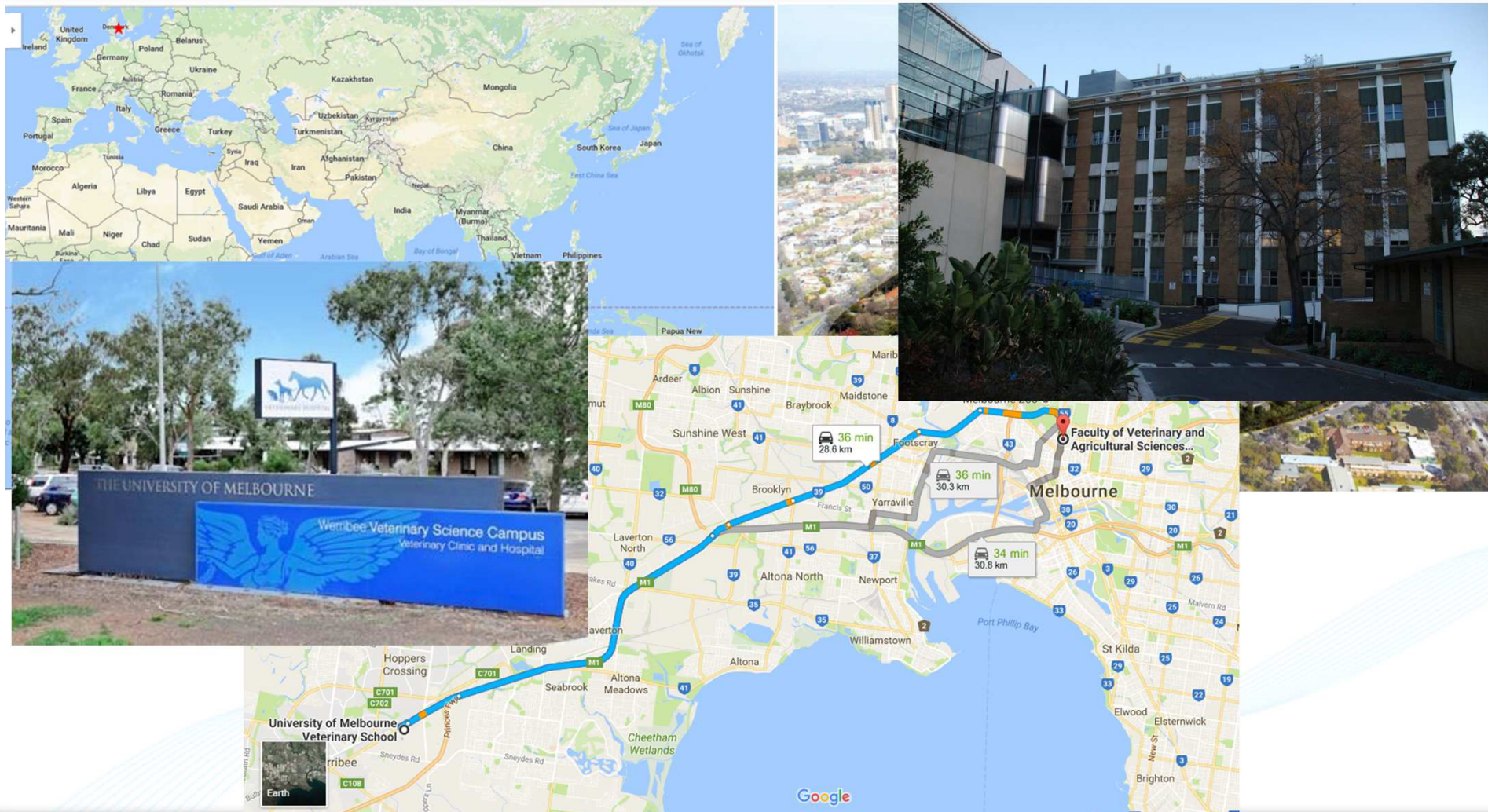


# ***Mycoplasma bovis* Diagnostics and New Developments in The Land Down Under**

**Nadeeka Wawegama (BVSc, PhD)**

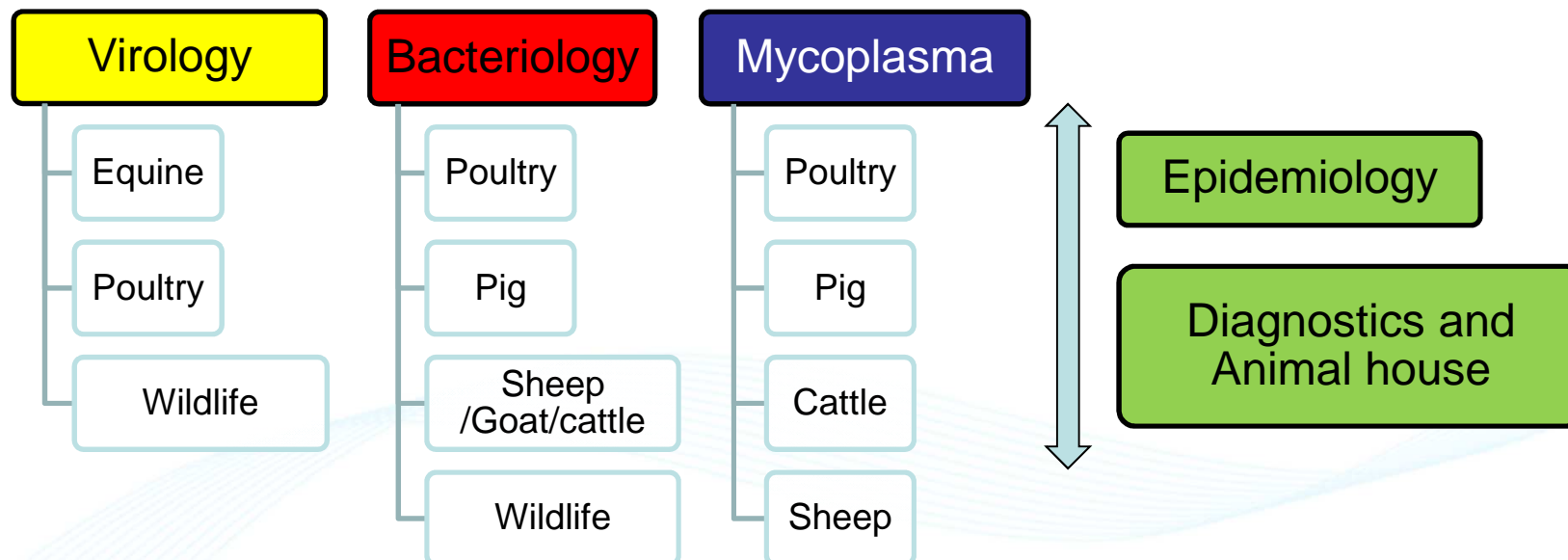
Faculty of Veterinary and Agricultural Sciences

University of Melbourne, Australia



*“...an internationally focused research and research training centre that aims to optimise animal production and product quality through improved control of infectious agents.”*

