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# *Mannheimia haemolytica* in Feedlot Cattle: Prevalence of Recovery and Associations with Antimicrobial Use, Resistance, and Health Outcomes

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**Background:** *Mannheimia haemolytica* is an important etiological agent in bovine respiratory disease.

**Objectives:** Explore risk factors for recovery of susceptible and resistant *M. haemolytica* in feedlot cattle and explore associations with health outcomes.

**Animals:** Cattle (n = 5,498) from 4 feedlots sampled at arrival and later in feeding period.

**Methods:** Susceptibility of *M. haemolytica* isolates tested for 21 antimicrobials. Records of antimicrobial use and health events analyzed using multivariable regression.

**Results:** *M. haemolytica* recovered from 29% of cattle (1,596/5,498), 13.1% at arrival (95% CI, 12.3–14.1%), and 19.8% at second sampling (95% CI, 18.7–20.9%). Nearly half of study cattle received antimicrobial drugs (AMDs) parenterally, mostly as metaphylactic treatment at arrival. Individual parenteral AMD exposures were associated with decreased recovery of *M. haemolytica* (OR, 0.2; 95% CI, 0.02–1.2), whereas exposure in penmates was associated with increased recovery (OR, 1.5; 95% CI, 1.05–2.2). Most isolates were pan-susceptible (87.8%; 95% CI, 87.0–89.4%). AMD exposures were not associated with resistance to any single drug. Multiply-resistant isolates were rare (5.9%; 95% CI, 5.1–6.9%), but AMD exposures in pen mates were associated with increased odds of recovering multiply-resistant *M. haemolytica* (OR, 23.9; 95% CI, 8.4–68.3). Cattle positive for *M. haemolytica* on arrival were more likely to become ill within 10 days (OR, 1.7; 95% CI, 1.1–2.4).

**Conclusions and Clinical Importance:** Resistance generally was rare in *M. haemolytica*. Antimicrobial drug exposures in penmates increased the risk of isolating susceptible and multiply-resistant *M. haemolytica*, a finding that could be explained by contagious spread.

**Key words:** Antibiotic resistance; *Pasteurella haemolytica*; morbidity; mortality.

Bovine respiratory disease (BRD) is a major economic burden to feedlot operators. It is estimated that BRD-associated morbidity and mortality result in annual loss of one billion USD for North American feedlots.[1] BRD-related costs can account for 7% of total production costs, and per-calf revenue losses associated with treatment of BRD are estimated at up to $292 USD for animals requiring 3 antimicrobial treatments.[2,3]

Although the etiology of BRD is multifactorial, *M. haemolytica* is arguably the most important associated bacterial pathogen, primarily because of virulence factors that induce severe morbidity. *M. haemolytica* is typically the most common agent isolated from necropsy samples of cattle with BRD.[4,5]

Treatment of BRD in large commercial feedlots is focused on antimicrobial treatment in clinically ill animals and antimicrobial metaphylactic treatment of high-risk animals. Sick animals that fail to respond to initial treatment typically are retreated with a different antimicrobial (personal communication: Calvin Booker). Recently, BRD treatment strategies have come under scrutiny because of a perception of antimicrobial resistance (AMR) in *M. haemolytica* isolates recovered from feedlot cattle, including multiply-resistant isolates.[6] Despite the putative importance of BRD and *M. haemolytica* for feedlot economics and animal health, ambiguity persists regarding colonization dynamics of *M. haemolytica* and associations with clinical disease.

**Abbreviations:**

| | |
|---|---|
| ALR | alternating logistic regression |
| AMD | antimicrobial Drug |
| AMR | antimicrobial resistance |
| AMU | antimicrobial use |
| BRD | bovine respiratory disease |
| CI | confidence interval |
| FHMS | Feedlot Health Management Services |
| GEE | generalized estimating equations |
| VIF | variance inflation factor |

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*Sample collection and record management took place at 4 beef feedlot operations in Alberta, Canada. Sample processing and testing were undertaken at the Agriculture and Agri-Food Canada Lethbridge Research Station, Lethbridge, Alberta. Resistance testing was performed at Agriculture and Agri-Food Canada Lethbridge Research Station, Lethbridge, Alberta and the Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Saint-Hyacinth, Quebec. Data analysis and manuscript preparation took place at Colorado State University in Fort Collins, Colorado.*

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Primary objectives of this study were to describe the prevalence of *M. haemolytica* in isolates obtained from commercial feedlot beef cattle, to describe resistance prevalence and patterns in isolates, and to investigate associations between antimicrobial use (AMU) and resistant isolates. A secondary objective was to investigate associations between *M. haemolytica* isolation and morbidity and mortality outcomes.

