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Changes in the rates of field isolation and antimicrobial susceptibility of bacterial pathogens collected from fall-placed feedlot steers between arrival at the feedlot and 90 to 120 days on feed

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Abstract

The change in bacterial recovery and phenotypic antimicrobial susceptibility of 3 important bovine respiratory disease bacteria (*Mannheimia haemolytica, Pasteurella multocida*, and *Histophilus somni*) between arrival and 90 to 120 days was observed in 295 healthy fall-placed feedlot calves in western Canada using deep nasal swabs. At the arrival sampling, 28%, 28%, and 9% of calves were culture positive for *Mannheimia haemolytica, Pasteurella multocida*, and *Histophilus somni*, respectively; these rates changed to 23%, 14%, and 16%, respectively, in the post-arrival period. A decrease in antimicrobial susceptibility was observed between the arrival and post-arrival sample periods, with numerically fewer pan-susceptible isolates and little change in multi-drug resistant isolates. Between the 2 sampling periods, statistically significant decreases in susceptibility to tilmicosin and tulathromycin were observed for *Mannheimia haemolytica* and tildipirosin for *Pasteurella multocida* isolates. Results of this study suggest that changes to both the proportions of bacteria isolated and the antimicrobial susceptibility of isolates occur between arrival and ~90 days post-arrival.

Key words: BRD, antimicrobial, susceptibility, pneumonia, feedlot

Résumé

La fréquence d'isolement des bactéries et la susceptibilité antimicrobienne phénotypique de trois bactéries importantes causant des maladies respiratoires chez les bovins (*Mannheimia haemolytica, Pasteurella multocida* et *Histophilus somni*) ont été observées à l'aide d'écouvillons nasaux profonds chez 295 veaux en santé dans un parc d'engraissement en automne dans l'ouest Canadien entre leur arrivée et de 90 à 120 jours plus tard. Au moment de l'échantillonnage à l'arrivée, le pourcentage de veaux avec des cultures positives était de 28% pour *Mannheimia haemolytica*, de 28% pour *Pasteurella multocida* et de 8% pour *Histophilus somni*; les pourcentages après l'arrivée (*note du traducteur : environ 90 jours après l'arrivée*) étaient de 23%, 14% et 16%, respectivement. Il y a eu une baisse de la susceptibilité antimicrobienne entre l'échantillonnage à l'arrivée et celui après l'arrivée, avec numériquement moins d'isolats susceptibles à tous les médicaments et peu de changements dans les isolats résistants à plusieurs médicaments. Entre les deux périodes d'échantillonnage, une baisse statistiquement significative de la susceptibilité à la tilmicosine et à la tulathromycine a été observée pour les isolats de *Mannheimia haemolytica* et une baisse de la susceptibilité à la tildipirosine a été observée pour les isolats de *Pasteurella multocida*. Les résultats de cette étude suggèrent des changements tant au niveau des proportions de bactéries isolées que de la susceptibilité antimicrobienne des isolats entre l'arrivée et près de 90 jours plus tard.

Introduction

Bovine respiratory disease (BRD) is an important cause of morbidity and mortality in the feedlot industry and is associated with multiple risk factors including weaning, mixing of calves through livestock auctions, weather events, transpor-

tation, commingling at the feedlot, and exposure to viral and bacterial respiratory pathogens.[21] Bacteria most frequently implicated in BRD (*Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*) are typically carried as part of the normal flora of the upper respiratory tract of cattle.[21] The recovery of these bacteria in recently weaned calves upon arrival to a feedlot varies from 13 to 60%, and is typically controlled through the use of vaccination and metaphylaxis.[2,6,10,22]

Metaphylaxis is the control of BRD in high-risk calves with antimicrobials upon arrival to feedlots in order to decrease the morbidity and mortality associated with this disease complex.[11] Despite interventions such as metaphylaxis and vaccination, BRD mortality continues to increase by 0.05% per year in some US feedlots.[9] The detection of this trend serves to highlight the continued importance of BRD morbidity and mortality in feedlots. As a result of the challenges in managing this disease, the majority of industry professionals agree that metaphylaxis remains a viable intervention for managing BRD.[4]

In North America, a number of antimicrobials labeled for control of BRD are within the macrolide family, including gamithromycin, tilmicosin, tildipirosin, and tulathromycin. These macrolides are either 15 member ring (gamithromycin, tulathromycin) or 16 member ring (tildipirosin, tilmicosin) derivatives of erythromycin that interfere with protein synthesis at the bacterial 50S ribosomal subunit.[7]

As macrolide use in the feedlot industry has increased, there has been a corresponding rise in studies examining the susceptibility of *M. haemolytica*, *P. multocida*, and *H. somni* to commercial products within this class of antimicrobials. A recent study determined that the susceptibility of these 3 organisms to various macrolides has either been decreasing or shifting toward a higher minimum inhibitory concentration (MIC).[20] Additional studies have also attributed the detection of resistance to multiple antimicrobials to integrative and conjugative elements (ICE).[8,13,17,18] The discovery of ICE raises questions about susceptibility profiles and if resistance to 1 antimicrobial can co-select for resistance to other antimicrobials.