- Director; Professor Glenn Browning
- More than 50 staff and students



- Development of novel vaccines to control disease in poultry
  - *Mycoplasma gallisepticum* - chronic respiratory disease
  - *Mycoplasma synoviae* - infectious synovitis

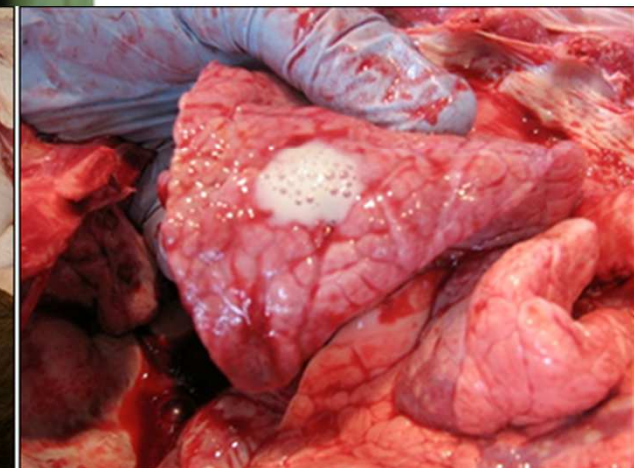


- Novel diagnostic antigens for serological diagnosis of mycoplasmosis in poultry