## Materials and Methods

### *Study Overview*

Isolates evaluated in this study were collected as part of a project to develop and evaluate surveillance methods of AMR in feedlots.[7] The study population, sampling methods and laboratory procedures, and interpretive criteria for antimicrobial susceptibility have been described.[7] Briefly, 5,968 individual cattle were enrolled using a 2-stage random sampling as they entered 4 feedlots in Alberta, Canada. Morbidity, mortality and antimicrobial treatment events were tracked throughout the study. Deep nasopharyngeal swabs were collected at arrival to the feedlot ("arrival sample") and again later in the feeding period ("second sample") and cultured for *M. haemolytica*. Isolates with morphologic characteristics of *M. haemolytica* were confirmed using biochemical tests and PCR.[7,8] Confirmed isolates were evaluated for resistance to antimicrobial drugs (AMDs; Table 5) using broth microdilution (Table S1), disk diffusion (Table S2) or both. Prevalence of and risk factors for isolation of *M. haemolytica* were described, and multivariable logistic regression was used to investigate associations between AMU and AMR in *M. haemolytica* isolates and between *M. haemolytica* isolation and health outcomes.

### *Study Population*

Four feedlots in Alberta, Canada with one-time holding capacities of between 15,000 and 20,000 cattle were purposely selected based on their ability to track AMU and other health data as well as their willingness to participate. Production conditions were typical for North American commercial cattle feedlots, and veterinary care was managed by Feedlot Health Management Services (FHMS). Cattle handling and sampling procedures were approved by the Animal Care Committee of the University of Calgary (Protocol Number M07031).

Cattle were sourced from across Canada through auction markets, and entered the feedlots at a range of weights (225–400 kg), ages, frame sizes, and sexes. Based upon these factors and historical patterns of illness in similar cattle, arriving groups were assigned an ordinal category of perceived risk for developing BRD (low risk to very high risk), which was used to employ prevention and treatment protocols. All cattle received a growth implant, vaccines against selected pathogens, and topical anthelmintic upon arrival. Very high risk cattle received *M. haemolytica* anti-leukotoxin vaccine, and cattle with assigned risk status of very high or high received AMDs as metaphylaxis for respiratory disease, whereas lower risk, non-clinical cattle did not (Table 1). Cattle in higher risk categories received drugs shown to have greater efficacy for prevention and treatment of respiratory disease.[9] Cattle were fed a diet that met or exceeded the National Research Council requirements for beef cattle until reaching a body weight of 550–650 kg, at which time they were sent to slaughter, typically 120–250 days after arrival in the feedlot.[10]

Trained feedlot personnel evaluated cattle for signs of illness at arrival and daily thereafter. Animals exhibiting systemic illness (eg, dyspnea, lack of response to stimulation, reluctance to move, abnormal carriage or posture of the head, or some combination of these signs) were assigned a diagnosis of "undifferentiated systemic illness" with or without fever based on a body temperature of higher or lower than 40.5°C, respectively, and treated using antimicrobial protocols formulated specifically for their diagnosis and risk status. All cattle that died underwent necropsy by a FHMS veterinarian, who used clinical history and physical findings to classify the cause of death as either BRD, bovine viral diarrhea-associated disease, disease caused by *Histophilus somni*, diseases of the appendicular skeleton, metabolic disease, and miscellaneous heath events (eg, trauma).

**Table 1.** Antimicrobial drugs used in this study population.

| Antimicrobial Drug and Dosage | Primary Reason for Use | Class |
|---|---|---|
| Parenteral | | |
| Ceftiofur sodium 1 mg/kg BW | BRD Treatment | Beta lactam |
| Ceftiofur crystalline free acid 6.6 mg/kg BW | BRD Treatment | Beta lactam |
| Ceftiofur hydrochloride 1.1 mg/kg BW | BRD Treatment | Beta lactam |
| Enrofloxacin 7.7 mg/kg BW | Relapse BRD Treatment | Quinolone |
| Florfenicol 40 mg/kg BW | BRD Treatment | Phenicol |
| Florfenicol 40 mg/kg BW & Flunixin meglumine 2.2 mg/kg BW | BRD Treatment | Phenicol |
| Oxytetracycline | | |
| 10 mg/kg BW | BRD Prevention/Treatment | Tetracycline |
| 20 mg/kg BW | BRD Prevention/Treatment | Tetracycline |
| 30 mg/kg BW | BRD Prevention/Treatment | Tetracycline |
| Tilmicosin 10 mg/kg BW | BRD Prevention/Treatment | Macrolide |
| Trimethoprim and sulfadoxine 16 mg/kg BW | BRD Treatment | Sulfonamide |
| Tulathromycin 2.5 mg/kg BW | BRD Prevention/Treatment | Macrolide |
| Tylosin tartrate 29 mg | Implant Site Abscess Prevention | Macrolide |
| In-Feed | | |
| Chlortetracycline @ | | |
| 35 mg/kg diet dry matter | Liver Abscess Prevention | Tetracycline |
| 1 g/head/day | Histophilosis Prevention/Treatment | Tetracycline |
| 3 g/head/day | Histophilosis Prevention/Treatment | Tetracycline |
| 6 g/head/day | Histophilosis Prevention/Treatment | Tetracycline |
| Tylosin phosphate @ 11 mg/kg diet dry matter | Liver Abscess Prevention | Macrolide |

### *Animal and Pen Record Management*

A computerized data collection system[a] was used to track the date each animal arrived at the feedlot, the number of cattle in the pen, the BRD risk status of each animal, and all health events, including treatments (date, drug administered, dose, and route of administration) and clinical and necropsy diagnoses. Only in-feedlot AMD exposures were included in this study because of a lack of information on management of cattle before arrival in the feedlot. Most cattle's pen assignments did not change after arrival, exceptions being pens that were split or mixed for the purposes of marketing homogenous groups of cattle; such pens were excluded from analysis because of an inability to accurately characterize AMU for penmates. Nasopharyngeal sample collection dates, culture results, *M. haemolytica* isolate identification numbers, and resistance testing results were compiled and linked using the unique animal identification assigned to each animal upon arrival.