The objective of this study was to compare the proportion of calves culture-positive for BRD-related bacterial organisms at arrival to a commercial feedlot to the proportion of the same cohort of calves culture-positive for these same bacteria at ~90 days post-arrival. These isolates were then used to study the changes in phenotypic antimicrobial susceptibility to 8 antimicrobials from arrival to the post-arrival period. Our data will provide clinical veterinarians with an understanding of the proportional change of BRD pathogen presence between arrival and ~90 days post-arrival, and the proportional change of antimicrobial susceptibility during this same period and after exposure to metaphylactic antimicrobials. Clinical feedlot veterinarians are challenged with determining how to monitor pathogen presence and antimicrobial effectiveness; these results will aid the veterinarian in both of these tasks.

Materials and Methods

Experimental Use of Animals

University of Saskatchewan Animal Research Ethics Board granted a Certificate of Approval for this project on December 10, 2015 and it can be found under AUP#20150075 at the Research Ethics Office.

Sample Collection

Deep nasopharyngeal swabs (DNS) were collected from 295 freshly weaned steer calves upon arrival to a commercial feedlot in central Saskatchewan, Canada in the fall of 2015. These 295 calves were a subset of a larger group of approximately 1000 calves, and were enrolled in the study by sampling every third calf through the chute at processing. Calves were weighed upon arrival and placed into 1 of 5 pens, depending on their weight, and remained in their weight sorted pens for the duration of the trial. The calves were housed in outdoor dirt floor pens with 20% porosity wind fencing, a shared central feed alley, and automatic watering bowls. Individual animals remained in the same home pen throughout the study period. On arrival, calves received a standard protocol including a BRD pathogen vaccine,[a] a clostridial/*Histophilus* vaccine,[b] a metaphylactic antimicrobial,[c] a growth implant,[d] and topical avermectin.[e] At approximately 15 days-on-feed, calves began receiving tylosin phosphate for liver abscess control[f] in their ration, and this continued for the remainder of the study. At 90 to 120 days after arrival sample collection, DNS were once again collected from the study calves (post-arrival samples). Post-arrival samples were collected when the cattle were scheduled for reapplication of a growth-promoting implant in order to avoid unnecessary movement of cattle through a handling system in accordance with university animal care recommendations.

During the collection procedure, each individual calf was restrained in a chute, with the animal's head held steady by the chute neck extender. The left nares of each calf was cleaned externally using a fresh paper towel. A double guarded 32.68 inches (83 cm) nasal swab[g] was inserted into the ventral meatus of the nasal cavity a distance of approximately 5.91 inches (15 cm). The cotton tip swab was extended 0.79 to 1.57 inches (2 to 4 cm) out of its guarding and rotated a minimum of 10 times, then retracted back into the guards. The entire device was removed from the nasal passage and the cotton tipped swab pulled from the end of the guards. The cotton tip swab was immediately removed and placed into Amies media, then stored in a cooler at approximately ~41°F (~5°C). On the same day they were collected all samples were transported for 1.5 hours to the laboratory for culture and were processed within 2 hours of arrival.

Bacterial Culture

The nasal swabs were cultured on 5% Columbia sheep blood and Chocolate agar plates[h] and incubated at ~95°F (~35°C) for 18 hours in an environment containing 5% CO_2, for

isolation of *Histophilus somni*, *Mannheimia haemolytica*, *Pasteurella multocida*, and *Bibersteinia trehalosi*. Bacterial colonies were examined for cultural characteristics such as production of yellow pigment (*H. somni*), β-hemolysis (*M. haemolytica*), and mucoid appearance (*P. multocida*), at 18 and 48 hours of incubation. The microorganisms of interest were identified using the Matrix Assisted Laser Desorption and Ionization Time of Flight (MALDI-TOF) Mass Spectrometry System.[i]

Briefly, individual bacterial colonies were transferred onto the stainless steel MALDI-TOF target in duplicate. Each target well was overlaid with 1 µl of α-cyano-4-hydroxycinnamic acid (HCCA) matrix,[j] and the mass spectra were acquired using MALDI-TOF MF, Microflex LT system in a linear positive mode.[j] Instrument calibration was performed using standard reference BTS *Escherichia coli*.[j] For bacterial identification MALDI Biotyper 3.1.66 was used.[j] The cut-off scores used for bacterial identification were ≥2.0. Only isolates that positively identified with scores equal or greater than 2.0 were included in this study.