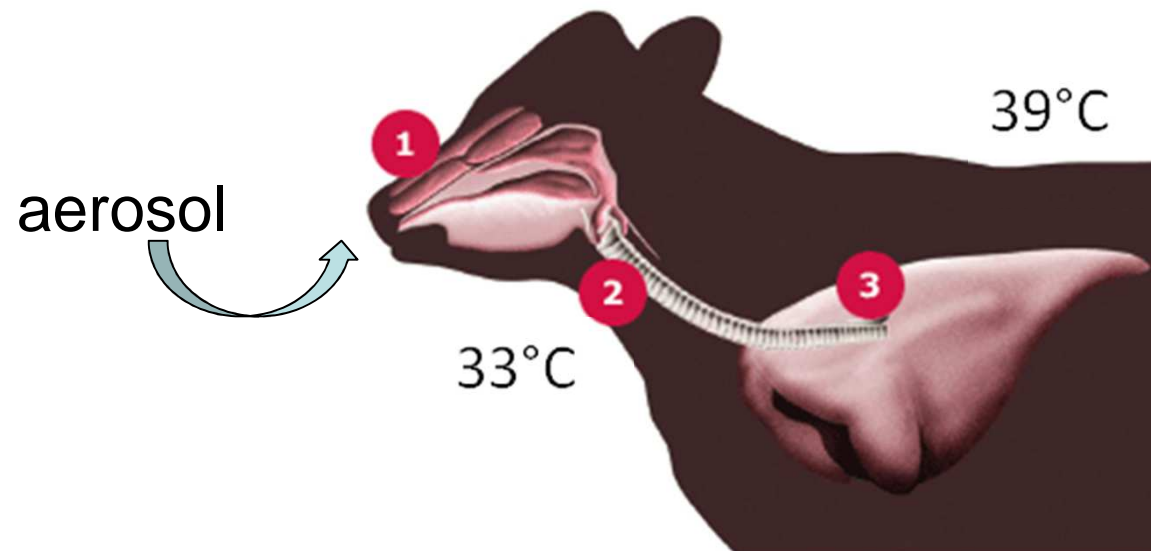
- Development of a novel vaccine for *Mycoplasma hyopneumoniae* – respiratory disease in pigs
  - registered in Mexico and currently undergoing field trials in Australia
- Development of a novel vaccine and serological diagnostic test for *Mycoplasma bovis*

# Development of a novel vaccine for *Mycoplasma bovis*

- Targeted to control respiratory tract infection in calves
- Developed an infection model to reproduce natural infection