### *Data Analysis*

Descriptive analyses and data distributions were explored graphically and using numerical summaries. Adjusted CI for binomial proportions (adding 2 successes and 2 failures) were estimated.[11] Crude prevalence of susceptible and resistant *M. haemolytica* at arrival and second sampling was calculated and compared. When drugs were tested by both broth microdilution and disk diffusion (eg, ampicillin, ceftiofur), isolates were classified as resistant if either test result indicated resistance. "Pan-susceptibility" was defined as phenotypic susceptibility to all drugs tested. "Multiple resistance" was defined as phenotypic resistance to ≥2 antimicrobials, regardless of drug class and whether results were obtained from broth microdilution or disk diffusion, because the drugs included in these panels differed. McNemar's test was used to detect significant differences in isolation of *M. haemolytica* within individual cattle between the 2 sampling points. Least-square means estimates from generalized estimating equations (GEE) were used to determine significant changes in the overall prevalence of *M. haemolytica* between the 2 sampling points, with cattle ID specified as a repeated measure.

Inferential analyses were performed with commercial software[b] using logistic regression with GEE to control for clustering within pens, specifying an exchangeable correlation structure. Feedlot was included in all models as a fixed effect. The distributions of AMU were strongly right-skewed and zero-inflated, and therefore were modeled dichotomously (ie, no exposure versus any exposure). Each antimicrobial class (tetracycline, macrolide, beta-lactam, phenicol, sulfonamide, and quinolone) and route of exposure (in-feed versus parenteral) was modeled separately. Parenteral drugs were grouped into a single variable if exposures were too sparse for model convergence.

The primary study outcome was isolation of *M. haemolytica* (yes or no) in the second sample. Primary exposure variables of interest were previous exposure to parenteral antimicrobials of any type, and in-feed macrolides and tetracyclines. Exposures were classified as direct (ie, administered directly to the enrolled individual) or indirect (ie, administered to penmates of the enrolled individual). Furthermore, exposures were dichotomized as occurring >7 or ≤7 days from sample collection. Arrival sample *M. haemolytica* status (positive or negative) and cattle risk level also were risk factors of interest. Pen size was added as a potential confounder.

Secondary outcomes were resistance in second sample *M. haemolytica* isolates, both to each of the 21 drugs tested in the 2 panels, as well as to ≥2 drugs, that is multiply-resistant (ie, multiply-resistant versus singly-resistant or susceptible). Isolates tested by broth microdilution and disk diffusion were considered multiply-resistant if either method showed multiple resistance. Primary exposure variables included individual and penmate parenteral exposure to betalactams, sulfonamides, phenicols, quinolones, macrolides, and tetracyclines and in-feed macrolides and tetracyclines at any point before sample collection. For the outcome of multiply-resistant *M. haemolytica,* all parenterally administered drugs were grouped together into a single exposure variable. *M. haemolytica* status of cattle at arrival was the primary risk factor of interest for resistance outcomes. Secondary risk factors included the season of feedlot arrival and sample collection (Jan–Mar, Apr–Jun, Jul–Sept, Oct–Dec), the risk status assigned to each animal (low, medium, high, or very high), and the number of cattle in the pen (<100, 101–200, 201–300, 301–400, or >400). The number of days cattle had been in the feedlot at sample collection was forced into all models as a potential confounder. To account for repeated measures on samples from testing of multiple isolates, we specified "sample" as a subcluster with a "1-(nested log odds ratio)" structure using GEE with alternating logistic regression (ALR).[12] Some isolates were tested by both broth microdilution and disk diffusion, and therefore "test type" was added as a fixed effect.

To model isolation of susceptible and resistant *M. haemolytica,* each AMU variable was first modeled individually. Variables exhibiting a $P$ value of ≤.20 were included in multivariable modeling, which proceeded in a backwards stepwise fashion with a critical alpha for retention of 0.05. Variables with a relatively large effect size and biological relevance also were retained in the final model. Confounding (defined as parameter estimate change of ≥20%) was assessed for all excluded variables. Collinearity was evaluated using the variance inflation factor and Chi-square test for continuous and categorical variables, respectively.[13]

A third set of outcomes included BRD-associated mortality and morbidity (diagnosis of systemic illness with fever at arrival, at any time during the study period, and within 10 days after sample collection). For these 4 outcomes, the primary risk factor of interest was arrival sample *M. haemolytica* status. Secondary a priori risk factors included in all models were BRD risk status, number of cattle in the pen, and the season of arrival.

## Results

### *Samples*

A total of 5,968 cattle from 288 pens housing 56,080 cattle were enrolled in the study. During the study period, 71 cattle died and were not sampled a second time. Approximately 7.9% (470/5,968) of arrival samples and 15.6% (918/5,897) of second samples were excluded from analysis, resulting in 10,477 samples available for analyses. The majority of exclusions occurred as a result of split/mixed pens, but 1.4% (20/1,388) were excluded because of missing sample numbers and laboratory results.

