abdomen very tucked up 	<ul style="list-style-type: none"> ▪ Any signs described for scores 1, 2, or 3

BRD Case Definition	
BRD Score	Criteria
1 – 2	<ul style="list-style-type: none"> ▪ AND a rectal temperature ≥ 40 °C
≥ 3	<ul style="list-style-type: none"> ▪ Regardless of rectal temperature ▪ With <u>NO</u> other obvious signs of disease (e.g., lameness, diarrhea, swollen limbs, etc.)

Table S1. Overview of statistical analyses for calves sampled near 36DOF for all culture and susceptibility (C/S) test results considered associated with C/S results for samples collected at 1DOF and 13DOF for individual calf-level, pen-level, and contextual analysis.

	Individual outcomes & risk factors	Pen-level outcomes & risk factors
Risk period for outcome of interest:	Outcome DNP C/S - Calf status 36DOF	Outcome DNP C/S - Pen results 36DOF
36DOF	Individual calf results from C/S at 1DOF	Pen results from calf C/S at 1DOF
36DOF	Individual calf results from C/S at 13DOF	Pen results from calf C/S at 13DOF

Culture and susceptibility (C/S) results considered:

- recovery of *M. haemolytica*, *P. multocida*, or *H. somni*
- co-isolation of two or more bacteria
- recovery of any BRD bacteria with: any AMR, macrolide resistance, or tetracycline resistance

Table S2. For calves sampled near 36DOF, associations between culture and susceptibility (C/S) results for calves at 1DOF and likelihood of subsequent C/S results near 36DOF. $n=309$ calves (tag identification not available for one calf at 1DOF). Analysis adjusted for year and injectable metaphylaxis administered and accounted for clustering by pen cohort.

Risk Factor: Culture Positive at 1DOF	Outcome: C/S result near 36DOF	OR	95% CI		P-value
			Lower	Upper	
<i>M. haemolytica</i>	<i>M. haemolytica</i>	1.0	0.6	1.8	0.96
<i>P. multocida</i>	<i>P. multocida</i>	2.0	1.1	3.7	0.02
<i>H. somni</i>	<i>H. somni</i>	0.6	0.2	1.4	0.23
Any bacteria with AMR	Any bacteria with AMR	1.2	0.3	4.3	0.83
Any bacteria with macrolide resistance*	Any bacteria with macrolide resistance		Not estimable		
Any bacteria with tetracycline resistance**	Any bacteria with tetracycline resistance	16	2.4	∞	0.008

*Zero calves sampled near 36DOF had bacteria with macrolide resistance recovered at 1DOF.

**Exact logistic regression equation (SAS® version 9.4, Cary, NC, USA).

Table S3. For calves sampled near 36DOF, associations between culture and susceptibility (C/S) for calves at 13DOF and likelihood of subsequent C/S at 36DOF. Analysis adjusted for year and injectable metaphylaxis administered and accounted for clustering by pen cohort. AST data were not available for one calf at time point 3 (pen 16); $n=309$ calves for AMR analyses (N=16 pens).

Risk Factor: Culture Positive at 13DOF	Outcome: C/S result near 36DOF	OR	95% CI		P-value
			Lower	Upper	
<i>M. haemolytica</i>	<i>M. haemolytica</i>	1.2	0.7	1.9	0.48
<i>P. multocida</i>	<i>P. multocida</i>	6.8	3.1	15	<0.001
<i>H. somni</i>	<i>H. somni</i>	1.0	0.3	3.4	0.97
Any bacteria with AMR	Any bacteria with AMR	1.9	0.8	4.2	0.13
Any bacteria with macrolide resistance*	Any bacteria with macrolide resistance	1.2	0.5	2.7	0.62
Any bacteria with tetracycline resistance	Any bacteria with tetracycline resistance	3.1	0.9	11	0.08

*Unconditional GEE reported as zero calves sampled near 36DOF from year 2021/oxytetracycline cohorts had bacteria with macrolide resistance recovered.

Table S4. For calves sampled near 36DOF, associations between culture and susceptibility (C/S) results at 36DOF given pen-level prevalence of C/S results at 1DOF and 13DOF. Analysis adjusted for year and metaphylaxis and accounted for clustering by pen cohort. $n=1,599$ calves at 1DOF and 1,596 calves at 13DOF (N=16 pens) (SAS® version 9.4, Cary, NC, USA).

Risk Factor: Pen-level C/S Prevalence	Outcome Pen Probability	OR	95% CI		P-value
			Lower	Upper	
Increase in prevalence at 1DOF	C/S at 36DOF				
<i>M. haemolytica</i> (5%)	<i>M. haemolytica</i>	1.0	0.9	1.2	0.53
<i>P. multocida</i> (5%)	<i>P. multocida</i>	1.0	0.9	1.0	0.39
<i>H. somni</i> (5%)	<i>H. somni</i>	1.0	0.9	1.1	0.91
Tetracycline resistance (5%)	Tetracycline resistance	1.5	1.2	1.9	0.0007
Macrolide resistance (5%)*	Macrolide resistance	8.8	0.8	103	0.08
Any AMR (5%)	Any AMR	1.0	0.6	1.8	0.92
Increase in prevalence at 13DOF	C/S at 36DOF				
<i>M. haemolytica</i> (5%)	<i>M. haemolytica</i>	1.0	0.9	1.0	0.23
<i>P. multocida</i> (5%)	<i>P. multocida</i>	1.2	1.2	1.3	<0.0001
<i>H. somni</i> (5%)	<i>H. somni</i>	1.0	0.8	1.3	0.93
Tetracycline resistance (5%)	Tetracycline resistance	1.0	1.0	1.1	0.04
Macrolide resistance (5%)*	Macrolide resistance	1.2	1.1	1.3	<0.0001
Any AMR (5%)	Any AMR	1.0	1.0	1.1	0.01

*Unconditional GEE reported as zero calves sampled near 36DOF from year 2021/oxytetracycline cohorts had bacteria with macrolide resistance recovered.

Table S5. Summary of associations between culture and susceptibility (C/S) results at 1DOF and 13DOF and subsequent C/S for calves at 36DOF.

Individual calf level models		
Predictor	Outcome	Significant risk factors
C/S result at 1DOF	C/S result at 36DOF	<i>P. multocida</i> , tetracycline resistance
C/S result at 13DOF	C/S result at 36DOF	<i>P. multocida</i>
Pen-level data		
Risk factor: Pen-level C/S prevalence	Outcome	Significant risk factors
Pen prevalence at 1DOF	C/S result at 36DOF	Tetracycline
Pen prevalence at 13DOF	C/S result at 36DOF	<i>P. multocida</i> , any AMR, macrolide resistance, or tetracycline resistance