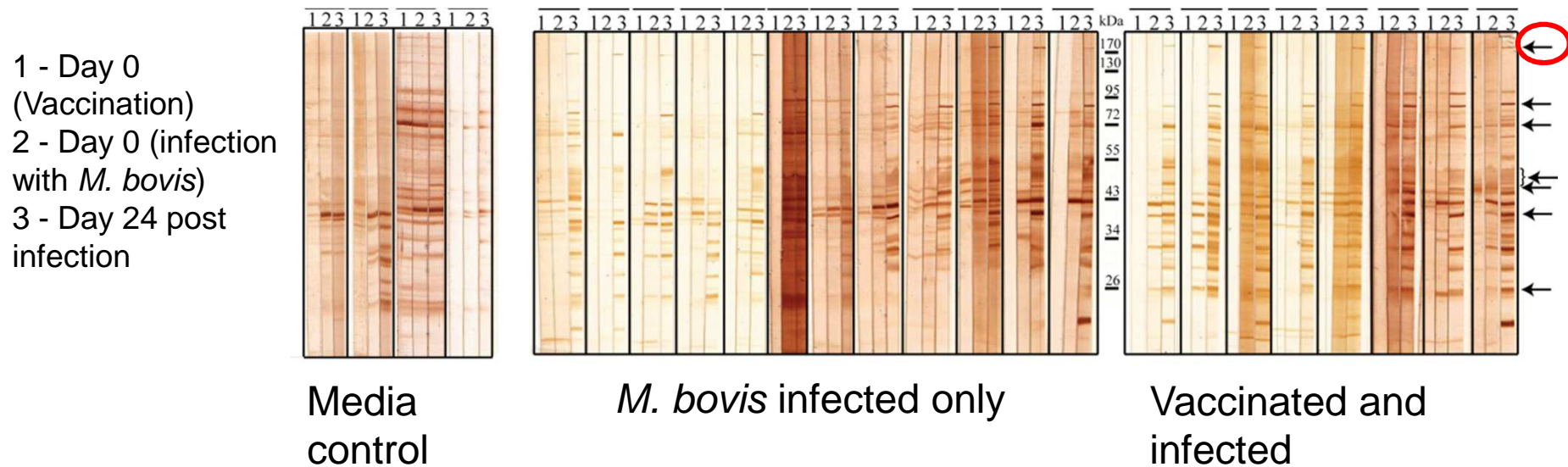


- Live temperature sensitive mutant (ts mutant)



- Patent process ongoing between Unimelb-  
APCAH-Zoetis Australia

- Identification of novel diagnostic antigens



- New protein- Mycoplasma immunogenic lipase A (MilA)



- Developed and optimised the MilA ELISA



Substrate- ABTS

Sheep anti-bovine antibody

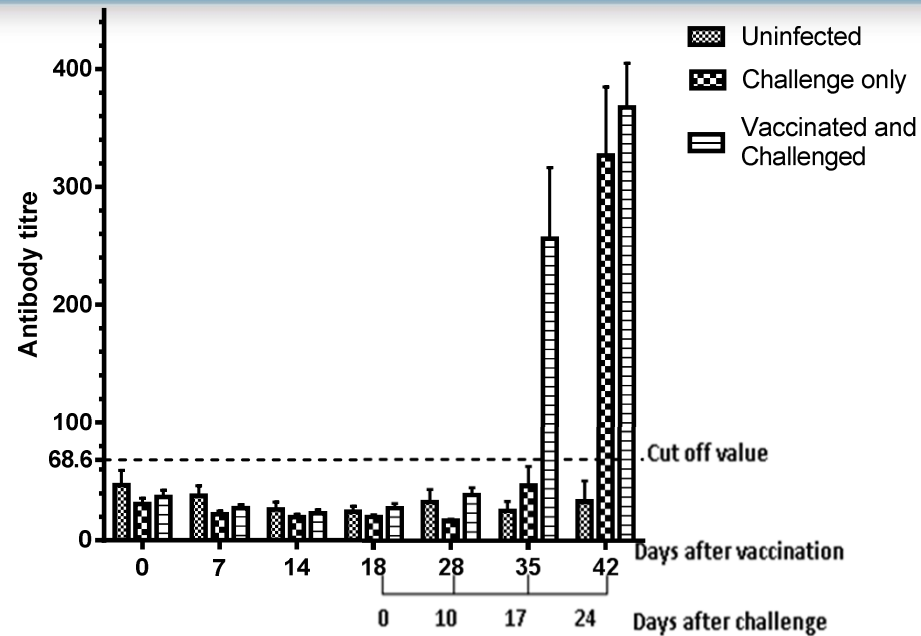
Test Calf serum

Antigen

- Plate design

	1	2	3	4	5	6	7	8	9	10	11	12
A	POSITIVE SERA STANDARD CURVE		SAMPLES 1/300									
B												
C												
D												
E												
F												
G												
H	BLANK											

