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Subclinical mastitis caused by *Mycoplasma*-like bacteria in dairy cattle in South Australia

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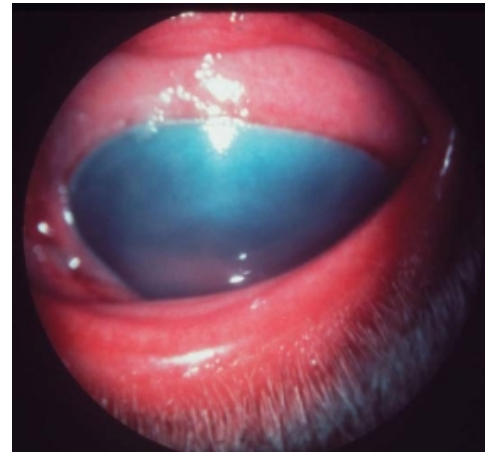
Mycoplasma

- **Smallest bacteria**
- **Mollicutes**
- **Cattle-associated**
 - *M. bovis*
 - *M. californicum*
 - *M. bovigenitalium*
 - *M. alkalescence*
 - *M. bovoculi*
 - *M. mycoides mycoides*
 - *M. dispar*
 - *Acholeplasma spp.*
 - *Ureaplasma diversum*



Disorders in cattle^{3,4}

- Pneumonia
- Mastitis
- Arthritis
- Keratoconjunctivitis
- Otitis media
- Urogenital tract disorders



Issues with *Mycoplasma*

- **Slow growing bacteria**
- **Special culture requirements**
- **Not part of routine mastitis culture**
 - **Do not grow on routine culture media**
- **Difficulties in survival**



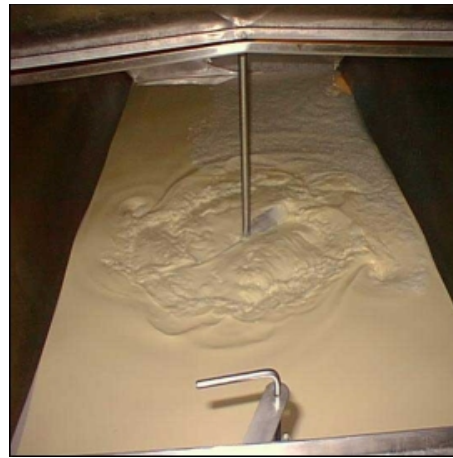
Mastitis

- **Clinical: acute, subacute and chronic**
- **sub-clinical**
- **Mycoplasma mastitis, undifferentiated mastitis**
- **SCC**
- **Milk production**