Second samples were collected throughout the feeding period: 14.5% (721/4,979) were obtained between 30 and 60 DOF; 49.0% (2,441/4,979) between 61 and 90 DOF; 10.1% (502/4,979) between 91 and 120 DOF; 17.5% (873/4,979) between 121 and 150 DOF; 6.0% (300/4,979) between 151 and 180 DOF; and 2.9% (142/4,979) at >180 DOF.

### *Study Population*

The 5,498 cattle represented a diversity of BRD risk categories (Table 2). Most cattle were housed with 101–300 animals (59.4%, 3,267/5,498), and entered the feedlot in the summer and fall (68.6%, 3,771/5,498).

**Table 2.** Demographics of study population.

| | No. of Cattle | % of Cattle |
|---|---|---|
| Risk status of cattle | | |
| Low risk | 2,420 | 44.0 |
| Medium risk | 832 | 15.1 |
| High risk | 1,356 | 24.7 |
| Very high risk | 890 | 16.2 |
| Arrival season of cattle | | |
| Winter (Jan–Mar) | 876 | 15.9 |
| Spring (Apr–Jun) | 851 | 15.5 |
| Summer (Jul–Sept) | 1,623 | 29.5 |
| Fall (Oct–Dec) | 2,148 | 39.1 |
| Pen size | | |
| <101 | 459 | 8.4 |
| 101–200 | 1,858 | 33.8 |
| 201–300 | 1,409 | 25.6 |
| 301–400 | 1,173 | 21.3 |
| >400 | 599 | 10.9 |

### *Prevalence of Mannheimia haemolytica Recovery*

A total of 10,477 nasopharyngeal samples were obtained, and *M. haemolytica* was isolated from 16.6% (1,744/10,477; 95% CI, 15.9–17.4%). Overall, 29% of cattle (1,596/5,498) were culture-positive for *M. haemolytica* at least once, and there was significant discordance in recovery likelihood between arrival and second samples (McNemar's $P < .001$), that is, a majority of positive cattle (90.7%; 1,448/1,596) were culture-positive only once. There was a significant increase ($P < .001$) in the likelihood of recovery from arrival to second sample (13.1%; 95% CI, 12.3–14.1% and 19.8%; 95% CI, 18.7–20.9, respectively).

### *Antimicrobial Use*

All enrolled cattle received tetracycline and 9.6% (477/4,979) received macrolides in-feed for liver abscess control before second sampling (Table 3). Parenteral drugs were given to 47.5% (2,611/5,498) of enrolled cattle, most commonly during initial processing as metaphylaxis for respiratory disease. Tetracyclines and macrolides were the most common parenterally administered antimicrobials, with 31% (1,563/4,979) and 23% (1,158/4,979) of enrolled cattle exposed during the study, respectively. Other parenterally administered AMDs were each given to <2% of study cattle.

**Table 3.** Drug use before the time of second sampling, by class.

| Drug Class | Total ADD's Analyzed[a] | % of ADD's | No. of Cattle Exposed | % of Cattle Exposed |
|---|---|---|---|---|
| Parenteral Betalactam | 211 | 0.4 | 73 | 1.5 |
| Parenteral Quinolone | 57 | 0.1 | 19 | 0.4 |
| Parenteral Phenicol | 81 | 0.2 | 27 | 0.5 |
| Parenteral Macrolide | 3,166 | 5.7 | 1,158 | 23.3 |
| Parenteral Sulfonamide | 51 | 0.1 | 17 | 0.3 |
| Parenteral Tetracycline | 4,540 | 8.2 | 1,563 | 31.4 |
| In-feed Tetracycline | 47,178 | 85.2 | 4,979 | 100.0 |
| In-feed Macrolide | 63 | 0.1 | 477 | 9.6 |
| Total | 55,346 | | | |

[a]ADD = animal daily dose, defined as the number of days that a single treatment remains in the target tissue(s) at therapeutic concentrations.

### *Risk Factors for Recovery of M. haemolytica*

Odds of isolating *M. haemolytica* in second samples from cattle that received any parenterally administered drug ≤7 days preceding sample collection were about 5 times lower than for cattle that did not receive parenterally administered drugs in this same timeframe (OR, 0.2; 95% CI, 0.02–1.2; $P = .006$; Table 4). Nontreated enrolled cattle housed in a pen with cattle that received injections >7 days before sample collection were about 1.5-times more likely to be colonized with *M. haemolytica* than study cattle that did not have treated penmates (OR, 1.5; 95% CI, 1.05–2.2; $P = .02$; Table 4). Arrival sample *M. haemolytica* status was not significantly associated with second sample *M. haemolytica* status. BRD risk status was collinear with AMD exposure and could not be modeled.