# MiA ELISA performance in experimental *M. bovis* infections



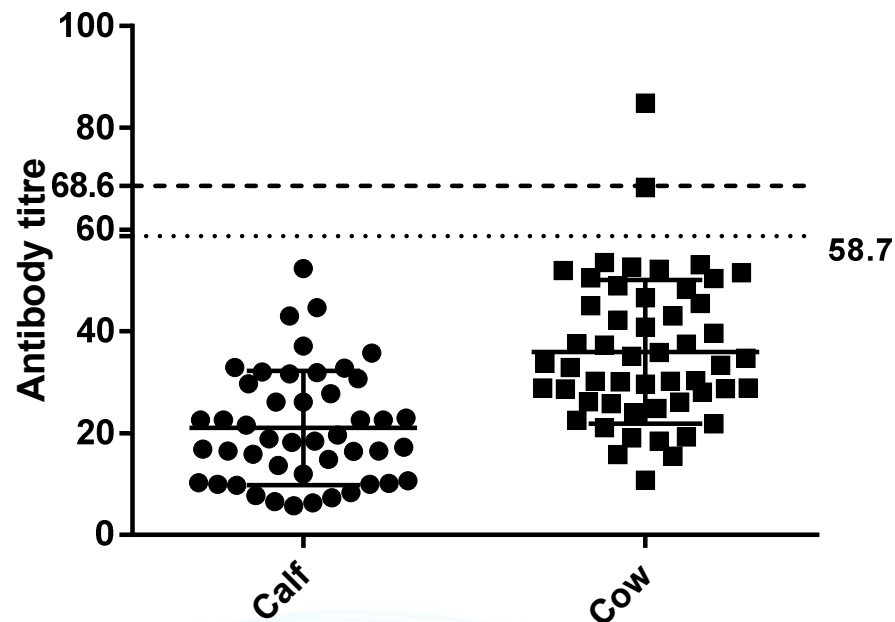
Group	No. calves	No. calves positive on day:							
		0	7	14	18	28	35	42	
		Days after challenge					10	17	24
Uninfected	9	2	0	0	0	0	0	0	
Challenged only	42	3	0	0	0	0	5	36	
Vaccinated and challenged	39	5	2	0	3	6	23	38	

# Comparison of the performance with the commercial ELISAs

	BIO K302	BIO K260	MiIA 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)

# MiA ELISA performance in the field/ adult cattle

- Serum samples from a farm in NSW – no previous exposure to *M. bovis* - 46 calves and 52 adult dairy cattle



- Specificity of 96%

# MiA ELISA performance in the field- Feedlot cattle

- *M. bovis* responsible for BRD in Feedlot cattle
- Prevalence unknown

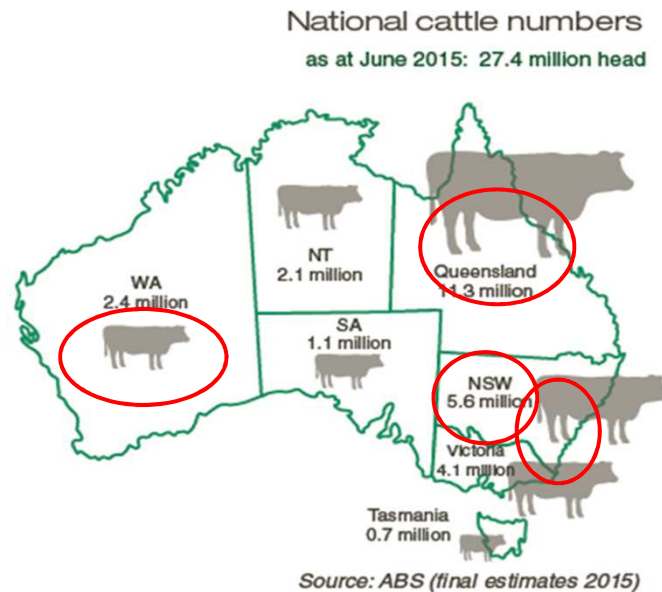


# MiA ELISA performance in the field- Feedlot cattle



# MiA ELISA performance in the field- Feedlot cattle

- Paired serum samples from 7448 feedlot cattle from 14 feedlots across Australia (NSW, QLD and WA)



