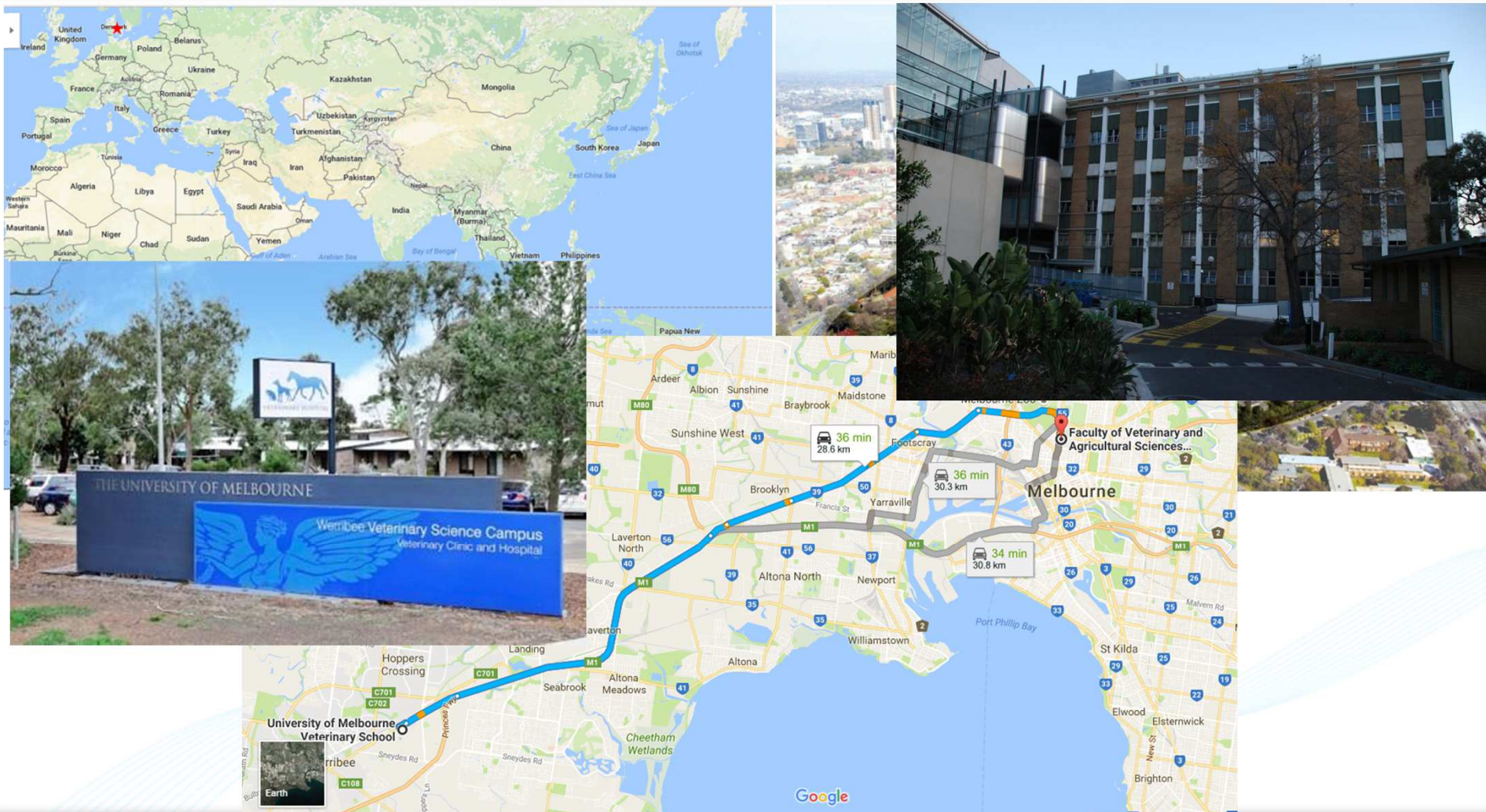# *Mycoplasma bovis* Diagnostics and New Developments in The Land Down Under