### *Antimicrobial Resistance*

Susceptibility testing was performed on 2,989 isolates taken from 1,744 culture-positive nasopharyngeal samples. A total of 1,200 isolates were tested with only broth

**Table 4.** Risk factors associated with the isolation of *M. haemolytica* in second samples.

| Predictor | Level | Odds Ratio | 95% CI | *P* Value |
|---|---|---|---|---|
| Parenteral drugs given to sampled individual within 7 days of sample collection | Any exposure | 0.16 | 0.02–1.23 | .006 |
| | No exposure | Reference | Reference | Reference |
| Parenteral drugs given to penmates of sampled individual at least 7 days before sample collection | Any exposure | 1.52 | 1.05–2.19 | .023 |
| | No exposure | Reference | Reference | Reference |
| Pen size | Confounded | Confounded | Confounded | Confounded |

microdilution, 215 with only disk diffusion, and 1,574 with both methods. Over 87% of isolates (2,623/2,989; 95% CI, 87.0–89.4%) were pan-susceptible. Most single-drug phenotypes exhibited crude prevalence ≤2.0%, with insufficient occurrence to support logistic regression modeling (Table 5). The relatively low prevalence of resistance across all drugs is also reflected in the distributions of minimum inhibitory concentration (MIC) and zone diameter data (Tables S3, S4). Spectinomycin exhibited the highest resistance prevalence at 4.5% (81/1,789), followed by tetracycline (4.4%, 204/4,622), streptomycin (4.3%, 119/2,833), and kanamycin (3.8%, 108/2,833; Table 5). No AMD exposures were significantly associated with resistance to any of these 4 drugs.

A subset of the 2,989 isolates tested for susceptibility (8.6%, 415/2,989) was excluded from inferential analyses of resistant *M. haemolytica* because of missing AMD exposure information when pens were split or mixed before sampling. A small proportion of remaining isolates was multiply-resistant (5.9%; 152/2,573; 95% CI, 5.1–6.9%), comprising 3.8% of arrival isolates (47/1,225; 95% CI, 2.9–5.1%) and 7.8% of second sample isolates (105/1,348; 95% CI, 6.5–9.4%). Combined kanamycin and streptomycin resistance was the most common multiple-resistant phenotype at 47.1% (80/170; 95% CI, 39.7–54.5%; Table 6). Although multiple resistance was rare, odds of recovery was much greater when penmates of sampled individuals received parenterally administered AMDs (OR, 23.9; 95% CI, 8.4–68.3; $P < .001$; Table 7). The wide CI for this estimate indicates a predictable lack of precision given the relatively rare occurrence of multiple resistance. Parenteral AMU in sampled cattle also was associated with increased odds of recovering multiply-resistant *M. haemolytica* from second samples, but was collinear with parenteral exposure in penmates; individual exposures were removed from the model because of a weaker magnitude of effect on recovery of multiply-resistant *M. haemolytica*. Multiple-resistance status was

**Table 5.** Crude prevalence of resistance of *M. haemolytica* isolates (n = 2,989).[a]

| Resistance Phenotype | No. of Isolates | % (95% CI)[e] |
|---|---|---|
| Pan-susceptible | 2,623 | 87.8 (87.0–89.4) |
| Amikacin[b] | 3 | 0.1 (0.0–0.3) |
| Amoxicillin-clavulanate[c] | 34 | 0.7 (0.5–1.0) |
| Ampicillin[c] | 70 | 1.5 (1.2–1.9) |
| Cefoxitin[b] | 5 | 0.2 (0.1–0.4) |
| Ceftiofur[c] | 2 | 0.0 (0.0–0.2) |
| Ceftriaxone[b] | 1 | 0.0 (0.0–0.2) |
| Chloramphenicol[b] | 0 | 0.0 (0.0–0.1) |
| Ciprofloxacin[b] | 0 | 0.0 (0.0–0.2) |
| Enrofloxacin[d] | 1 | 0.0 (0.0–0.3) |
| Florfenicol[d] | 2 | 0.1 (0.0–0.4) |
| Gentamicin[c] | 0 | 0.0 (0.0–0.1) |
| Kanamycin[b] | 108 | 3.8 (3.2–4.6) |
| Nalidix acid[b] | 4 | 0.1 (0.0–0.3) |
| Streptomycin[b] | 119 | 4.2 (3.5–5.0) |
| Sulfonamide[b] | 12 | 0.4 (0.2–0.8) |
| Spectinomycin[d] | 81 | 4.5 (3.7–5.6) |
| Danofloxacin[d] | 35 | 2.0 (1.4–2.7) |
| Tilmicosin[d] | 5 | 0.3 (0.1–0.7) |
| Tulathromycin[d] | 2 | 0.1 (0.0–0.4) |
| Tetracycline[c] | 204 | 4.4 (3.9–5.1) |
| Trimethoprim-sulfadiazine[c] | 9 | 0.2 (0.1–0.4) |

[a]Isolates can be listed more than once if they were multiply resistant; 1,574 isolates were tested by both broth microdilution and disk diffusion, 1,200 isolates were tested by only broth microdilution, and 215 isolates tested only by disk diffusion, for a total of 2,833 test results from broth microdilution and 1,789 from disk diffusion (4,622 total test results).

[b]Tested by broth microdilution only.

[c]Tested by both broth microdilution and disk diffusion.

[d]Tested by disk diffusion only.