Antimicrobial Susceptibility Test

Susceptibility of isolates to 8 antimicrobial agents was determined by the standard disk diffusion technique of Kirby-Bauer,[3] after isolation and identification. Briefly, 4 to 5 isolated colonies were inoculated into Tryptic Soy Broth[j] for *M. haemolytica* and *P. multocida* or Todd Hewitt Broth[k] for *H. somni* and incubated at ~95°F (~35°C) in 5% CO_2 environment for approximately 2 to 3 hours to obtain 0.5 McFarland turbidity. Susceptibility test was done using Mueller Hinton agar (MHA) supplemented with 5% sheep blood[k] and incubated for 18 hours, aerobically for *M. haemolytica* and *P. multocida*, or in 5% CO_2 environment for *H. somni*. *M. haemolytica* ATCC 33396 was used as quality control for each batch of MHA media used.

Selection of disk concentrations and interpretation of zone diameter results was done according to the Clinical Laboratory Standards Institute (CLSI) recommendations.[5] The following antimicrobial agents and disk potencies (µg) were used: ceftiofur (XNL, 30), enrofloxacin (ENR, 5), florfenicol (FFC, 30), gamithromycin (GAM, 15), spectinomycin (SPT, 100), tildipirosin (TIP, 60), tilmicosin (TIL, 15), and tulathromycin (TUL, 30).

The diameter of the zone of inhibition was measured to the nearest millimeter and results were recorded as either susceptible or non-susceptible based on CLSI-approved zone measurements;[5] for the purposes of this study non-susceptible categorization includes both resistant and intermediate isolates. Results from antimicrobials without CLSI-approved measurements were not used in the analysis of this study.

Statistical Analysis

Susceptibility results were entered into a spreadsheet for import into commercially available statistical software[l] for analysis.

Descriptive statistics were used to examine the differences between the proportion of calves culture-positive for *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* observed in this study upon arrival and at second sampling (post-arrival). Ninety-five percent confidence intervals were calculated for each of the proportions. Only 1 calf was found to be positive for *Bibersteinia trehalosi*, therefore this bacterial species was not included in any analysis. Also, given the relative infrequent occurrence of cultures positive for multiple pathogens, the proportions were not analyzed further. The isolates cultured in combination from a single animal were included in the study as single isolates within each bacterial species analyzed.

Descriptive statistics were also used to examine differences between the proportional susceptibility of bacteria to the 8 antimicrobials tested, with a 95% CI calculated for each bacteria, antimicrobial, and sample period combination. The statistical significance of antimicrobial susceptibility results was performed using Fisher's exact test to determine whether the changes in proportional susceptibility were significantly different ($P < 0.05$) between the 2 sampling periods.

Results

The mean body weights for each of the 5 enrolled pens were as follows: Pens 1 and 2 – 586 lb (266 kg), Pens 3 and 4 – 667 lb (303 kg), and Pen 5 – 748 lb (340 kg). Deep nasopharyngeal swabs were collected from 295 healthy steer calves upon arrival to a commercial feedlot. Of these animals sampled, 287 were available for swab collection approximately 90 to 120 days later (post-arrival) when the calves were brought through the chute for reapplication of a growth promotant implant. The 8 calves lost from the study were the result of either mortality or removal from their home pen and into a convalescence pen; animals in the convalescence pen were not available on the post-arrival collection day and were excluded.

At the arrival sampling, 44% of the calves studied were negative for all 3 bacterial pathogens tested, 9% were culture-positive for *H. somni*, 28% were culture-positive for *M. haemolytica*, and 28% of calves were culture-positive for *P. multocida* (Table 1). The confidence intervals for each of the culture proportions were moderately narrow, indicating that the true mean for each is likely within the 95% CI (Table 1). Of 295 calves sampled upon arrival, 5% were culture-positive for both *M. haemolytica* and *P. multocida*, 2% for both *M. haemolytica* and *H. somni*, and 2% for both *P. multocida* and *H. somni*. There were no calves that cultured positive for all 3 bacteria.

Of the 287 calves presented for sample collection during the post-arrival period (90 to 120 days post arrival), 52% were culture-negative for all 3 bacteria, and 16%, 23%, and 14% were culture-positive for *H. somni*, *M. haemolytica*, and *P. multocida*, respectively (Table 1). Moderately narrow 95% CI indicate that the true mean proportions of culture-positive

for each bacteria is likely within the confidence interval calculated (Table 1).