Nadeeka Wawegama (BVSc, PhD)
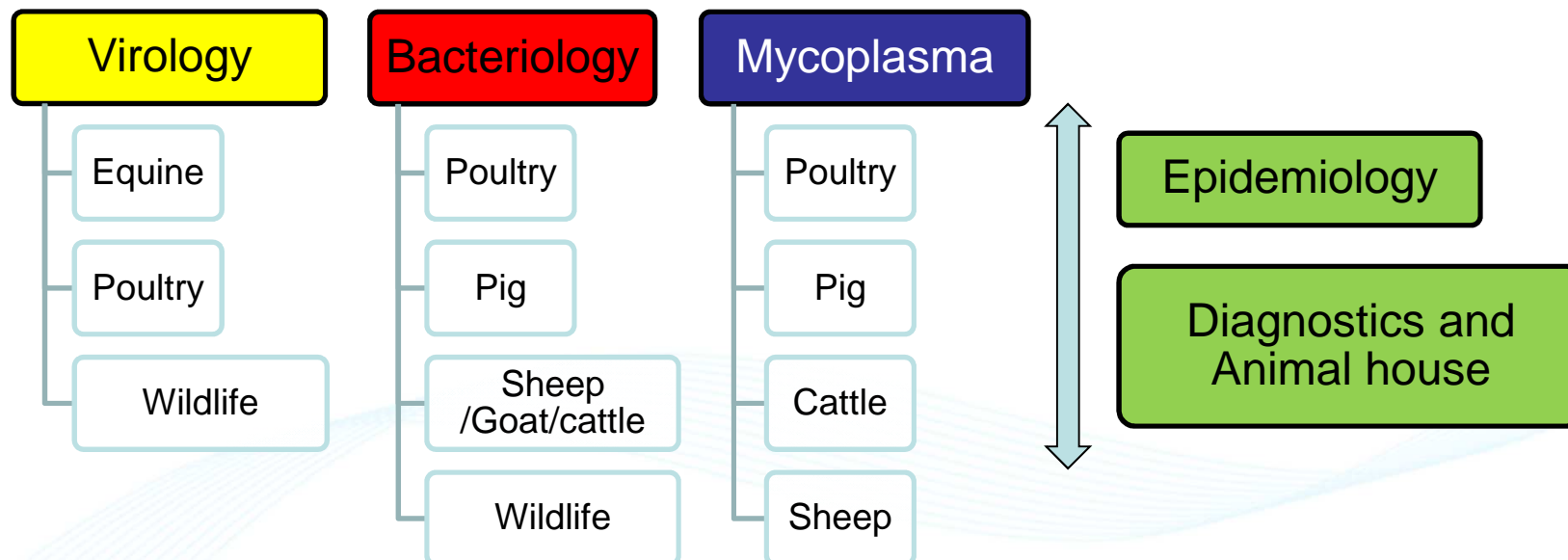
Faculty of Veterinary and Agricultural Sciences

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

Virology	Bacteriology	Mycoplasma
Equine	Poultry	Poultry
Poultry	Pig	Pig
Wildlife	Sheep /Goat/cattle	Cattle
	Wildlife	Sheep