Induction



6 weeks

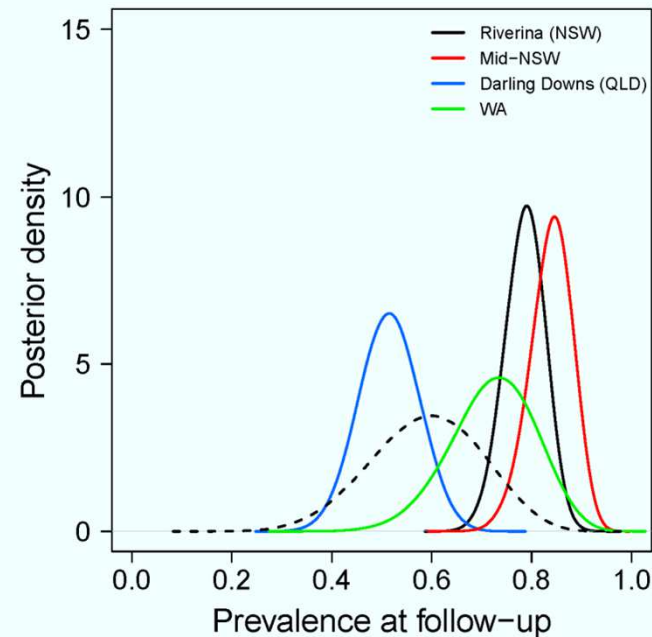
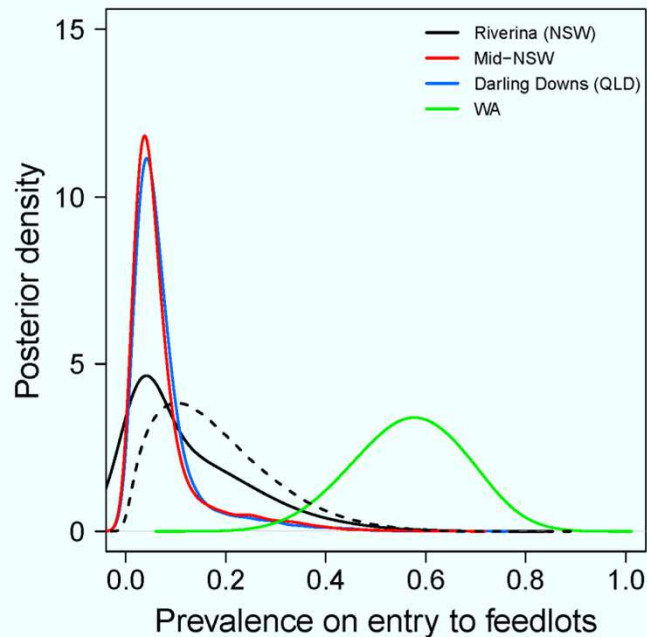
# MiA ELISA performance in the field- Feedlot cattle

- Test using MiA ELISA- total of 14896 serum samples
- Applied Bayesian latent class modelling to the results
- Calculated the globally optimum cut-off; 135 antibody titre
- Diagnostic sensitivity 94.3%, diagnostic specificity 94.4%





# MiA ELISA performance in the field- Feedlot cattle



- 13.1% of cattle were seropositive for infection with *M. bovis* on entry into feedlots, 73.5% were seropositive six weeks later
- Suggests a high risk of infection shortly after entry into feedlots