There was a significantly higher proportion of calves positive for *H. somni* ($P = 0.02$) during the post-arrival period, a significantly lower proportion of calves positive for *P. multocida* ($P < 0.01$) during the post-arrival period, and no difference in the proportion positive for *M. haemolytica* ($P = 0.2$; Table 1). Combination cultures were also observed in the post-arrival period, with 1% of calves positive for *M. haemolytica* and *P. multocida*, 2% positive for *M. haemolytica* and *H. somni*, and 1% for *P. multocida* and *H. somni*. Again, during this observation period, there were no calves that cultured positive for all 3 bacteria.

A small proportion of calves were found to be positive to the same bacteria in both sample periods. Of the 26 calves culture-positive for *H. somni* in the arrival period, *H. somni* was recovered from 27% of these animals in the post-arrival period. Of the 82 calves culture-positive for *M. haemolytica* in the arrival period, 23% were positive on the second sample; identical results were observed for *P. multocida* with 82 culture-positive at arrival and only 23% remaining positive on the second sample. This indicates that the majority of calves that cultured positive to a particular bacteria on arrival were either culture-positive for a different bacteria or were culture-negative upon the post-arrival sample period.

H. somni had relatively low susceptibility rates, <80%, for 3 of the 4 macrolide antimicrobials tested during both sample periods, and there were no statistically significant differences between samplings for any of the antimicrobials tested (Table 2). The 95% CI for the 3 macrolides (tildipirosin, tilmicosin, and tulathromycin) which had low susceptibility rates were also relatively wide, indicating that the mean proportion of susceptible isolates is less likely to be within the confidence interval.

Mannheimia haemolytica had similar proportional susceptibility rates between the 2 sample periods for most of the antimicrobials tested; however, statistically significant decreases in susceptibility were observed for tilmicosin ($P < 0.01$) and tulathromycin ($P = 0.01$; Table 3). Overall, the confidence intervals for proportion susceptibility were wide.

P. multocida also had similar proportional susceptibility rates between the 2 sampling periods for most antimicrobials tested. The only statistically significant difference was a decrease in susceptibility to tildipirosin between sampling periods ($P = 0.04$), and again the 95% CI for all 4 macrolides were greater than 10% apart, indicating measured proportion susceptibility of these antimicrobials is less reliable than if the 95% CI had been narrower (Table 4).

Multiple antimicrobial non-susceptibility was also examined by comparing the proportion of isolates non-susceptible to a single antimicrobial, to the proportion of isolates that were non-susceptible to 2 or more antimicrobials for each bacteria and within each sample period (Table 5). At the time of arrival sampling, a relatively small proportion of *H. somni*, *M. haemolytica*, and *P. multocida* isolates (0%, 3%, and 2%, respectively) were observed to be non-susceptible to ≥2 antimicrobials. Multiple non-susceptibility increased for *H. somni* and *M. haemolytica* in the post-arrival sample period, with 5% of isolates observed as non-susceptible to ≥2 antimicrobials. Non-susceptibility to multiple antimicrobials

Table 1. Isolation rates of BRD pathogens from the nasopharynx of healthy feedlot calves.

Sample period	Bacterial species	No. calves	No. isolates	Proportion of calves	CI 95%	Fisher's Exact (*P*-value)
Arrival	*H. somni*	295	26	9%	5.8% - 13%	—
	M. haemolytica	295	82	28%	23% - 33%	—
	P. multocida	295	82	28%	23% - 33%	—
~90 days post-arrival	*H. somni*	287	45	16%	12% - 20%	0.02
	M. haemolytica	287	66	23%	18% - 28%	0.2
	P. multocida	287	40	14%	10% - 19%	<0.01

Table 2. A comparison of the change in *Histophilus somni* antimicrobial susceptibility rates from nasopharyngeal cultures of healthy feedlot calves at arrival and ~ 90 days post-arrival.

Antimicrobial	*Histophilus somni* Proportional Antimicrobial Susceptibility				Fisher's Exact (*P*-value)
	Arrival		Post-arrival		
	Proportion	CI 95%	Proportion	CI 95%	
Ceftiofur	96%	0.804 - 0.999	98%	0.891% - 0.999%	1.0
Enrofloxacin	100%	0.868 - 1.00	98%	0.891% - 0.999%	1.0
Florfenicol	100%	0.868 - 1.00	100%	0.920% - 1.00%	N/A
Spectinomycin	100%	0.868 - 1.00	98%	0.880% - 0.999%	1.0
Gamithromycin	100%	0.868 - 1.00	100%	0.920% - 1.00%	N/A
Tildipirosin	77%	0.564 - 0.910	68%	0.524% - 0.814%	0.6
Tilmicosin	58%	0.369 - 0.766	55%	0.388% - 0.696%	1.0
Tulathromycin	62%	0.406 - 0.798	57%	0.410% - 0.717%	0.8

in *P. multocida* decreased between the 2 sample periods, with no post-arrival multiple non-susceptibility being observed compared to 2% at arrival. Overall multiple antimicrobial non-susceptibility was low and the rates were not analyzed further.