Economic impacts

- **US= US\$ 108 million annually**
- **Europe= US\$ 130 million annually**



http://www.overthecounter.cc/training_modules_view.asp?module=Cattle&id=69



Aims

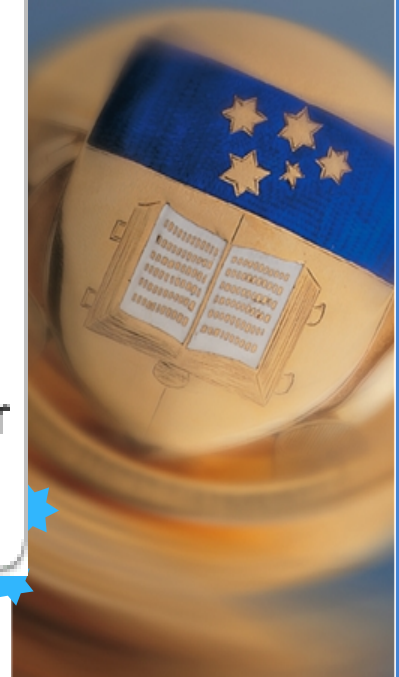
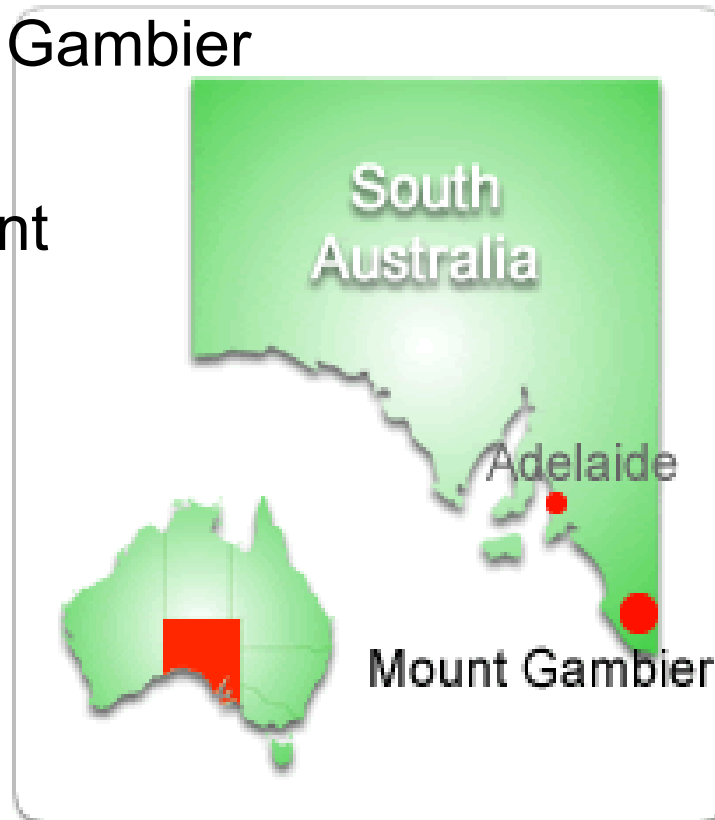
- * Identify and isolate *Mycoplasma* species by microscopic culture method
- * Examine the effects of *Mycoplasma*-like organisms compared to other mastitis pathogens on the test-day SCC and milk production
- * Develop and compare between different PCR detection methods for *Mycoplasma*
- * Evaluate the survival of *Mycoplasma* under different freezing conditions