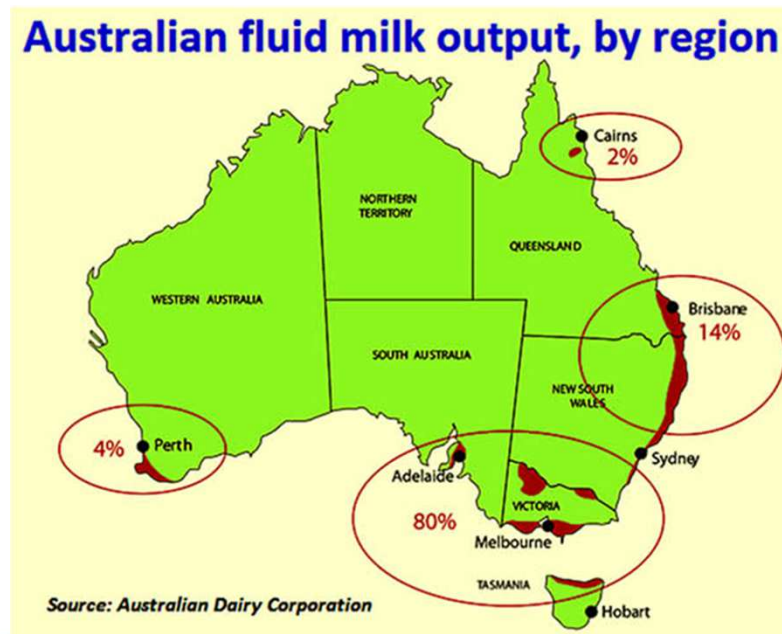
# Prevalence of *M. bovis* in Australian dairy herds



- National Dairy Herd: 1.74 million cows
- Average Herd Size: 284 cows
- *M. bovis* prevalence; ~3.5 % (Pathoproof PCR)

# Prevalence of *M. bovis* in Australian dairy herds

- Random cross-sectional study - similar proportions of dairy herds within each region within each state
- Repeat sampling three/two times



- Use bulk tank milk samples collected daily

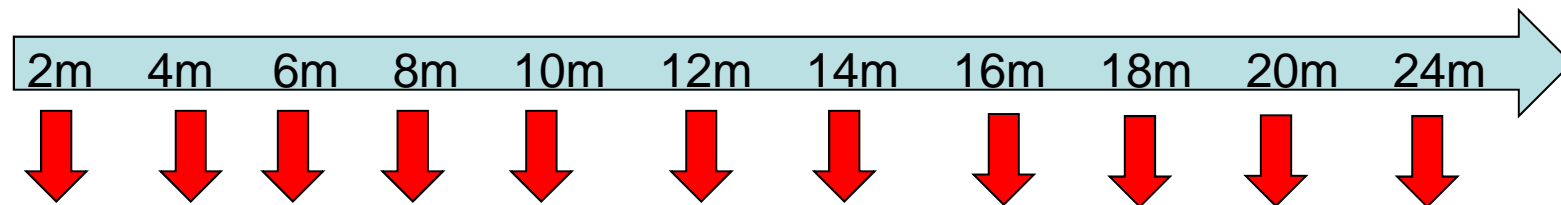
# Prevalence of *M. bovis* in Australian dairy herds

- Use optimised MiA ELISA to test bulk tank milk samples

	1	2	3	4	5	6	7	8	9	10	11	12	
A	POSITIVE SERA STANDARD CURVE		SAMPLES 1/20									NEGATIVE CONTROL	
B													
C													
D													
E													
F													
G													
H												BLANK	

- Calculate a global cut-off for bulk tank milk
- PCR (*uvrc* gene) for *M. bovis* positive samples

- Repeat sampling of a calf herd (n=90) from 2 months of age until they enter the dairy herd (*M. bovis* positive)



- Blood ; MiA ELISA
- Nasal swabs ; Culture and Universal mycoplasma PCR, *M. bovis* PCR
- Isolation of bovine mycoplasmas

# Thank you

- APCA, Uni Melbourne
  - Prof. Glenn Browning
  - Assoc. Prof. Phil Markham
  - Ms. Anna Kanci
  - Dr. Simon Firestone
- FVAS, Uni Melbourne
  - Assoc. Prof. Peter Mansell
  - Dominic Siu
- University of Queensland
  - Dr. Tamsin Barnes
  - Dr. Timothy Mahony
  - Dr. Megan Schibrowski
- Zoetis, Australia
  - Dr. Sally Oswin
- Warrnambool Veterinary clinic
  - Dr. Charles Blackwood
- Meat and Livestock Australia
- Dairy Australia