Article

Antimicrobial resistance in bovine respiratory disease: Auction market- and ranch-raised calves

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Abstract — This study compared changes in prevalence and antimicrobial susceptibility of *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* in feedlot calves derived from the auction market (AUCT; *n* = 299) and from a single-ranch source (RANCH; *n* = 300). In the AUCT calves, the prevalence of *Mannheimia haemolytica* decreased, whereas *Histophilus somni* increased over the feeding period. The AUCT calves showed an increase in isolates not susceptible to tulathromycin for all bovine respiratory disease (BRD) pathogens, an increase in *Pasteurella multocida* and *Histophilus somni* isolates not susceptible to oxytetracycline, and an increase in *Pasteurella multocida* isolates not susceptible to florfenicol. In the RANCH calves, the prevalence of all 3 BRD pathogens was high at feedlot entry and decreased significantly during the study period. In RANCH calves, there was a significant increase in *Pasteurella multocida* isolates not susceptible to oxytetracycline, tulathromycin, and florfenicol. Surprisingly, there was a significant decrease in *Mannheimia haemolytica* isolates that were not susceptible to oxytetracycline, tilmicosin, and tulathromycin.

Résumé — Résistance aux antimicrobiens lors de maladies respiratoires bovines : veaux provenant de marché aux enchères et ceux élevés en ranch. Cette étude a comparé les changements dans la prévalence et la sensibilité aux antimicrobiens de *Mannheimia haemolytica, Pasteurella multocida* et *Histophilus somni* isolés de veaux en parc d'engraissement provenant du marché aux enchères (AUCT; *n* = 299) et d'un seul ranch (RANCH; *n* = 300). Chez les veaux AUCT, la prévalence de *M. haemolytica* a diminué, tandis que celle d'*H. somni* a augmenté au cours de la période d'alimentation. Les veaux AUCT ont montré une augmentation des isolats non sensibles à la tulathromycine pour tous les agents pathogènes des maladies respiratoires bovines (BRD), une augmentation des isolats de *P. multocida* et *H. somni* non sensibles à l'oxytétracycline, et une augmentation des isolats de *P. multocida* non sensibles au florfénicol. Chez les veaux du RANCH, la prévalence des 3 agents pathogènes BRD était élevée à l'entrée du parc d'engraissement et a diminué de manière significative au cours de la période d'étude. Chez les veaux RANCH, il y a eu une augmentation significative des isolats de *P. multocida* non sensibles à l'oxytétracycline, à la tulathromycine et au florfénicol. Étonnamment, il y a eu une diminution significative des isolats de *M. haemolytica* qui n'étaient pas sensibles à l'oxytétracycline, à la tilmicosine et à la tulathromycine.

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Introduction

Bovine respiratory disease (BRD) is the leading cause of sickness and death in feedlot cattle in North America (1), and a lack of effective antimicrobials has the potential to further increase that impact. The available research on antimicrobial resistance in cattle on arrival at a feedlot is limited, but growing (1–4). A lack of effective antimicrobials to treat BRD could have serious consequences on cattle welfare and affect the sustainability of cattle production. Understanding how resistant BRD bacteria are entering the feedlot and why resistance

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is increasing is important to improving cattle health and welfare.

Increasing resistance to antimicrobials has been reported in the literature for the major BRD pathogens: *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* (1,5–8). Resistance to tetracyclines is the most common, particularly in *M. haemolytica* and *P. multocida,* with multiple studies since 1988 showing that < 50% of isolates are susceptible to tetracycline (5–7,9). The next most common resistance is to macrolide antimicrobials, including tilmicosin, gamithromycin, tulathromycin, and tildipirosin. Susceptibility to drugs in this class has been steadily decreasing since 1990 when tilmicosin, the first macrolide available for livestock in Canada, was introduced (6,9). For the most part, susceptibility of the major BRD pathogens to florfenicol, the fluoroquinolones, and ceftiofur remains high, most often between 90 and 100% (6,7,9,10).

As antimicrobials become less effective, an increased focus on alternatives will be required to maintain animal health and welfare. Improved diagnostic procedures, more effective vaccines, and alternative treatment modalities are areas that are being explored (11,12). Another way to potentially reduce the risk of BRD in feedlot calves, is by acquiring calves for the feedlot directly from a known single source instead of multiple unknown sources. Step et al (13) showed that single-source calves were less likely to be treated for BRD than auction market-derived calves, and calves that were weaned 45 d before feedlot entry also were less likely to require treatment. In a large Canadian study, Ribble et al (14) demonstrated that mixing and commingling of calves from multiple ranches at the auction market increased the risk of fatal pneumonia in the feedlot. Acquiring calves from a single source can be a challenge in that Canada has over 72 000 (15) beef cattle farms that funnel cattle into a much smaller number of feedlots, resulting in a large amount of mixing (14). Making strategic management decisions on the source of calves being purchased and how much commingling those calves have experienced seems to be an important factor in the development of BRD.