Materials and methods

➤ Source of isolates

- Single farm from Mt Gambier
- High SCC
- High rate of treatment failure



Materials and methods

➤ Milk samples

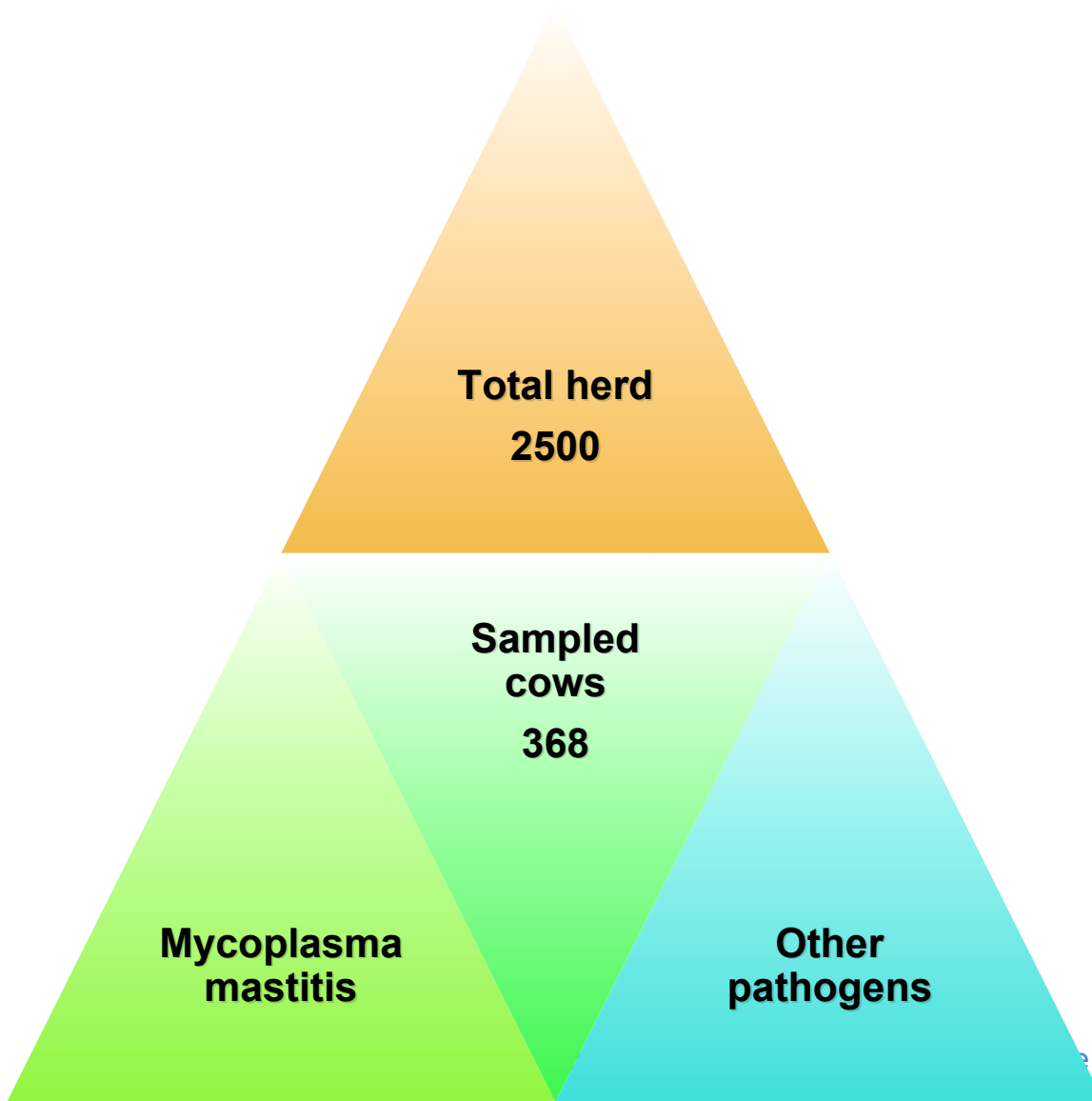
➤ Cow level

➤ 2 occasions

February 2015

September 2015



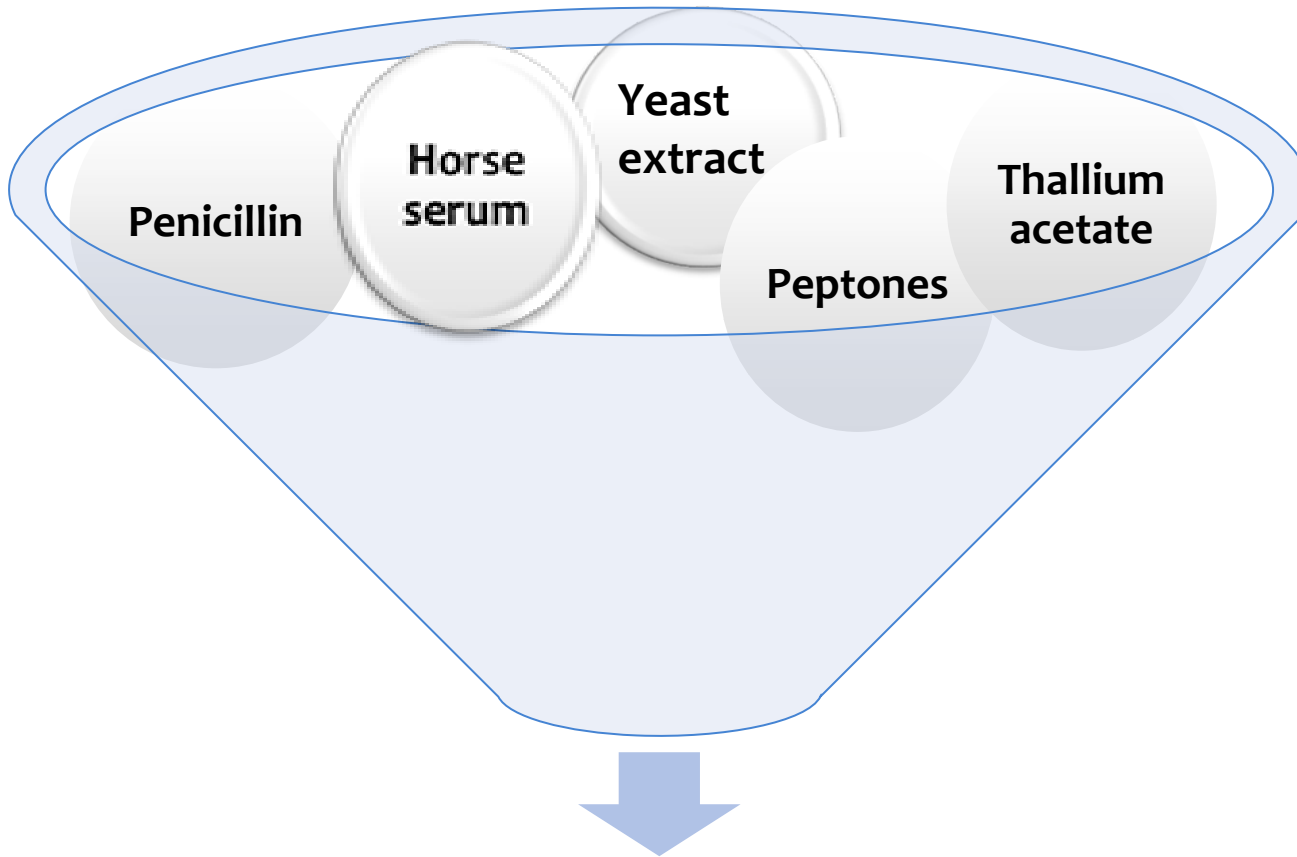