[e]Adjusted CI for binomial proportions (adding 2 successes and 2 failures) were estimated as previously described.[11]

**Table 6.** Most common phenotypes among multiply-resistant isolates (n = 152).

| Frequency of Resistance Phenotype[a] | % (95% CI)[b] | Phenotype |
|---|---|---|
| 80 | 47.1 (39.7–54.5) | Kanamycin, Streptomycin |
| 11 | 6.5 (3.6–11.4) | Ampicillin, Amoxicillin-Clavulanate |
| 8 | 4.7 (2.3–9.2) | Kanamycin, Streptomycin, Tetracycline |
| 8 | 4.7 (2.3–9.2) | Ampicillin-Clavulanate, Tetracycline |
| 7 | 4.1 (1.9–8.5) | Spectinomycin, Danofloxacin |
| 7 | 4.1 (1.9–8.5) | Spectinomycin, Danofloxacin, Tetracycline |
| 6 | 3.5 (1.5–7.7) | Kanamycin, Streptomycin, Ampicillin-Clavulanate |
| 25 | 16.4 (10.5–22.4) | 25 other multiply-resistant phenotypes |

[a]From a total of 32 multiply-resistance phenotypes; the phenotypes listed had a frequency of >2% among multiply-resistant *M. haemolytica* isolates.

[b]Adjusted CI for binomial proportions (adding 2 successes and 2 failures) were estimated as previously described.[9]

**Table 7.** Final multivariable model for risk factors associated with recovery of multiply-resistant *M. haemolytica* in second sample (multiply-resistant versus singly-resistant or susceptible).

| Predictor | Level | Odds Ratio | 95% CI | *P* Value |
|---|---|---|---|---|
| Parenteral drugs given to penmates of sampled individual at any time before sample collection | Any exposure | 23.9 | 8.4–68.3 | <.0001 |
| | No exposure | Reference | Reference | |
| Arrival season | Fall (Oct–Dec) | 1.2 | 0.5–3.1 | .07 |
| | Summer (Jul–Sept) | 0.6 | 0.2–1.9 | |
| | Spring (Apr–Jun) | 0.2 | 0.1–1.0 | |
| | Winter | Reference | Reference | |

not associated with BRD risk status, pen size, or diagnosis of systemic illness or fever.

### *Morbidity and Mortality*

Overall, 7% of enrolled cattle (401/5,498) were diagnosed with systemic illness requiring treatment, 19% of which were febrile at arrival (75/401), 50% of which became systemically ill and febrile while in the feedlot (200/401), and 31% of which became ill during the feeding period but were not febrile (126/401). Of the 401 sick cattle, 41% (164/401) were diagnosed as ill <10 days after sample collection, the majority (95.7%; 157/164) <10 days after arrival sampling.

Approximately 1.3% of enrolled cattle died during the study (71/5,498), with 21% (15/71) attributed to metabolic disease, 21% (15/71) to *Histophilus somni*, 13% (9/71) to lameness, 11% (8/71) to BRD, and 3% (2/71) to mucosal disease caused by bovine viral diarrhea virus. The remaining 31% (22/71) succumbed to miscellaneous causes.

### *M. haemolytica* Isolation as a Risk Factor for Respiratory Morbidity and Mortality

Isolation of *M. haemolytica* at arrival was not a significant predictor of mortality, arrival diagnosis with systemic illness and fever, or diagnosis of systemic illness and fever later in the feeding period (Table 8). However, cattle that were culture-positive for *M. haemolytica* on arrival had almost twice the likelihood of being identified as systemically ill and febrile <10 days after arrival as compared to culture-negative cattle (OR, 1.7; 95% CI, 1.1–2.4; $P = .07$).

## Discussion

*Mannheimia haemolytica* prevalence in this study was similar to that of previous reports,[3,14] but recovery was significantly lower at arrival than later in the feeding period. Almost 90% of isolates were susceptible to all AMDs evaluated (21 AMDs from 9 different drug classes), and only approximately 6% were multiply-resistant. The likelihood of recovery was decreased in cattle that received AMDs parenterally, but increased in untreated cattle whose penmates received antimicrobials parenterally, which may be an indicator of contagious transmission within pens. In addition, parenteral AMD exposures in penmates greatly increased the odds of recovering multiply-resistant *M. haemolytica* in study subjects.

These findings are especially relevant to producers and their veterinarians, because they stem from a longitudinal study conducted in commercial cattle under typical feedlot conditions. Deep nasopharyngeal sampling was done on live feedlot cattle regardless of clinical signs, as opposed to sampling necropsy lung tissues, and therefore provides a potentially more relevant picture of *M. haemolytica* transmission dynamics. Although live animal sampling is unique, it must be noted that cattle were sampled only twice and outcomes therefore represent only a snapshot of *M. haemolytica* feedlot dynamics.