Epidemiology

Diagnostics and Animal house

- 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
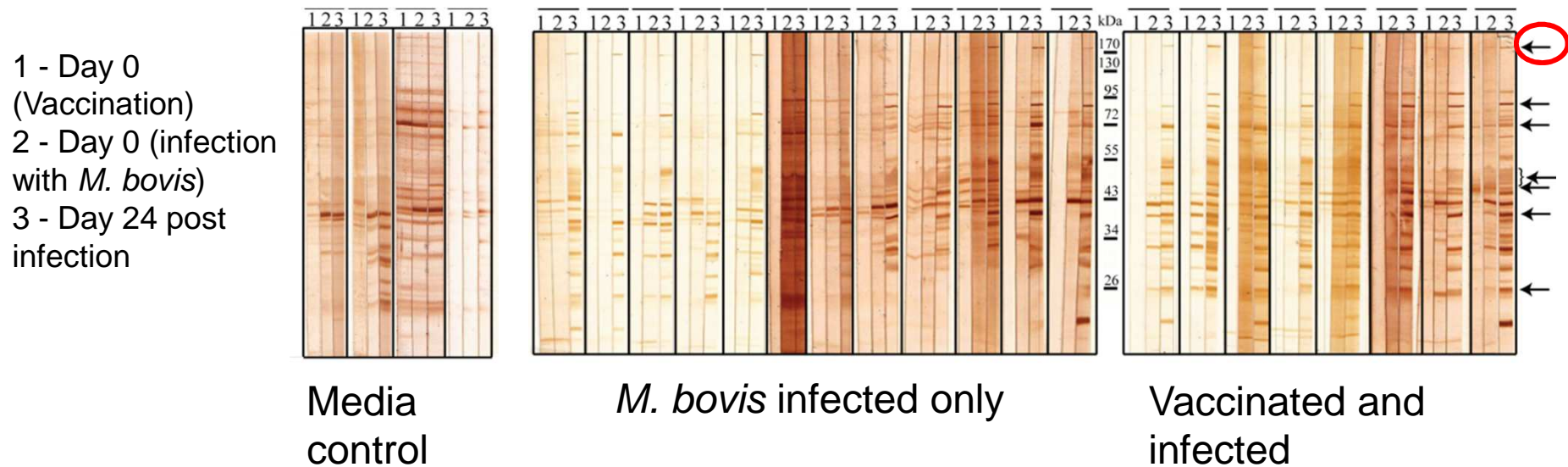- Developed an infection model to reproduce natural infection
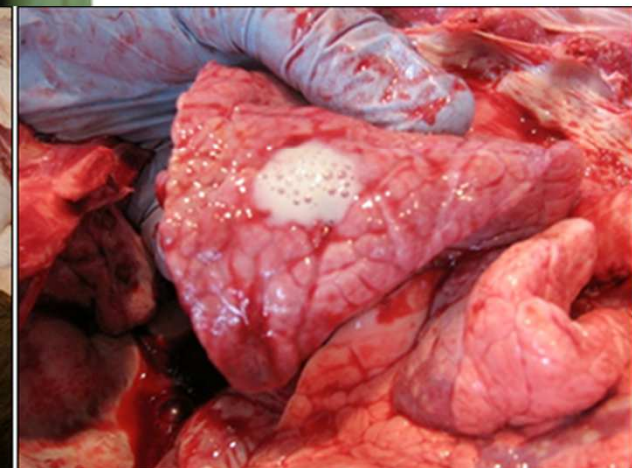
- Live temperature sensitive mutant (ts mutant)

aerosol

39°C

33°C
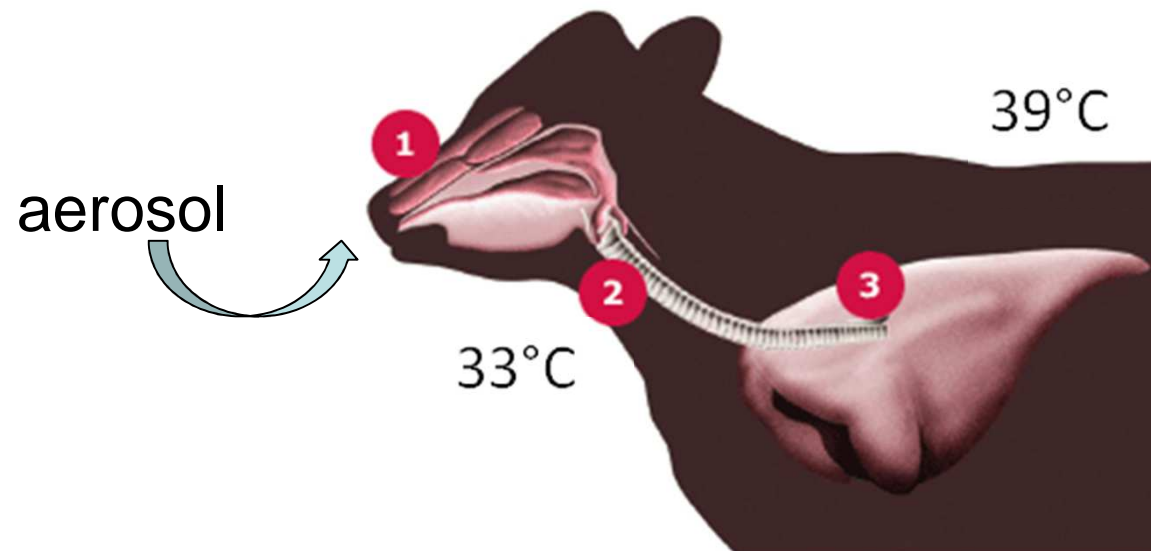
- Patent process ongoing between Unimelb-APCAH-Zoetis Australia