The objective of this study was to describe the prevalence and antimicrobial susceptibility of 3 major bovine respiratory pathogens *(Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni)* in calves derived from the auction market and those from a single source. A secondary objective was to compare the prevalence and antimicrobial susceptibility of the BRD pathogens in these calves at arrival and again later in the feeding period.

Materials and methods

Ethics statement

The research protocol was reviewed and approved by the University of Saskatchewan Animal Care Committee, AUP #20140003.

Animals

Recently weaned steers and heifers of various mixed beef breeds were used for this study. The study was conducted at a commercial feedlot with an 8000-head one-time capacity. The feedlot typically buys auction market-derived, recently weaned calves each fall. The feedlot also feeds calves from its own commercial cow-calf herd. A sample of 299 auction-market derived calves (AUCT) and 300 calves from the abovementioned commercial cow-calf herd (RANCH) were sampled on feedlot arrival. The calves all entered the feedlot in November and December 2017.

Study design

Each calf had a deep nasopharyngeal swab taken at the time of processing on entry to the feedlot (first test) by the same person (Wennekamp). The double-guarded swab (Reproduction Provisions LLC, Walworth, Wisconsin, USA) was advanced up the ventral meatus of the nose to the level of the medial canthus of the eye, a polystyrene cotton-tipped swab was fully advanced into the deep nasopharynx, and the swab was swirled on the mucosa approximately 6 to 10 times, as described elsewhere (3). Samples were collected from every 2nd or 3rd calf through the chute at processing, depending on group size, in order to collect approximately 100 samples per day. Samples were collected in AUCT calves from 5 separate management groups, with the number of samples collected ranging from 13 to 102. By looking for unique numbers in the first 6 digits of the radio frequency identification (RFID) tags, this represents a minimum of 170 different herds of origin. Samples were collected in RANCH calves from 2 large groups, one of heifers and one of steers, with 150 samples from each group.

The samples were placed into Amies media (Copan Diagnostics, Marrieta, California, USA) and transported overnight in cooled containers to Prairie Diagnostic Services laboratory in Saskatoon, Saskatchewan. Bacterial cultures were initiated the following morning by inoculating 5% Columbia sheep blood and Chocolate agar plates with the swab tips followed by incubation. Plates were incubated at 35°C for 24 h in an environment containing 5% CO_2 for isolation of *H. somni, M. haemolytica,* and *P. multocida.* 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 24 and 48 h of incubation. The microorganisms of interest were identified using a Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) system (Bruker Daltonics, Billerica, Massachusetts, USA). Briefly, individual bacterial colonies were transferred onto a stainless steel MALDI-TOF MS target in duplicate. Each target spot was overlaid with 1 μL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix, and the mass spectra were acquired using a MALDI-TOF MS Microflex LT system (Bruker Corporation, Billerica, Massachusetts, USA) in a linear positive mode. Instrument calibration was performed using Bruker Bacterial Test Standard (Bruker Corporation). For bacterial identification, Bruker Compass v.3.1.66 software with BDAL library was used. Only isolates that positively identified with scores ≥ 2.0 were included in this study. Confirmed isolates of *M. haemolytica, P. multocida,* and *H. somni* were purified and cryopreserved at −80°C for later antimicrobial susceptibility testing, as described elsewhere (3).

Antimicrobial susceptibility testing was performed by Sensititre (ThermoFisher Scientific, Nepean, Ontario) serial broth microdilution method using a commercially available BOPO6F plate (ThermoFisher Scientific). These plates are

