

Prevalence of *Mycoplasma bovis* in a Southern NSW feedlot during high and low risk periods for bovine respiratory disease

R. J. Barnewall^{A,B,C,E}, I. B. Marsh^C, N. Sales^C, L. J. Stroud^C, T. M. Williams^B, M. A. Campbell^{A,B}, P. M. V. Cusack^{A,D} and J. C. Quinn^{A,B}

^A School of Animal and Veterinary Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga NSW 2678

^B Graham Centre for Agricultural Innovation (Charles Sturt University and NSW DPI), Wagga Wagga NSW 2678.

^C NSW DPI, Elizabeth Macarthur Agricultural Institute. PMB 4008, Narellan NSW 2567.

^D Australian Livestock Production Services, Cowra, New South Wales, 2794.

^E Email: rbarnewall@csu.edu.au

Bovine respiratory disease (BRD) is a multifactorial disorder in which the proliferation of bacterial pathogens may be influenced by pre-exposure to viral pathogens and behavioural and environmental stressors. The role of *Mycoplasma bovis* in the pathogenicity of BRD, as a non-commensal bacterium, is poorly understood and its recalcitrant nature complicates the detection in clinical samples. To overcome the limitations of conventional culture, we have utilised an absolute quantitative PCR approach (Kishimoto *et al.* 2017) to improve the detection of *M. bovis* in feedlot steers. To better understand the prevalence of this organism in the Australian feedlot system, we compared induction to hospital pen animals using qPCR and compared two DNA extraction techniques.

Nasal swabs were collected at feedlot induction, and/or processing of hospital pen steers in a Southern NSW commercial feedlot. Fifty-one nasal swabs were collected at induction during a low risk (October 2018) and 220 during a high BRD risk period (April 2019) (Barnes *et al.* 2015). A further 54 hospital pen animals were sampled during the high risk BRD period (Charles Sturt University ACEC Protocol A18070). Swabs were stored in Nucleic Acid Preservation (NAP) buffer (Camacho-Sanchez *et al.* 2013) for DNA extraction using a Qiagen BioSprint®, One-for-All Vet Kit (Cat No. 947057) and/or Phosphate Buffered saline (PBS) for direct heat extraction (boiling in PBS at 100°C for 10min). PCR was performed on all samples to identify the presence of *M. bovis* according to Tsuchiaka *et al.* (2016) with minor modifications.

Mycoplasma bovis was not detected in any induction animals from either BRD risk periods, compared to a prevalence of 66.7% of hospital pen animals. Fisher's exact test resolved a significance difference ($p < 0.05$) in the detection rate of *M. bovis* when comparing BioSprint® and direct heat extraction methods (Figure 1).

Sample inhibition did not cause the reduction in detection by the Biosprint® method as a 1:10 dilution of BioSprint® extracted samples did not increase detection of *M. bovis* (results not shown). Together this data suggests that direct heat extraction of field samples is the most effective method for accurate molecular detection of *M. bovis* in feedlot cattle.

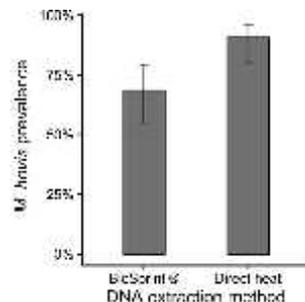


Figure 1. Prevalence of *M. bovis* in nasal swabs from hospital pen feedlot cattle sampled during a high risk period for bovine respiratory disease using two DNA extraction techniques (n=54). Error bars denote confidence intervals.

In conclusion, this study is the first to report a high prevalence of *M. bovis* detected in feedlot hospital pen animals compared to induction animals in an Australian feedlot by comparison of two different DNA extraction techniques. This suggests *M. bovis* may be playing a more critical role in BRD pathogenesis than previously considered. These findings will better inform treatment and management strategies for *M. bovis* associated respiratory disease and help minimise its economic impacts in Australian feedlots.

References

- Barnes T, Hay K, Morton J and Mahony T (2015) Project code: B.FLT.0225 *Meat and Livestock Australia*.
Camacho-Sanchez M, Burraco P, Gomez-Mestre I and Leonard JA (2013) *Molecular ecology resources*. **13**, 663-673.
Kishimoto M, Tsuchiaka S, Rahpaya SS, Hasbe A, Otsu K, Sugimura S, Kobayashi S, Komatsu N, Nagai M and Omatsu T (2017) *Journal of Veterinary Medical Science*. **79**, 517-523.
Tsuchiaka S, Masuda T, Sugimura S, Kobayashi S, Komatsu N, Nagai M, Omatsu T, Furuya T, Oba M and Katayama Y (2016) *Journal of Veterinary Medical Science*. **78**, 383-389.

This project was funded by the Meat and Livestock Donor Company under Project P.PSH.0873.