- Identification of novel diagnostic antigens

1 - Day 0
(Vaccination)
2 - Day 0 (infection
with *M. bovis*)
3 - Day 24 post
infection

Media
control

M. bovis infected only

Vaccinated and
infected

- New protein- Mycoplasma immunogenic lipase A (MilA)

- Developed and optimised the MiIA 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			SAMPLES 1/300									
B												
C												
D	POSITIVE SERA											
E	STANDARD CURVE											
F												
G											NEGATIVE CONTROL	
H											BLANK	

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

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Comparison of the performance with the commercial ELISAs

	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)

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

- Specificity of 96%

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

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

National cattle numbers
as at June 2015: 27.4 million head

WA
2.4 million

NT
2.1 million

Queensland
4.3 million

SA
1.1 million

NSW
5.6 million

Victoria
4.1 million

Tasmania
0.7 million
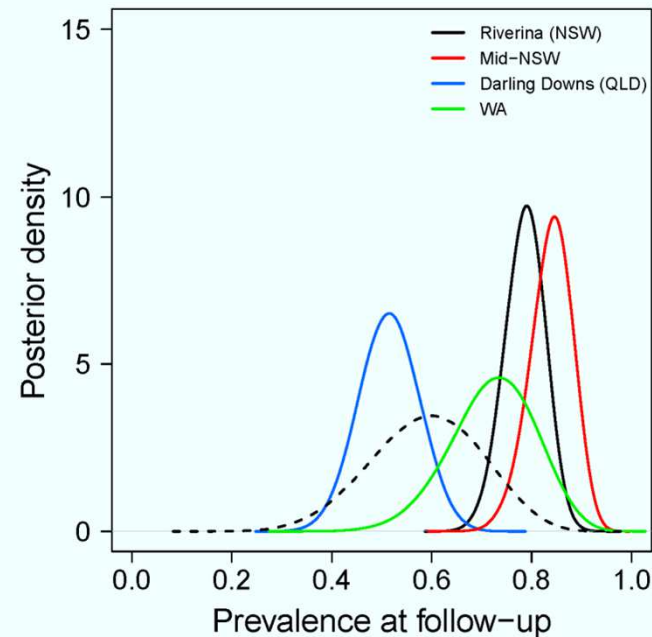
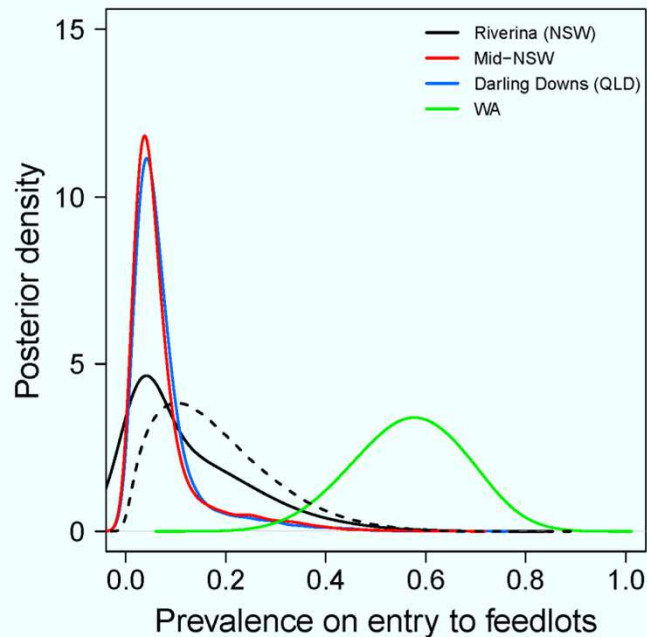Source: ABS (final estimates 2015)
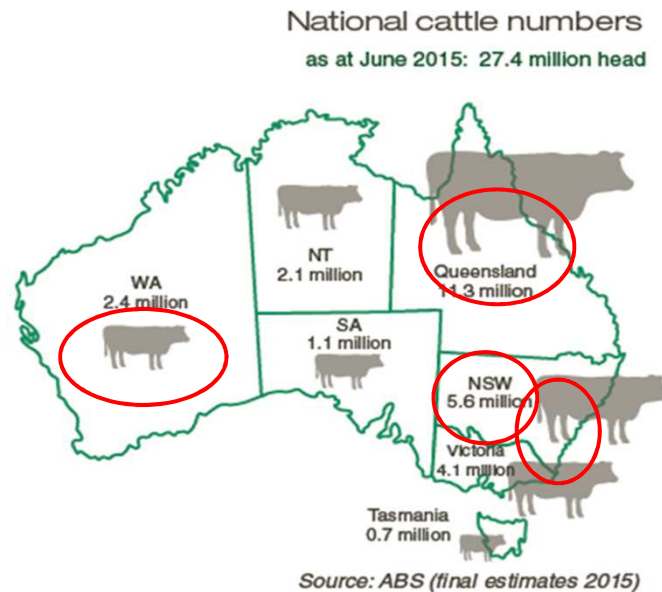
Induction