Pan-susceptibility to the antimicrobials tested was also observed (Table 5). Few *H. somni* isolates (54%) were observed to be pan-susceptible to the antimicrobials tested on arrival isolates, compared to *M. haemolytica* and *P. multocida*, which were 85% and 95% pan-susceptible, respectively. Pan-susceptibility was found to be numerically reduced for all 3 of the bacterial species at the post-arrival sampling. At post-arrival sampling, 39%, 67%, and 80% of *H. somni*, *M. haemolytica*, and *P. multocida*, respectively, were observed to be pan-susceptible to the antimicrobials tested.

Discussion

The proportional detection of *H. somni*, *M. haemolytica*, and *P. multocida* from DNS of healthy cattle upon arrival and during the post-arrival sample period indicated a change in the recovery rate of these important BRD pathogens over time. Previous work conducted using DNS for similar arrival study purposes or on calves clinically ill with BRD has been inconsistent in its agreement between nasal presence, lung presence, and risk of BRD development.[6,14,19,22] The predictive nature of DNS for disease outcome may depend on whether the animal is clinically ill at the time of collection; DeRosa et al[6] found that DNS cultures from acutely ill animals were predictive of lung culture results, and cultures had a high degree of genetic similarity. However, Noyes et al[19] observed

Table 3. A comparison of the change in *Mannheimia haemolytica* antimicrobial susceptibility rates from nasopharyngeal cultures of healthy feedlot calves at arrival and ~ 90 days post-arrival.

***Mannheimia haemolytica* Proportional Antimicrobial Susceptibility**					
Antimicrobial	**Arrival**		**Post-arrival**		**Fisher's Exact (*P*-value)**
	Proportion	**CI 95%**	**Proportion**	**CI 95%**	
Ceftiofur	100%	0.955 - 1.00	100%	0.946 - 1.00	N/A
Enrofloxacin	99%	0.933 - 1.00	97%	0.896 - 0.996	0.6
Florfenicol	98%	0.914 - 0.997	99%	0.920 - 1.00	1.0
Spectinomycin	100%	0.955 - 1.00	100%	0.946 - 1.00	N/A
Gamithromycin	95%	0.878 - 0.986	96%	0.875 - 0.991	1.0
Tildipirosin	95%	0.878 - 0.986	87%	0.760 - 0.937	0.1
Tilmicosin	99%	0.933 - 1.00	72%	0.593 - 0.820	<0.01
Tulathromycin	94%	0.863 - 0.980	80%	0.688 - 0.882	0.01

Table 4. A comparison of the change in *Pasteurella multocida* antimicrobial susceptibility rates from nasopharyngeal cultures of healthy feedlot calves at arrival and ~ 90 days post-arrival.

***Pasteurella multocida* Proportional Antimicrobial Susceptibility**					
Antimicrobial	**Arrival**		**Post-arrival**		**Fisher's Exact (*P*-value)**
	Proportion	**CI 95%**	**Proportion**	**CI 95%**	
Ceftiofur	100%	0.956 - 1.00	100%	0.912 - 1.00	N/A
Enrofloxacin	98%	0.914 - 0.997	100%	0.912 - 1.00	1.0
Florfenicol	99%	0.934 - 1.00	100%	0.912 - 1.00	1.0
Spectinomycin	99%	0.934 - 1.00	90%	0.763 - 0.972	0.04
Gamithromycin	99%	0.934 - 1.00	95%	0.831 - 0.994	0.3
Tildipirosin	99%	0.934 - 1.00	90%	0.763 - 0.972	0.04
Tilmicosin	99%	0.934 - 1.00	95%	0.831 - 0.994	0.3
Tulathromycin	100%	0.956 - 1.00	95%	0.831 - 0.994	0.1

Table 5. Comparison of arrival and post-arrival BRD pathogen rates of multiple non-susceptibility and pan-susceptibility.