designed to test for minimum inhibitory concentration (MIC) of the following antimicrobials: ampicillin, ceftiofur, chlortetracycline, clindamycin, danofloxacin, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, spectinomycin, sulphadimethoxine, tiamulin, tilmicosin, trimethoprim/sulfamethoxazole, tylosin tartrate, and tulathromycin. Briefly, the pure bacterial isolate colony was suspended in a Sensititre Cation Adjusted Muller-Hinton Broth w/TES (ThermoFisher Scientific) to achieve a 0.5 McFarland turbidity as measured by the Sensititre Nephelometer (ThermoFisher Scientific). For *M. haemolytica* and *P. multocida,* dosing broth was prepared by adding 10 μL of the suspension to 11 mL of a Sensititre Cation Adjusted AutoRead Muller-Hinton Broth w/TES w/Lysed Horse Blood (ThermoFisher Scientific). For *H. somni,* dosing broth was prepared by adding 50 μL of the suspension to 11 mL of a Sensititre Veterinary Fastidious Medium (ThermoFisher Scientific). BOPO6F plates were immediately inoculated by adding 50 μL of the dosing broth into each well using the Sensititre AIM Automated Inoculation System (ThermoFisher Scientific). For *M. haemolytica* and *P. multocida,* plates were sealed with a non-perforated adhesive seal and incubated for 24 h at 35°C in a regular aerobic environment. For *H. somni,* plates were sealed with a perforated adhesive seal and incubated for 24 h at 35°C in an environment containing 5% CO_2. The MICs were determined using a BioMic V3 system (Giles Scientific, Santa Barbara, California, USA) and a manual mirror box confirmation if required to observe the lowest concentration of antimicrobial agent that completely inhibits growth of the organism (16,17).

Isolates were categorized as resistant to an antimicrobial according to MIC defined by the Clinical and Laboratory Standards Institute (CLSI) for ampicillin (≥ 0.25 μg/mL), ceftiofur (≥ 8 μg/mL), enrofloxacin (≥ 2 μg/mL), danofloxacin (≥ 1 μg/mL, for *M. haemolytica* and *P. multocida*), florfenicol (≥ 8 μg/mL), penicillin (≥ 1 μg/mL), oxytetracycline (≥ 8 μg/mL), tilmicosin (≥ 32 μg/mL, for *M. haemolytica*), and tulathromycin (≥ 64 μg/mL) (18). The CLSI breakpoints were not available for chlortetracycline, danofloxacin (for *H. somni*), gentamycin, spectinomycin, tiamulin, tilmicosin (for *P. multocida* and *H. somni*), trimethoprim/sulfamethoxazole and neomycin; therefore, these antimicrobials were not included in the analysis.

Both AUCT and RANCH calves were treated on arrival with a subcutaneous injection of Draxxin, a long-acting macrolide compound containing tulathromycin, at a dose of 2.5 mg/kg to control BRD (Zoetis, Kirkland, Quebec), as per standard procedures at this feedlot. Calves were then weighed and vaccinated against infectious bovine herpes virus-1 (BHV-1), bovine viral diarrhea virus (BVDV; types I and II), bovine parainfluenza-3 (PI3), bovine respiratory syncytial virus (BRSV) and *M. haemolytica* (Bovishield Gold One Shot; Zoetis), 2 cc SQ, as well as *H. somni* and clostridial pathogens (Vision 8 Somnus; Merck Animal Health, Kirkland, Quebec), 2 cc SQ. The calves were also treated with an anthelminthic (Bimectin; Bimeda-MTC, Cambridge, Ontario). In addition, auction market-derived heifers received cloprostenol (Estrumate; Merck Animal Health), 1 mg, IM to induce abortion.

Table 1. Comparison of positive isolates on deep nasopharyngeal swabs for each bovine respiratory disease pathogen at first test on arrival at the feedlot between auction market-derived and ranch-raised calves.

Positive isolates	Auction market calves *n* = 299 (%)	Ranch-raised calves *n* = 300 (%)	*P*-value
M. haemolytica	39.5%	29.7%	0.57
P. multocida	28.1%	60.0%	< 0.001
H. somni	14.8%	38.7%	0.02

Steers and heifers were housed separately and fed in large outdoor dirt-floor pens in groups of 100 to 250. The study cattle remained in the larger groups in which they entered the feedlot; that is, AUCT cattle and RANCH cattle did not mix. All cattle received 2 treatments of chlortetracycline (aureomycin; Zoetis, Kirkland, Quebec), 6 g daily in the feed for 5 d within the first 28 d on feed (DOF) as per standard feedlot procedure. Calves were identified by radio frequency ear tag.

A second deep nasalpharyngeal swab was collected from enrolled calves (second test) as permitted by routine feedlot operation. Calves with both a first and second test sample collected were considered test matched. The AUCT calves were sampled at the time of another vaccination against BHV-1 and PI3 (Bovi-shield IBR-Pi3; Zoetis). This was an average of 76 d after the first swab. A total of 217 AUCT calves were resampled. The RANCH calves did not receive a follow-up vaccination and there was a change in feedlot protocol which delayed resampling until an average of 153 d after the first swab. A total of 279 RANCH calves were resampled. Collection, shipping, and processing was the same as described above for the first test. Eighty-two AUCT and 21 RANCH calves were dropped from the study due to death (AUCT = 10, RANCH = 5), or being sold from the feedlot (AUCT = 72, RANCH = 16).