6 weeks

MiIA ELISA performance in the field- Feedlot cattle

- Test using MiIA 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%

- 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)

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

Australian fluid milk output, by region

Source: Australian Dairy Corporation
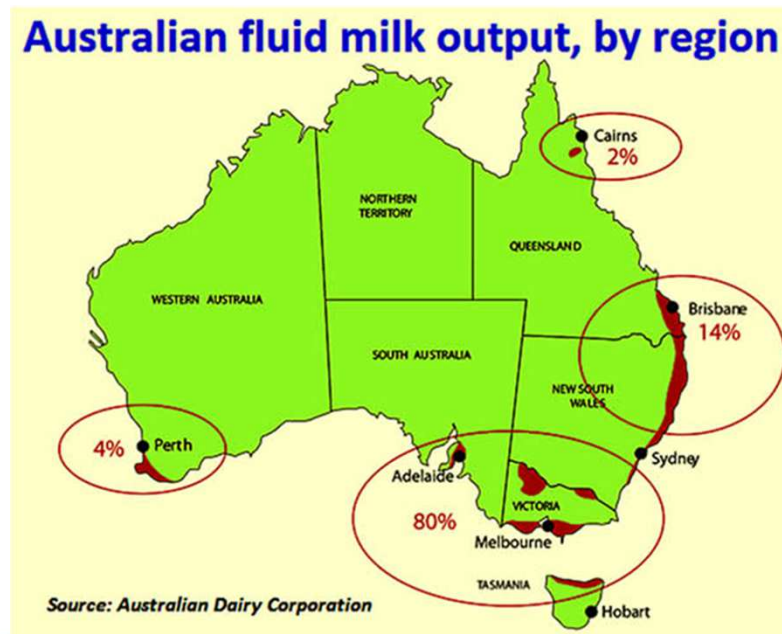
- Use bulk tank milk samples collected daily

- Use optimised MilA ELISA to test bulk tank milk samples

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

2m   4m   6m   8m   10m   12m   14m   16m   18m   20m   24m
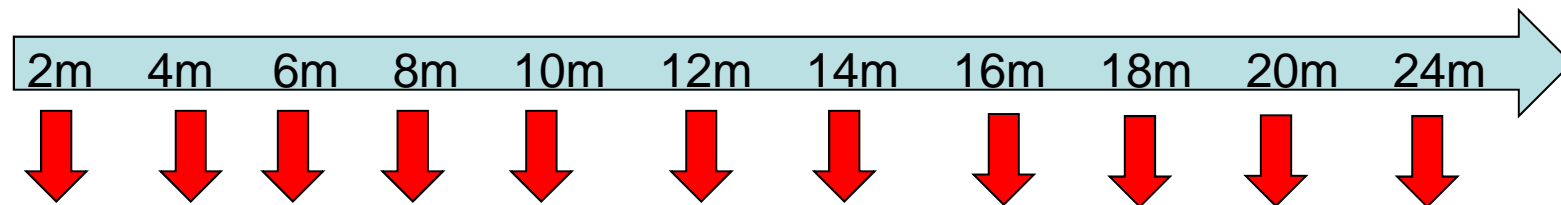
- Blood ; MilA ELISA
- Nasal swabs ; Culture and Universal mycoplasma PCR, *M. bovis* PCR
- Isolation of bovine mycoplasmas

Thank you

- APCAH, Uni Melbourne
  - Prof. Glenn Browning
  - Assoc. Prof. Phil Markham
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  - Dr. Timothy Mahony
  - Dr. Megan Schibrowski

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- Meat and Livestock Australia
- Dairy Australia