Period	**Species**	**No. of isolates**	**% of isolates non-susceptible to:**		**Percent isolates pan-susceptible**
			One antimicrobial	**Two or more antimicrobials**	
Arrival	*H. somni*	26	46%	0%	54%
	M. haemolytica	81	12%	3%	85%
	P. multocida	82	2%	2%	95%
Post-arrival	*H. somni*	44	57%	5%	39%
	M. haemolytica	67	31%	4%	67%
	P. multocida	40	20%	0%	80%

that isolation of *M. haemolytica* from calves upon arrival to a feedlot was not predictive of the risk of clinical disease or BRD mortality. With the evidence provided from the study reported here, it is important to consider that the antimicrobial susceptibility results of isolates recovered by DNS should not be used to determine clinical outcomes of diseased feedlot calves because the DNS culture results of healthy cattle may not be predictive of the population of bacteria found in the lungs. Rather, results of the current study should be used as a consideration of the current proportional antimicrobial susceptibility of isolates from calves arriving to a feedlot in Saskatchewan, and how this proportion changes with exposure to antibiotics and over the first ~90 days-on-feed. Clinical feedlot veterinarians could use DNS samples routinely on their client's calves to determine the antimicrobial susceptibility rates early in the filling period of the feedlot.

Deep nasopharyngeal swabs have been used previously to detect the proportional presence of BRD pathogens in the upper respiratory tract of feedlot calves. Earlier studies have shown that between 13% and 30% of recently weaned calves are DNS culture-positive for *M. haemolytica* upon arrival at the feedlot, whereas post-arrival rates range between 19 and 28%.[2,19,22] The current study agrees with these previous findings for both the arrival and post-arrival proportions of calves shedding *M. haemolytica*. Fewer comparisons from previous research were available with regards to *P. multocida* and *H. somni*. Only 1 previous study was found that had both arrival and post-arrival DNS culture results for *P. multocida*, which were 25% and 63%, respectively.[22] The current study observed similar arrival results; however, the post-arrival proportion was considerably lower at 14%. These differences may be due to a lack of metaphylactic antibiotic use and an absence of bacterial pathogen vaccine in the processing protocol of the previous study. With regards to *H. somni*, only 1 trial was found that observed the proportion of calves culture-positive upon arrival to a feedlot; however, this trial did not include a post-arrival sampling period. The previous trial found that 5% of calves were positive for *H. somni* on arrival, compared to 9% in the current study.[2] A review article was found that reported *H. somni* arrival prevalence at 15 to 50%.[10]

Histophilus somni proportions in the current study increased significantly ($P = 0.02$) from 9% on arrival to 16% post-arrival; unfortunately no comparisons are available in the literature. The relative increase in proportion of calves that were culture-positive for *H. somni* may be related to the low macrolide susceptibility observed for these isolates. The calves were treated metaphylactically with tulathromycin upon arrival; however, only 62% of the *H. somni* isolates collected were susceptible to tulathromycin. It is probable that the low rate of susceptibility of *H. somni* isolates to tulathromycin in combination with the relatively high susceptibility of *M. haemolytica* and *P. multocida* (94% and 100%, respectively) resulted in a competitive advantage for *H. somni* isolates during the arrival period, and the reduction in competition at that time led to a greater frequency of recovery during the post-arrival period. While this probable increase in *H. somni* competiveness may be hypothesized, it should also be noted that the frequency of re-culture of the same species from the same individual upon arrival and post-arrival was low. Only 27% of calves positive for *H. somni* upon arrival were also positive for *H. somni* at the post-arrival sample, indicating that most of the previously shedding calves were no longer shedding and that most of the post-arrival positive calves were new carriers. The current study's findings of low rates of agreement between arrival and post-arrival culture status is similar to the work of Noyes et al,[19] which observed less than 10% of calves being positive for *M. haemolytica* at both the arrival and post-arrival samples.

Practitioners should note these changes in bacterial populations as surveillance or routine sampling for BRD pathogen presence of cattle upon arrival is common in commercial feedlots, and the results of this study indicate that the bacterial population in nasal passages changes over time. If results from arrival sampling are used alone as a prediction of potential pathogen presence, it is likely that the frequency of specific bacteria will be over or under-estimated as cattle move through the risk period for BRD. Collecting and analyzing cultures from healthy controls and BRD treatments may be a more accurate method of observing the frequency of pathogen presence, and the importance of pathogen presence to BRD treatment rates.

For example, low susceptibility of the current study's arrival *H. somni* isolates to tulathromycin may have allowed survival of these isolates; however, if this were true it would be reasonable to expect that significantly more of the calves positive to *H. somni* on arrival would still be positive post-arrival, compared to the other bacteria monitored, and this was not the case. However, had samples been collected from healthy controls and treatments during the high disease period, these culture results may have shown a better indication of the influence of tulathromycin-resistant *H. somni* isolates on the prevalence of BRD treatment.