All cattle were exposed to in-feed AMDs and 50% to parenterally administered AMDs, primarily for BRD metaphylaxis. Most AMDs used in this population were macrolides and tetracyclines, while other classes were relatively infrequently used, including antimicrobials germane to AMR in *M. haemolytica* (eg, ceftiofur). Although these AMD use patterns reflect real world practices, they can also hamper analytic analysis because of sparse data distributions for AMD exposure measures, as well as rare resistance outcomes. Given low parenteral AMU rates, randomized controlled trials may be necessary to evaluate specific hypotheses regarding the impact of use on *M. haemolytica* recovery and AMR, particularly for specific resistances that pose substantial human, animal, or economic health risk. However, we believe that the observational nature of this study better reflects real world ecological impact of AMU on *M. haemolytica* recovery and AMR.

A striking finding of this study is that parenteral AMU in penmates not only modestly increased the odds of isolating any *M. haemolytica* but also dramatically increased the likelihood of recovering rare multiply-resistant isolates (Tables 4, 7). Parenteral treatment is a marker of disease occurrence under the management strategy used in this population, and therefore this finding could suggest that contagious spread is predicted by disease occurrence in penmates. If this is true, the use of arrival metaphylaxis in high-risk populations may be effective in controlling disease in clinically ill cattle, as well as preventing colonization of healthy penmates.[9,15,16] However, it might also suggest that treatment selects for more resistant bacterial populations, which spread among penmates. Indeed, this theory is supported by the large effect of parenteral treatment on increasing the likelihood of isolating multiply-resistant *M. haemolytica*. Together, these findings suggest that metaphylaxis treatment protocols may be striking a delicate balance between the competing interests of animal health and antimicrobial resistance. This ecological impact warrants further investigation given the importance of *M. haemolytica* in feedlot cattle.

We also found that feedlot-of-origin exhibited a strong and consistent association with the 4 single-resistance outcomes that could be modeled (ie, spectinomycin, tetracycline, kanamycin, and streptomycin). If *M. haemolytica* undergoes contagious spread, we would expect resistance patterns to be strongly associated with geographic location (ie, feedlot). Indeed, the contagious nature of *M. haemolytica* previously has been suggested based on evidence of BRD clustering within transport trucks and pens.[17]

This finding also suggests that *M. haemolytica* subpopulations may undergo clonal expansion, and that resistant strains are maintained at low levels within a feedlot. Previous studies have shown a link between resistance patterns and *M. haemolytica* subtype.[18] Furthermore, the significant increase in prevalence of *M. haemolytica* from arrival to second samples (13–20%) could suggest that phenotypic characteristics (eg, virulence) of

**Table 8.** Odds ratio, 95% CI,[a] and *P*-value for a priori risk factors of respiratory morbidity and mortality.

| | Outcome | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Risk Factor | Mortality at Any Time During the Feeding Period | *P* Value | Diagnosis of Fever[b] on Arrival | *P* Value | Diagnosis of Fever[b] After Arrival, at Any Time in Feeding Period | *P* Value | Diagnosis of Fever[b] After Arrival, Within 10 days After Sample Collection | *P* Value |
| *M. haemolytica* status of arrival sample | | | | | | | | |
| Negative | Reference | | Reference | | Reference | | Reference | |
| Positive | 1.1 (0.3–2.9) | .93 | 1.4 (0.7–1.7) | .38 | 1.2 (1.0–1.6) | .24 | 1.7 (1.1–2.4) | .07 |
| Risk status of cattle | | | | | | | | |
| Low risk | Reference | .03 | Reference | .008 | Reference | <.001 | Reference | <.001 |
| Medium risk | 3.5 (0.4–90.2) | | 2.9 (2.1–7.0) | | 1.7 (1.7–3.2) | | 2.1 (1.5–4.0) | |
| High risk | 13.7 (2.2–285.7) | | 2.7 (1.4–4.3) | | 5.6 (4.1–7.8) | | 2.5 (1.5–4.1) | |
| Very high risk | 3.8 (0.4–111.7) | | 0.5 (0.2–0.9) | | 2.7 (1.8–3.8) | | 0.3 (0.1–0.6) | |
| Arrival season of cattle | | | | | | | | |
| Winter (Jan–Mar) | Reference | .21 | Reference | .09 | Reference | .002 | Reference | .002 |
| Spring (Apr–Jun) | 0.3 (0.01–2.7) | | 5.2 (2.2–11.4) | | 1.3 (0.6–1.7) | | 1.2 (0.5–2.5) | |
| Summer (Jul–Sept) | 0.3 (0.03–2.0) | | 2.3 (0.9–4.7) | | 2.5 (1.4–3.0) | | 1.2 (0.5–1.0) | |
| Fall (Oct–Dec) | 1.3 (0.3–5.2) | | 2.7 (1.1–5.2) | | 3.9 (2.5–5.1) | | 3.1 (1.6–5.1) | |
| Pen size | | | | | | | | |
| <101 | Reference | .23 | Reference | .38 | Reference | .24 | Reference | .97 |
| 101–200 | 3.1 (0.54–56.6) | | 1.2 (0.6–2.7) | | 1.7 (1.2–2.8) | | 1.0 (0.5–2.0) | |
| 201–300 | 1.6 (0.1–37.0) | | 0.4 (0.1–1.5) | | 1.6 (1.0–2.8) | | 1.0 (0.5–2.4) | |
| 301–400 | 0.4 (0.02–15.4) | | 1.8 (0.9–6.1) | | 1.6 (1.0–2.9) | | 0.8 (0.4–2.2) | |
| >400 | 1.6 (0.2–92.6) | | 0.6 (0.3–2.5) | | 2.8 (1.6–5.2) | | 0.9 (0.3–2.7) | |

[a]95% CI represent likelihood ratio-based CI.
[b]Systemic illness with fever (bovine respiratory disease).