Statistical analysis

Data were collected for each calf on the BRD pathogens prevalence *(M. haemolytica, P. multocida,* and *H. somni)*. Based on the available CLSI breakpoints for each antimicrobial, BRD pathogens were classified as susceptible, resistant, or intermediate. These data were entered into a spreadsheet (Excel 2017; Microsoft, Redmond, Washington, USA), then imported into a statistical software package (Stata/IC version 15.1, Stata, College Station, Texas, USA) for analysis. Isolates that were classified as intermediate or resistant were grouped together and termed not susceptible, as compared to susceptible isolates. The prevalence of BRD pathogens and susceptibility data were summarized in 2 ways: i) the prevalence of positive BRD isolates and isolates that were not susceptible to antimicrobials; and ii) calves as matched pairs from the first to the second test.

The AUCT and RANCH calves were compared at first test for the prevalence of positive isolates for the 3 BRD pathogens, and the prevalence of isolates and calves not sensitive to each antimicrobial for the 3 BRD pathogens using random effects logistic regression; accounting for clustering within management group. The prevalence of calves with positive isolates for each of the 33 BRD pathogens and calves with isolates not sensitive to each antimicrobial for the 3 BRD pathogens were compared

Table 2. Positive isolates on deep nasopharyngeal swabs in auction market-derived and ranch-raised calves for each of the bovine respiratory disease pathogens *M. haemolytica, P. multocida,* and *H. somni,* both on arrival at the feedlot (first test) and later in the feeding period (second test), looking at matched pairs only.

M. haemolytica results	Positive *M. haemolytica* isolates on first test (%)	Positive *M. haemolytica* isolates on second test (%)	*P*-value for matched calves[a]
AUCT calves (n = 217)	37.8%	20.3%	< 0.001
RANCH calves (n = 279)	29.4%	20.4%	0.011
P. multocida results	Positive *P. multocida* isolates on first test (%)	Positive *P. multocida* isolates on second test (%)	
AUCT calves (n = 217)	30.9%	23.5%	0.07
RANCH calves (n = 279)	60.0%	43.4%	< 0.001
H. somni results	Positive *H. somni* isolates on first test (%)	Positive *H. somni* isolates on second test (%)	
AUCT calves (n = 217)	17.1%	30.4%	0.001
RANCH calves (n = 279)	39.8%	6.1%	< 0.001

[a] Calves with both a first test and a second test sample collected were designated as test matched.

from the first test to the second test. Random effects logistic regression was used, accounting for repeated measures within individual calves and clustering of calves within management group for AUCT calves and then for RANCH calves.

Results

Prevalence of positive isolates of *M. haemolytica, P. multocida,* and *H. somni*

At the first test, there was not a significant difference in culture prevalence for *M. haemolytica* in AUCT calves at 39.5% compared to 29.7% for RANCH calves (P = 0.57; Table 1). However, the RANCH calves had higher culture prevalence for *P. multocida* and *H. somni* at 60.0% ($P < 0.001$) and 38.7% (P = 0.02) respectively compared to 28.1% and 14.8% for the AUCT calves. In the AUCT calves, prevalence of positive isolates of *M. haemolytica* decreased significantly from 37.8% at the first test to 20.3% at the second test ($P < 0.001$; Table 2). There was no significant change in *P. multocida* culture prevalence from the first to second tests in AUCT calves, but positive *H. somni* isolates increased significantly from 17.1 to 30.4% (P = 0.001). From the first to the second test, positive culture prevalence dropped significantly for all 3 pathogens in the RANCH calves (Table 2). Prevalence dropped from 29.4 to 20.4% for *M. haemolytica* (P = 0.011), from 60.0 to 43.4% for *P. multocida* ($P < 0.001$), and from 39.8 to 6.1% for *H. somni* ($P < 0.001$).

Antimicrobial susceptibility of *M. haemolytica, P. multocida,* and *H. somni* isolates to commonly used antimicrobials

In the AUCT calves at the first test, 26.7% of the *M. haemolytica* isolates were not susceptible to oxytetracycline and 27.6% were not susceptible to tilmicosin (Table 3). The RANCH calves had similar results on arrival with 20.3% of *M. haemolytica* isolates not susceptible to oxytetracycline and 19.0% not susceptible to each of tilmicosin and tulathromycin. In AUCT calves there was a significant increase in calves not susceptible to tulathromycin on the second test; from 1.4 to 7.4% (P = 0.006). In the RANCH calves, non-susceptible isolates of *M. haemolytica* decreased significantly by the second test from 5.7 to 0.4% for oxytetracycline (P = 0.005), and from 5.4 to 0% for both tilmicosin and tulathromycin (P = 0.001).

