Evaluating factors affecting recovery of *Mannheimia haemolytica* and *Pasteurella multocida*

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Introduction

Microbiological diagnosis is an important step in controlling and preventing bovine respiratory disease (BRD). Moreover, adequate transport storage type, elapsed time, and storage temperature before laboratory submission are critical for optimal results. The objective was to evaluate the effect of transport storage media, time and storage temperature on *Mannheimia haemolytica* (MH) and *Pasteurella multocida* (PM) yield using an in-vitro model simulation.

Materials and methods

Semi-quantitative (quadrant method) and quantitative culture methods using colony forming units per ml (CFU/ml) were used to recover MH or PM using an in-vitro model with swabs. In both approaches, samples were grown in 5% sheep blood agar plates. In each trial, a sterile PBS solution was inoculated with MH or PM, achieving 0.5 McFarland using OD600 between 0.08-0.1. A total of 58 sterile cotton swabs were inoculated in a culture solution with MH or PM and placed in either: 1) sterile falcon tube (DRY); 2) Aimes culture media with charcoal (ACM); or 3) Cary-Blair transport Agar (CBA). Swabs were evaluated for recovery of MH or PM at 3 temperatures: 4, 23 and 36°C; and assessed at 4 time points 0 (baseline), 8, 24 and 48 hrs. A multivariate mixed model was fitted to analyze the data using lme4 and lmerTest packages of R. When normality was not rejected, the dependent variable was the CFU/ml. The independent variables were storage media (DRY, ACM and CBA), time points (8, 24 and 48 hrs), and the interaction between storage media and time points. Each swab was considered as an independent measure. When the normality was rejected, the non-parametric Dunn all-pairs approach was used to compare CFU/ml between storage media, with 1 model created for each temperature and time point combination.

Results

The CFU/ml recovery of PM on samples stored at 4°C was lower for ACM when compared to DRY at 8 hrs ($P = 0.05$) but higher at 48 hrs ($P < 0.01$). For samples stored at 23°C, ACM had a higher CFU/ml recovery than DRY at 24 hrs ($P < 0.01$), and at 48 hrs, ACM and CBA were higher than DRY ($P < 0.01$). At all-time points, samples stored at 36°C had a higher CFU/ml recovery in ACM and CBA than DRY ($P = 0.02$). The CFU/ml recovery of MH on samples stored at 4°C was higher for ACM and CBA than DRY at time points 24 ($P < 0.01$) and 48 ($P < 0.01$). Samples stored at 36°C had a higher CFU/ml recovery for ACM and CBA than DRY at time point 24 ($P < 0.01$).

Significance

These results support the value of ACM and CBA for the recovery of PM and MH isolates, especially if samples were not refrigerated properly. Also, the combination of longer elapsed time and higher temperatures can impair diagnostic accuracy.