The *H. somni* isolates from the current study are also interesting in terms of their antimicrobial susceptibility patterns; the *H. somni* isolates had relatively low susceptibility to 3 of the 4 macrolides tested (tildipirosin, tilmicosin, tulathromycin), and high proportions of susceptibility to the other macrolide (gamithromycin), as well as to all of the other 4 antimicrobials tested (ceftiofur, enrofloxacin, florfenicol, spectinomycin). The *H. somni* isolates collected at arrival were 100% susceptible to gamithromycin, and only 77%, 58%, and 62% susceptible to tildipirosin, tilmicosin, and tulathromycin, respectively. The difference in macrolide susceptibility may be the result of the presence of genes conferring resistance to only some macrolides, but not others. Integrative conjugative elements have been closely studied in members of the *Pasteurellaceae* family, and it has been demonstrated that some of these elements confer resistance for macrolides. Evidence also exists for the presence of ICE genes amongst isolates of *H. somni* collected from calves in the United States.[12] Work by

Klima et al[12] observed that conjugal transfer of these genes is possible between bacteria and between bacterial species. More work should be done on the isolates recovered in this study to determine if ICE genes are present and, if present, whether there is evidence of conjugal transfer of genes between species.

The *M. haemolytica* and *P. multocida* isolates in this study showed evidence of reduced antimicrobial susceptibility post-arrival; this reduction in susceptibility may be the result of conjugal transfer of resistance genes between bacteria or selection of a sub-population of bacteria already containing these genetic elements. Evidence of ICE transfer has been observed in previous work[15-18] and is likely the cause of the phenotypic expression of non-susceptibility in this study. In the current study, exposure to metaphylactic macrolide products likely caused sufficient pressure on the populations of bacteria to either express resistance genes or led to a competitive advantage for growth of bacteria carrying resistance genes. Exposure to low levels of tylosin phosphate may also have contributed; however, previous work has shown that the presence of tylosin phosphate at sub-therapeutic levels in feedlot rations does not cause a reduction in antimicrobial susceptibility.[25]

The current study observed a significant decrease in the susceptibility of *M. haemolytica* isolates post-arrival/post-exposure to macrolides, for both tilmicosin ($P < 0.01$) and tulathromycin ($P = 0.01$). While it is not unexpected to find decreased susceptibility post-exposure to an antibiotic, the decrease in susceptibility to both tulathromycin and tilmicosin does indicate phenotypic evidence for the presence of gene(s) conferring resistance to multiple macrolides. This evidence is further observed by the numerical decrease in the proportion of susceptible isolates for tildipirosin.

Pasteurella multocida isolates had only 1 statistically significant ($P = 0.04$) reduction in susceptibility, which was to tildipirosin; however, a numerical reduction in susceptibility to the 3 other macrolides was also observed. It should be noted that there were relatively few *P. multocida* isolates post-arrival, and this likely reduced the power of this portion of the study. Wide confidence intervals among the susceptibility results for all 3 bacteria studied in the post-arrival period, and also for *H. somni* in the arrival period, indicate a weakness in power and a lack of reliability of the mean proportions. The low power may also account for the observance of no statistical significant differences in antimicrobial susceptibility for the *H. somni* isolates tested.

Consistent high susceptibility to gamithromycin of isolates from all 3 bacteria studied is of interest. While the presence of a gene conferring non-susceptibility to 3 of the 4 macrolides studied may be speculated, it is possible that the high susceptibility to 1 macrolide over 3 others is the result of true differences between drugs within the macrolide class, test error or incorrect breakpoints being used. The breakpoints being used are CLSI approved and were recently established, therefore this should be unlikely.

Multiple antimicrobial non-susceptibility has been reported in previous studies.[13,20,24] This study found that the proportion of *H. somni*, *M. haemolytica*, and *P. multocida* isolates non-susceptible to 2 or more antimicrobials was 0%, 3%, and 2%, respectively, in the arrival period, and 5%, 5%, and 0%, respectively, in the post arrival period (Table 5). These results show low multiple antimicrobial non-susceptibility rates for all 3 bacteria. Only *H. somni* and *M. haemolytica* isolate's multiple antimicrobial non-susceptibility rates increased over the study period, and this was due to a reduction in susceptibility to the macrolide class of antimicrobials (Table 2). A general decrease in macrolide susceptibility is observed for all 3 bacteria studied, and this may be due to metaphylactic exposure to a macrolide antimicrobial upon arrival. Feedlot practitioners should note the reduced multiple macrolide susceptibility of these isolates as this indicates that it may be prudent to choose non-macrolide antimicrobials for treatment if the cattle received a macrolide upon arrival. This observation is tempered with the fact that these samples were from DNS samples of healthy cattle, and therefore may not be indicative of susceptibility patterns of bacteria cultured from the lungs of clinically ill animals.