For the *P. multocida* isolated from AUCT calves, prevalence of non-susceptible bacteria was relatively low in the first test (Table 4). All isolates were susceptible to florfenicol and tulathromycin, with only a few isolates not susceptible to oxytetracycline. The pattern was similar in the RANCH calves for *P. multocida*, with only a few non-susceptible isolates to each antimicrobial (Table 4). In the AUCT calves for the second test, the prevalence of calves with *P. multocida* isolates not susceptible to florfenicol increased significantly from 0 to 2.8% (P = 0.05). Similarly, non-susceptible oxytetracycline and tulathromycin isolates prevalence in calves increased from 0.5 to 3.7% (P = 0.047) and 0 to 3.2% (P = 0.05), respectively. RANCH calves also had significant increases in non-susceptible *P. multocida* from the first to the second test: from 0.4 to 9.0% in the case of florfenicol (P = 0.001), from 0.4 to 10.0% for oxytetracycline (P = 0.001), and from 0.4% to 9.7% for tulathromycin (P = 0.001).

Finally, for *H. somni*, all the isolates were susceptible to florfenicol on the first and second tests for AUCT and RANCH calves (Table 5). In AUCT calves on the first test, all the *H. somni* isolates were susceptible to oxytetracycline and tulathromycin. However, by the second test, 11.5% of calves had *H. somni* isolates that were not susceptible to oxytetracycline (P = 0.001) and 9.2% were not susceptible to tulathromycin (P = 0.002). In the RANCH calves on the first test, all the *H. somni* isolates were susceptible to oxytetracycline and tulathromycin. There was no significant increase in calves with non-susceptible *H. somni* isolates by the second test in the RANCH calves.

Comparison of antimicrobial susceptibility of *M. haemolytica, P. multocida,* and *H. somni* isolates on arrival at the feedlot (first test)

There was a significantly higher prevalence of AUCT calves compared to RANCH calves with *M. haemolytica* isolates on the first

Table 3. Prevalence of *M. haemolytica* isolates not susceptible to antimicrobials on arrival at the feedlot (first test) and later in the feeding period (second test) (isolates identified as intermediate or resistant are classified as not susceptible) and calves matched (first test to second test).

Auction market calves	*M. haemolytica* isolates not susceptible on first test (%) (n = 116)	*M. haemolytica* isolates not susceptible on second test (%) (n = 44)	Prevalence of matched calves with *M. haemolytica* isolates not susceptible on first test (%) (n = 217)	Prevalence of matched calves with *M. haemolytica* isolates not susceptible on second test (%) (n = 217)	*P*-value for matched calves[a]
Ampicillin	0%	0%	0%	0%	—
Ceftiofur	0%	0%	0%	0%	—
Danofloxacin	0%	0%	0%	0%	—
Enrofloxacin	0%	0%	0%	0%	—
Florfenicol	0%	0%	0%	0%	—
Oxytetracycline	26.7%	52.3%	10.6%	10.6%	0.99
Penicillin	1.7%	2.3%	0%	0.5%	0.99
Tilmicosin	27.6%	50%	11.1%	10.1%	0.76
Tulathromycin	4.3%	36.4%	1.4%	7.4%	0.006

Ranch-raised calves	*M. haemolytica* isolates not susceptible on first test (%) (n = 79)	*M. haemolytica* isolates not susceptible on second test (%) (n = 57)	Prevalence of matched calves with *M. haemolytica* isolates not susceptible on first test (%) (n = 279)	Prevalence of matched calves with *M. haemolytica* isolates not susceptible on second test (%) (n = 279)	*P*-value for matched calves[a]
Ampicillin	0%	0%	0%	0%	—
Ceftiofur	0%	0%	0%	0%	—
Danofloxacin	0%	0%	0%	0%	—
Enrofloxacin	1.3%	0%	0.4%	0%	0.99
Florfenicol	0%	0%	0%	0%	—
Oxytetracycline	20.3%	1.8%	5.7%	0.4%	0.005
Penicillin	0%	8.8%	0%	1.8%	0.06
Tilmicosin	19.0%	0%	5.4%	0%	0.001
Tulathromycin	19.0%	0%	5.4%	0%	0.001

[a] Calves with both a first test and a second test sample collected were designated as test matched.

test that were not susceptible to oxytetracycline (P = 0.02) and tilmicosin (P = 0.01) (Table 6). When looking at tulathromycin, there was a significantly higher prevalence of RANCH calves with non-susceptible *M. haemolytica* isolates than AUCT calves (P = 0.04). For both *P. multocida* and *H. somni,* there were no significant differences in the first test between the AUCT and RANCH calves neither in prevalence nor antimicrobial susceptibility of the colonizing pathogens.

