A novel serological assay for *Mycoplasma bovis*

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Mycoplasma bovis infection

- **Causes a range of diseases in cattle, including mastitis, arthritis and pneumonia**
- **Transmission** – fomites, contact, aerosols and consumption of infected milk
- Control
  - poor response and increasing resistance to antimicrobial agents
  - lack of an effective vaccine
- Diagnosis
  - sub-clinically infected calves are carriers for life and introduce infection into naive herds
  - Carriers shed intermittently and can remain undetectable in current commercially available diagnostic tests, such as PCR and culture
  - Serological diagnosis can be sensitive and has been proven to be reliable and effective as a method to indicate exposure within herds
- **We have developed an indirect ELISA and evaluated its performance in the field, as well as in experimentally infected calves.**

Methods and Results

- **Identification of an immunogenic protein**

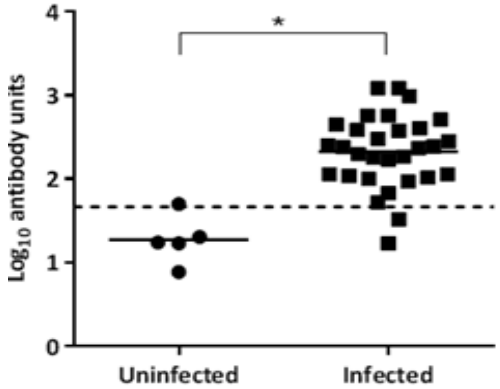
Mycoplasma immunogenic lipase A (MilA) was identified and the most immunogenic region was expressed as a recombinant protein

- **MilA ELISA**

An indirect ELISA was developed and optimised using the recombinant MilA protein as the capture antigen to detect antibodies specific for *M. bovis* in serum.

- **MilA ELISA performance in calves experimentally infected with *M. bovis***

Log10 antibody levels of calves on day 24 after infection.
bar = mean,
dashed line = cut-off,
* Significantly different



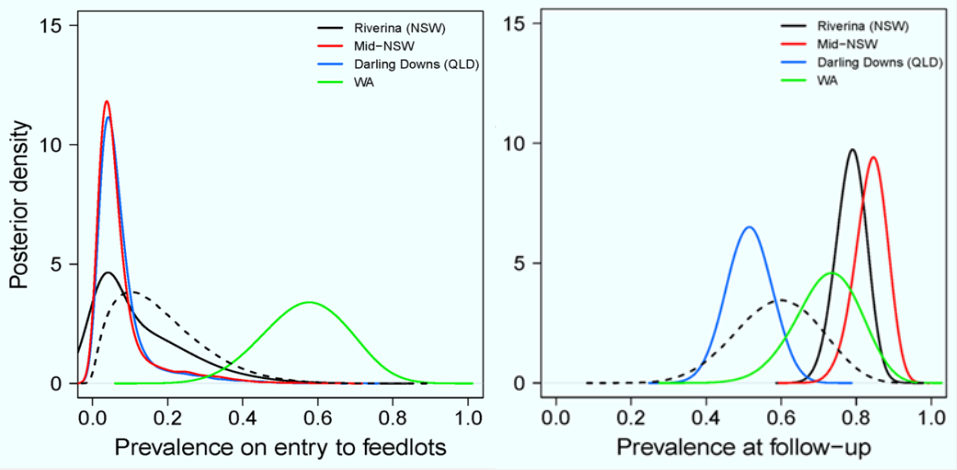
- **Comparison of the performance of commercial ELISAs and the MilA ELISA**

	True status		
	BIO K302	BIO K260	MilA ELISA
Relative sensitivity % (95% CI)	37 (22, 54)	13 (5, 30)	87 (70, 95)
Relative specificity % (95% CI)	95 (83, 99)	100 (91, 100)	90 (77, 96)

Methods and Results

• MilA ELISA performance in the field – Feedlot cattle

Paired sera from 7448 feedlot cattle from 14 feedlots across Australia (New South Wales, Queensland, South Australia, Western Australia). Bayesian latent class modelling applied to calculate the globally optimum cut-off.



Cut-off = 135 AU; Sensitivity = 94.3%; specificity = 94.4%
Cattle seropositive for infection with *M. bovis*: On entry = 13.1%; six weeks later =73.5%

• MilA ELISA for milk samples

Fifty blood samples from heifers (6-9 m olds) and milk from 100 cows from a Victorian dairy farm with a previous history of *M. bovis* outbreaks.

Seropositive
For exposure
To *M. bovis*:
Heifers = 54%
cows = 28%

Antibody titre

Future work

Investigations into the prevalence of *M. bovis* in Australian dairy herds using bulk tank milk samples.