*M. haemolytica* might influence treatment decisions and thus transmission dynamics. For example, arriving cattle with clinical signs might be colonized with a particularly virulent strain of *M. haemolytica*. Treatment of these cattle could then increase the likelihood that persistent *M. haemolytica* is resistant, and this resistant strain could then spread to untreated penmates, who subsequently exhibit a higher likelihood of colonization even in the absence of clinical illness. *M. haemolytica* serotypes have been shown to differ in virulence, and cattle exhibiting clinical BRD signs are more likely to be colonized with more virulent serotypes.[19] Future studies should include isolate typing to gain a clearer understanding of transmission dynamics.

Published rates of multiply-resistant *M. haemolytica* range from 0 to 50%, but it is difficult to compare the prevalence found in this study, because the only identified studies used small numbers of young animals in noncommercial settings (sample sizes ranging from 4 to 27).[20–23] One recent, larger study examined samples from over 350 cattle and found an increase in multiply-resistant *M. haemolytica* from 5 to 35% between 2009 and 2011, but evaluated only isolates from cattle with terminal respiratory disease.[6] As stated above, information regarding *M. haemolytica* susceptibility obtained from our study is particularly relevant to veterinarians and producers because isolates were obtained from randomly selected live cattle without considering treatment history or disease status.

Several lines of evidence indicate that isolates obtained in this study were representative of a highly susceptible bacterial population. The majority of *M. haemolytica* isolates (88%) were pan-susceptible, there was low resistance prevalence to all drugs, and distributions of susceptibility information (MICs and zones of inhibition) were highly suggestive of a largely susceptible population. The prevalence of multiply-resistant *M. haemolytica* isolates also was much lower than indicated by other recent research.[6]

In addition to a largely susceptible bacterial population, it should be noted that no associations were found between AMD exposures among enrolled cattle or their penmates, and resistance to single drugs. Furthermore, the most prevalent resistance phenotypes observed were for AMDs not used in the study population (eg, kanamycin, streptomycin, and spectinomycin). This finding is consistent with a recent study that showed no correlation between antemortem treatment regimens and resistance patterns in *M. haemolytica* recovered from necropsy lung samples.[24] Together, these findings highlight the complexity of AMR and suggest that AMU practices do not necessarily impact development of AMR in a predictable manner. Furthermore, these results support the contention that decreased efficacy of BRD treatment stems from chronic and repeatedly treated BRD cases, rather than from AMU practices. The association between treatment with parenterally administered antimicrobials and recovery of multiply-resistant *M. haemolytica* deserves closer study to determine whether this relationship affects BRD control or treatment efficacy in feedlot populations.

Recovery of *M. haemolytica* from 20% of cattle after arrival was higher than expected, as was the significant increase in prevalence over time. However, recovery of *M. haemolytica* in the second sample was not associated with increased morbidity or mortality, suggesting that post-arrival colonization is more likely to be subclinical and may not be as great a concern for feedlot operators. In contrast, isolation of *M. haemolytica* on arrival was associated with a short-term, significant increase in risk of clinical illness. Thus, although prevalence of *M. haemolytica* was lower at arrival, the clinical and economic relevance of such colonization was greater. In addition, these results showed that cattle receiving parenterally administered drugs were at decreased risk of *M. haemolytica* colonization in the short-term, a finding that supports AMU in high-risk individuals during a defined period of stress, such as arrival in the feedlot. These findings highlights the complexity of colonization, treatment and clinical illness, and support the belief that aggravating factors such as transport and handling stress are critical for causing cattle to develop overt disease.

## Footnotes

[a] *i*FHMS, FHMS, Okotoks, AB
[b] SAS 9.3, SAS Institute Inc, Cary, NC

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*Conflict of Interest Declaration*: Dr Booker is an owner and Dr Hannon is an employee at Feedlot Health Management Services, Okotoks, Alberta. This is a private company that provides expert consultation regarding management of feedlot cattle, including medical treatments such as use of antimicrobial drugs. They also conduct research on a fee-for-service basis regarding a variety of topics including the efficacy of different antimicrobial drugs.

*Off-label Antimicrobial Declaration*: The authors declare no off-label use of antimicrobials.

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## Supporting Information

Additional Supporting Information may be found online in Supporting Information:

**Table S1.** *M. haemolytica* broth microdilution interpretive criteria for minimum inhibitory concentration (μg/mL).

**Table S2.** *M. haemolytica* disk diffusion interpretive criterian for inhibition zone diameters (mm).

**Table S3.** Minimum inhibitory concentrations for *Mannheimia haemolytica* isolates recovered from deep nasopharyngeal swabs obtained from feedlot cattle (n = 2,833 isolates).

**Table S4.** Results of disk diffusion susceptibility testing of *Mannheimia haemolytica* isolates recovered from deep nasopharyngeal swabs obtained from feedlot cattle (n = 1,789 isolates).