Crude mortality in the AUCT group was 3.3% and in the RANCH group it was 1.7%, 14.4% of AUCT calves and 9.0% of RANCH calves were treated for BRD. For further information on the effect of treatment in this study see "Biosecurity and bovine respiratory disease on beef operations in western Canada" (19).

Discussion

To the authors' knowledge, this is the first study to look specifically at prevalence of BRD pathogens and their antimicrobial susceptibility in ranch-raised calves compared to auction market-derived calves. Most of the studies reported in the literature examined only auction market-derived cattle (3,13,20). One of the objectives of this study was to determine the effects of mixing cattle (AUCT calves) on the prevalence of BRD pathogens isolated and the antimicrobial sensitivity of those pathogens.

At 39.5%, this study has identified a higher prevalence of *M. haemolytica* isolates in auction market calves on arrival than has been seen in previous studies, in which prevalence ranged from 13 to 30% (3,21–23). At 29.7%, the prevalence of *M. haemolytica* isolates for RANCH calves is in close agreement with what has been recovered from auction market calves in other studies. The significant decrease in *M. haemolytica* culture prevalence for both AUCT and RANCH calves in the current study is not consistent with findings in other studies (3,22). One reason for this could be the timing of the second sample collection. Particularly in the RANCH calves, the samples were collected further along in the feeding period at an average of 153 d. Other studies have shown that isolation rates of *M. haemolytica* are higher when animals are affected with BRD; which is typically earlier in the feeding period (22,23). As well, some of the animals affected with BRD may be removed from the study due to death.

For *P. multocida* isolates, there was a prevalence of 28.1% on arrival for AUCT calves, similar to previous work (3,23). The culture prevalence of *P. multocida* in the RANCH calves on arrival in the current study is much higher at 60.0%. A similar pattern was observed for *H. somni* isolates on the first test. For AUCT calves on arrival, *H. somni* culture prevalence at 14.8% was similar to another study reporting 9% (3). Again,

Table 4. Prevalence of *P. multocida* isolates not susceptible to antimicrobials on arrival at the feedlot (first test) and later in the feeding period (second test) (isolates identified as intermediate or resistant are classified as not susceptible) and calves matched (first test to second test).

Auction market calves	*P. multocida* isolates not sensitive on first test (%) (n = 82)	*P. multocida* isolates not sensitive on second test (%) (n = 51)	Prevalence of matched calves with *P. multocida* isolates not sensitive on first test (%) (n = 217)	Prevalence of matched calves with *P. multocida* isolates not sensitive on second test (%) (n = 217)	*P*-value for matched calves[a]
Ampicillin	1.2%	5.9%	0.5%	1.4%	0.34
Ceftiofur	0%	0%	0%	0%	—
Danofloxacin	0%	0%	0%	0%	—
Enrofloxacin	0%	0%	0%	0%	—
Florfenicol	0%	11.8%	0%	2.8%	0.05
Oxytetracycline	1.2%	15.7%	0.5%	3.7%	0.047
Penicillin	0%	0%	0%	0%	—
Tulathromycin	0%	13.7%	0%	3.2%	0.05
Ranch-raised calves	***P. multocida* isolates not susceptible on first test (%) (n = 176)**	***P. multocida* isolates not susceptible on second test (%) (n = 121)**	**Prevalence of matched calves with *P. multocida* isolates not susceptible on first test (%) (n = 279)**	**Prevalence of matched calves with *P. multocida* isolates not susceptible on second test (%) (n = 279)**	***P*-value for matched calves[a]**
Ampicillin	0.6%	0%	0.4%	0%	0.99
Ceftiofur	0%	0%	0%	0%	—
Danofloxacin	0.6%	0.8%	0.4%	0.4%	0.99
Enrofloxacin	0%	0.8%	0%	0.4%	0.99
Florfenicol	1.7%	20.7%	0.4%	9.0%	0.001
Oxytetracycline	1.7%	23.1%	0.4%	10.0%	0.001
Penicillin	0%	0%	0%	0%	—
Tulathromycin	1.7%	22.3%	0.4%	9.7%	0.001

[a] Calves with both a first test and a second test sample collected were designated as test matched.

