Agreement of antimicrobial susceptibility testing of *Pasteurella multocida* and *Mannheimia haemolytica* found in pre-weaned dairy calves with bovine respiratory disease

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Introduction

Bovine respiratory disease (BRD) is one of the primary diseases affecting pre-weaned dairy calves and involves respiratory pathogens, such as *Mannheimia haemolytica* (Mh) and *Pasteurella multocida* (Pm). While agreement between methods for detection of bacterial pathogens in dairy calves with BRD is very good, there are limited data regarding how antimicrobial susceptibility (AS) of isolates collected via these methods agree. The objective was to evaluate agreement among the AS profiles of Mh or Pm obtained by transtracheal wash (TTW), nasal swab (NS), nasopharyngeal swab (DNP), and bronchoalveolar lavage (BAL) in pre-weaned dairy calves with BRD that were positive in all 4 samples.

Materials and methods

Holstein and Holstein-cross bull calves were observed daily for BRD by one trained observer using the Wisconsin Calf Respiratory Scoring Chart. Calves > 30 days old with naturally occurring BRD were sampled sequentially by NS, guarded DNP, TTW, and then BAL. Samples were cultured and Mh and Pm isolates were maintained at -80 °C until sent to a diagnostic laboratory for AS testing according to CLSI recommendations. For each antimicrobial, MIC_{50} and MIC_{90} were calculated, and isolates were categorized as susceptible or not. To evaluate agreement between AS obtained from TTW and AS obtained by other sampling methods, a McNemar's test was used to compare methods with respect to their marginal proportions of positive results. Then, agreement among diagnostic tests was evaluated by calculation of the kappa statistic and the percent positive agreement. Categorical discrepancies were recorded as minor, major and very major errors.

Results

For *P. multocida*, isolates were routinely susceptible to ceftiofur, spectinomycin and tulathromycin. In contrast, susceptibility to penicillin was inconsistent (24-39% intermediate across sampling methods) and isolates were routinely resistant to enrofloxacin and florfenicol. For *M. haemolytica*, isolates were routinely susceptible to penicillin, ceftiofur, enrofloxacin, danofloxacin, florfenicol and tulathromycin. In contrast, susceptibility to tilmicosin was inconsistent and isolates were routinely resistant to spectinomycin.

For *P. multocida,* minor errors were seen in up to 18.4% of classifications for enrofloxacin, 29% of classifications for florfenicol, 23.7% of classifications for penicillin, and 3.8% of classifications for tulathromycin. Additionally, very major and major errors were seen when comparing florfenicol and tulathromycin susceptibilities across sampling methods. For *M. haemolytica,* minor errors were seen in up to 10% of classifications for enrofloxacin, 8.3% of classifications for florfenicol and penicillin, and 10% of classifications for spectinomycin. Additionally, very major errors were seen when comparing spectinomycin across sampling methods.

Of the 13 animals that had *M. haemolytica* recovered from all 4 locations, complete susceptibility data for all 4 samples and all 8 drugs was only obtained from 7 animals. Within those 8 animals, only 3 had samples that yielded identical susceptibility interpretations across all 4 samples for all drugs considered. For the 4 animals with discordant results, there was overall good agreement across most of the drugs when comparing TTW to the other 3 sample types. However, spectinomycin had poor agreement among the sampling locations with 2 major errors for BAL vs. TTW, 2 major errors for NPS vs. TTW, and 3 major errors for NS vs. TTW.

In many instances, all samples agreed in terms of S or R within a single organism and a single drug in which case Kappa values were equal to 1 with no variability. Some variability was seen in agreement for enrofloxacin in *M. haemolytica* for several comparisons and for enrofloxacin, penicillin and tulathromycin for *P. multocida*.

Significance

This study highlights the complexity of the respiratory tract microbiome, the dynamic nature of the microbial ecology of the bovine respiratory tract, and our incomplete understanding of the pathogenesis of BRD.