Multiple non-susceptibility has been observed previously for *M. haemolytica*; however, the magnitude of the differences were much larger. Noyes et al[19] found 3.8% of *M. haemolytica* isolates showed multiple non-susceptibility at arrival, increasing to 7.8% post-arrival. The current study found a much lower rate of post-arrival multiple non-susceptibility, which was likely due to differences in analysis. The Noyes et al[19] study differs from the current study as they observed the susceptibility of 21 antimicrobials as compared to 8 in the current study. As well, Noyes et al did not indicate whether susceptibility was observed based on a family level or on an individual drug basis; these differences between the 2 studies may account for the differences in the results.

Pan-susceptibility was also observed in both the Noyes et al[19] and the current study; however, Noyes et al[19] reported a pan-susceptibility rate of 87.8% for all *M. haemolytica* isolates, arrival and post-arrival, together. The current study had a similar rate for *M. haemolytica* at arrival (85%), but this rate dropped to 67% post-arrival, indicating an increased frequency of non-susceptibility. The decrease in pan-susceptibility in the current study was due to an increase in macrolide non-susceptibility, which is likely due to macrolide exposure upon arrival.

Non-susceptibility to macrolides has been reported in the literature since the approval of the first long-acting macrolide, tilmicosin. A multi-year susceptibility study began 1 year prior to approval of tilmicosin, and observed US and Canadian *M. haemolytica*, *P. multocida*, and *H. somni* isolates from laboratory samples of animals that died acutely from BRD.[23] Within the first year of tilmicosin observation, the susceptibility rates were 82.8%, 80.6%, and 90.9% for *M. haemolytica*, *P. multocida*, and *H. somni*, respectively; however the susceptibility rates were variable over the 3-year

study, and the 3-year average rates were 69.1%, 58.9%, and 90.4%.[23] The trend observed in the current study was quite different, as tilmicosin was observed to have the lowest susceptibility rates for *H. somni* as opposed to the other 2 bacteria. It is interesting that the authors of the previous study noted that erythromycin use may have contributed to the early diminished susceptibility rates to tilmicosin, and speculated that non-use of erythromycin in Canada resulted in an observation of greater (90 to 100%) susceptibility of the isolates collected. This previous study may represent the first consideration of macrolide use resulting in multiple macrolide non-susceptibility.

The current study agrees with numerous previous studies that have demonstrated an increase in multiple non-susceptibility, and trends for decreasing susceptibility to macrolides.[1,20,24] The majority of the previous work studied the susceptibility trends for *M. haemolytica* and *P. multocida*, and little published work could be found with reference to susceptibility trends for *H. somni*. Most work does indicate a trend for increasing minimum inhibitory concentrations and a decrease in phenotypic susceptibility for numerous antimicrobials. This growing body of literature indicates that reduction of BRD-related pathogens' susceptibility to antimicrobials will continue, and that non-antimicrobial based management strategies for control of BRD should become the basis for control of this disease in the future.

Conclusion

The current study found a statistically significant decrease, over time, in the proportion of healthy calves culture-positive for *P. multocida* from DNS swabs at arrival and post-arrival. The study also found a statistically significant increase, over time, in the proportion of calves culture-positive for *H. somni*, and no difference in proportion of positive-culture for *M. haemolytica.* Overall, a trend for decreased pan-susceptibility to the antimicrobials was observed along with a decrease in the number of isolates susceptible to some macrolides. The reduction in susceptibility to some macrolides is likely the result of exposure to a macrolide through metaphylaxis. *Histophilus somni* was found to have poor susceptibility to 3 of 4 macrolides tested and 100% susceptibility to gamithromycin, in both the arrival and post-arrival periods, indicating probable resistance gene presence in these isolates. The presence of resistance genes should be explored further.

Endnotes

[a]Bovi-shield Gold One Shot®, Zoetis Canada Inc., Kirkland QC, Canada
[b]Ultrabac®7 Somnubac®, Zoetis Canada Inc., Kirkland QC, Canada
[c]Draxxin®, Zoetis Canada Inc., Kirkland QC, Canada
[d]Component® TE-G, Elanco, Division Eli Lilly Canada, Inc., Guelph, ON, Canada
[e]Bimectin®, Bimedia-MTC Animal Health Inc., Cambridge, ON, Canada
[f]Tylosin 40 Premix, Bio Agri Mix LP, Mitchell, ON, Canada
[g]Reproduction Resources, Walworth, WI, USA
[h]Oxoid, Nepean, ON, Canada
[i]Bruker Daltonics Ltd. East Milton, ON, Canada
[j]BD, Sparks, MD, USA
[k]Todd Hewitt Broth, Thermo Fisher Scientific
[l]Stata 13 for Windows, StataCorp LP, College Station, TX, USA

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