the RANCH calves had significantly higher culture prevalence of *H. somni* than the AUCT calves on arrival at 38.7%. The reason for this is not clear. The RANCH calves in this study had no previous exposure to calves or cows outside of their herd before entry into the feedlot. It is possible the herd from which the RANCH calves originated had a high level of BRD pathogens circulating within the herd. This herd had the same ownership as the feedlot and does have some contact during the year, such as use of facilities for pregnancy testing and shared feeding and bedding equipment. The harboring of BRD pathogens in adult cattle in a herd and their movement from dam to calf is an area needing further research. The AUCT calves had a significant increase in *H. somni* isolates to 30.4% at the time of the second test. In the RANCH cattle, prevalence of *P. multocida* and *H. somni* cultures dropped significantly on the second test. This could be from the RANCH calves having second samples taken later in the feeding period. Culture prevalence of *P. multocida* and *H. somni* were high at the first test in RANCH calves and this may have affected the second test results. Other research has also shown variations; whether the prevalence of these BRD pathogens increase or decrease does not seem to be consistent (3,23). Stroebel et al (20) showed that spending 24 h in an auction market did not increase the rates of these same respiratory pathogens being isolated from calves, and those authors suggested that BRD pathogens are not very transmissible between calves.

Antimicrobial resistance in *M. haemolytica* samples of auction market-derived calves on arrival at the feedlot is still relatively uncommon. *Mannheimia haemolytica* most commonly has resistance to tetracyclines, with studies showing 3 to 5% of *M. haemolytica* isolates resistant in auction market calves (22,24). The current study revealed that 26.7% of *M. haemolytica* isolates from AUCT calves were not susceptible to oxytetracycline on arrival. This is much higher than what is reported in other studies. It could be that the prevalence of resistant bacteria is increasing over time or that some of the cattle sampled in this study had been previously treated with tetracyclines. Interestingly, the RANCH calves had 20.3% of *M. haemolytica* isolates not susceptible to oxytetracycline; also much higher than reported elsewhere (4). The current study demonstrated that 27.6% of *M. haemolytica* isolated from AUCT calves and 19% from RANCH calves were not susceptible to tilmicosin. In contrast, Ericksen et al (3) determined only 1% of *M. haemolytica* samples resistant to tilmicosin in auction market calves on arrival, and other studies have shown similarly low levels of resistance in feedlot cattle (22,24). Two recent studies in the United States have reported higher levels of resistance to tilmicosin, in the 19 to 20% range, on arrival to the feedlot in small groups (1,2). As tilmicosin and the other macrolides are used extensively for metaphylaxis, this is an area of concern.

Many studies have shown an increase in antimicrobial resistance after cattle have been in the feedlot for a period; although

Table 5. Prevalence of *H. somni* isolates not susceptible to antimicrobials on arrival at the feedlot (first test) and later in the feeding period (second test) (isolates identified as intermediate or resistant are classified as not susceptible) and calves matched (first test to second test).

Auction market calves	*H. somni* isolates not susceptible on first test (%) (n = 44)	*H. somni* isolates not susceptible on second test (%) (n = 66)	Prevalence of calves with *H. somni* isolates not susceptible on first test (%) (n = 217)	Prevalence of calves with *H. somni* isolates not susceptible on second test (%) (n = 217)	*P*-value for matched calves[a]
Ampicillin	2.3%	1.5%	0.5%	0.5%	0.99
Ceftiofur	0%	0%	0%	0%	—
Enrofloxacin	0%	0%	0%	0%	—
Florfenicol	0%	0%	0%	0%	—
Oxytetracycline	0%	37.9%	0%	11.5%	0.001
Penicillin	2.3%	1.5%	0.5%	0.5%	0.99
Tulathromycin	0%	30.3%	0%	9.2%	0.002
Ranch-raised calves	***H. somni* isolates not susceptible on first test (%) (n = 102)**	***H. somni* isolates not susceptible on second test (%) (n = 17)**	**Prevalence of matched calves with *H. somni* isolates not susceptible on first test (%) (n = 279)**	**Prevalence of matched calves with *H. somni* isolates not susceptible on second test (%) (n = 279)**	***P*-value for matched calves[a]**
Ampicillin	0%	0%	0%	0%	—
Ceftiofur	0%	0%	0%	0%	—
Enrofloxacin	0%	0%	0%	0%	—
Florfenicol	0%	0%	0%	0%	—
Oxytetracycline	0%	11.8%	0%	0.7%	0.57
Penicillin	0%	0%	0%	0%	—
Tulathromycin	0%	11.8%	0%	0.7%	0.57

[a] Calves with both a first test and a second test sample collected were designated as test matched.

Table 6. Prevalence of *M. haemolytica* isolates and calves not susceptible to antimicrobials on arrival at the feedlot (first test) (samples classified as intermediate or resistant are classified as not susceptible).

M. haemolytica isolates	AUCT calves: prevalence of isolates not susceptible (%) (n = 116)	RANCH calves: prevalence of isolates not susceptible (%) (n = 79)	*P*-value	AUCT calves: prevalence of calves with not susceptible isolates (%) (n = 299)	RANCH calves: prevalence of calves with not susceptible isolates (%) (n = 300)	*P*-value
Ampicillin	0%	0%	—	0%	0%	—
Ceftiofur	0%	0%	—	0%	0%	—
Danofloxacin	0%	0%	—	0%	0%	—
Enrofloxacin	0%	1.3%	0.41	0%	0.3%	0.99
Florfenicol	0%	0%	—	0%	0%	—
Oxytetracycline	26.7%	20.3%	0.31	10.4%	5.3%	0.02
Penicillin	1.7%	0%	0.52	0.7%	0%	0.25
Tilmicosin	27.6%	19.0%	0.18	10.7%	5.0%	0.01
Tulathromycin	4.3%	19.0%	0.001	1.7%	5.0%	0.04

results can vary significantly. A recent Canadian study showed a significant increase in *M. haemolytica* resistant to tilmicosin and tulathromycin after 90 d in the feedlot (3), and other studies have shown resistance to macrolides approaching 100% after only 7 to 14 d in the feedlot (1,2). In the current study, the *M. haemolytica* isolates from AUCT calves showed a significant increase in resistance to tulathromycin while the cattle were in the feedlot, but not to the other antimicrobials. Interestingly, the resistance pattern in the isolates from RANCH calves was the opposite. The prevalence of *M. haemolytica* isolates not susceptible on the second sample was significantly less for oxytetracycline, tilmicosin, and tulathromycin. One possible reason for this is the longer interval between sample collection for RANCH calves compared to AUCT calves. However, this pattern was only observed in *M. haemolytica* isolates, not in *P. multocida* or *H. somni* isolates. The authors could not find any previous work demonstrating this pattern so it may warrant further investigation. Most research suggests steadily increasing resistance in *M. haemolytica* isolates (6,7), which is cause for concern.

There has been limited research into resistant *P. multocida* cultured from DNS on arrival, with most of the studies looking at animals that have BRD already. One relevant study by Ericksen et al (3) determined that less than 2% of *P. multocida* samples were not susceptible to antimicrobials on arrival at the feedlot, which is similar to our findings. Both AUCT and

RANCH calves had significant increases in *P. multocida* resistant to florfenicol, oxytetracycline, and tulathromycin on the second test. In the study by Ericksen et al (3) only spectinomycin and tildipirosin showed statistically significant increases in resistance. However, in studies on *P. multocida* in animals sick or dead from BRD, there is a very high prevalence of resistance to oxytetracycline, tulathromycin, tilmicosin, and florfenicol (7,10).

Like *P. multocida,* most of the research into antimicrobial resistance in *H. somni* has been done on animals that are sick or dead due to BRD, not based upon DNS on arrival. Again, the most relevant study was done by Ericksen et al (3) in which they demonstrated 42% of *H. somni* samples were not susceptible to tilmicosin and 38% were not susceptible to tulathromycin on arrival. After 90 d, similar prevalence of resistance persisted with 45% of samples not susceptible to tilmicosin and 43% not susceptible to tulathromycin. The current study did not discover such substantial antimicrobial resistance on arrival, although the number of samples not susceptible to oxytetracycline and tulathromycin increased significantly at the time of second sampling for the AUCT calves.

The RANCH calves having had second samples taken later in the feeding period than those taken from the AUCT calves is certainly a possible source of bias in the trial and may have affected the results. A second limitation of this study is that all the RANCH samples came from a single source and that source was connected to a feedlot. It is possible that the ranch calves selected for the study are not representative of calves on other ranches, and that if we had included samples from other ranch raised calves, the isolation rates of *P. multocida* and *H. somni* may not have been as high.

In conclusion, this study showed a significant difference between the prevalence of BRD bacteria isolated from DNS collected from auction market-derived cattle *versus* cattle acquired directly from a single ranch source. Although it might be reasonable to expect that the BRD pathogens examined in this study would be less prevalent in single source calves, this was not the case. This would seem to indicate that the transmission of BRD pathogens may be affected more by source than by mixing, but previous research suggests mixing may be more of a driver in development of disease (13,14). Further research is needed on how BRD pathogens transmit from adult cattle to calves and into non-medicinal BRD prevention such as altering cattle procurement. CVJ

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