Materials and methods

- **Enrichment**
- Anaerobically for 5 days

- **Culture**
- Anaerobically 7-10 days





***Mycoplasma* selective media**



Molecular detection

- Development of PCR
Mycoplasma in milk & culture samples
- Run all samples

1

- Differentiate between *Mycoplasma* spp.
- 16S rRNA gene sequencing

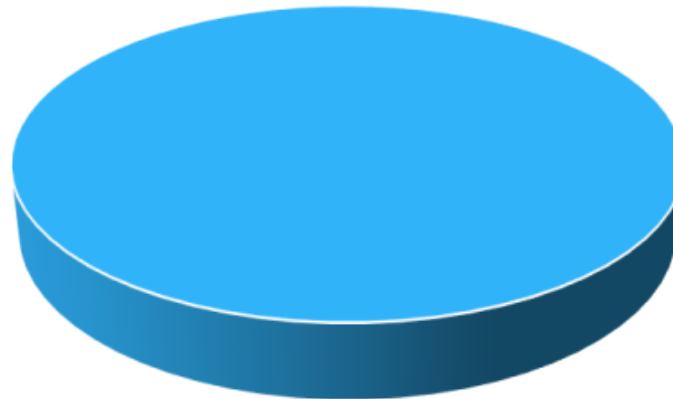
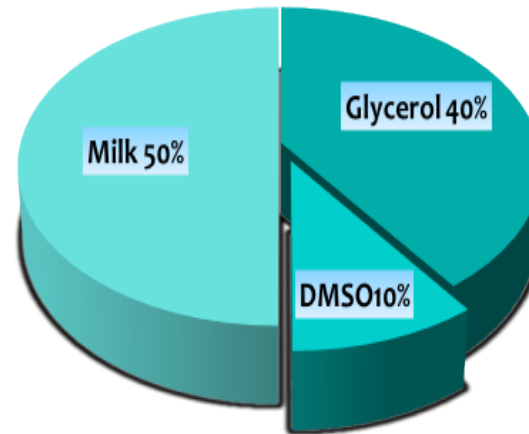
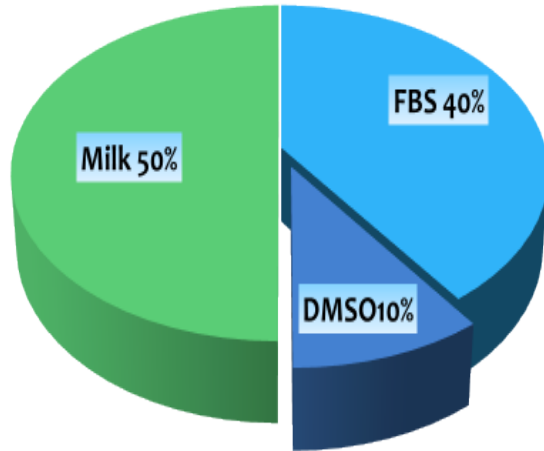
2

- Development of RT-PCR

3



Freezing Techniques



■ Milk -20 ■ ■ ■

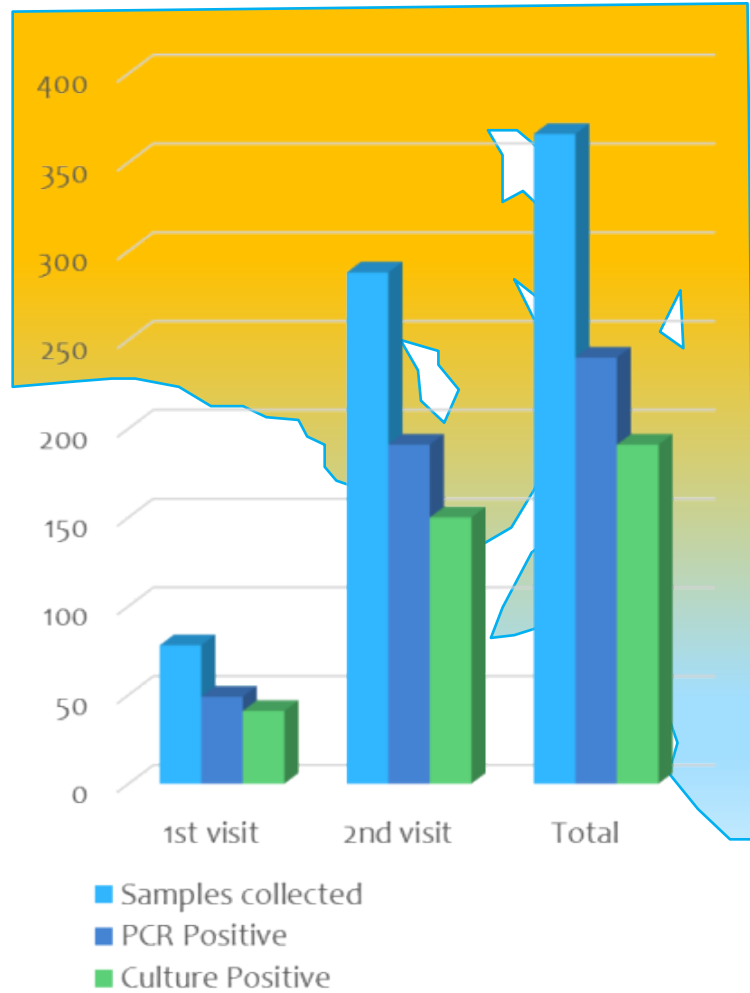




Results



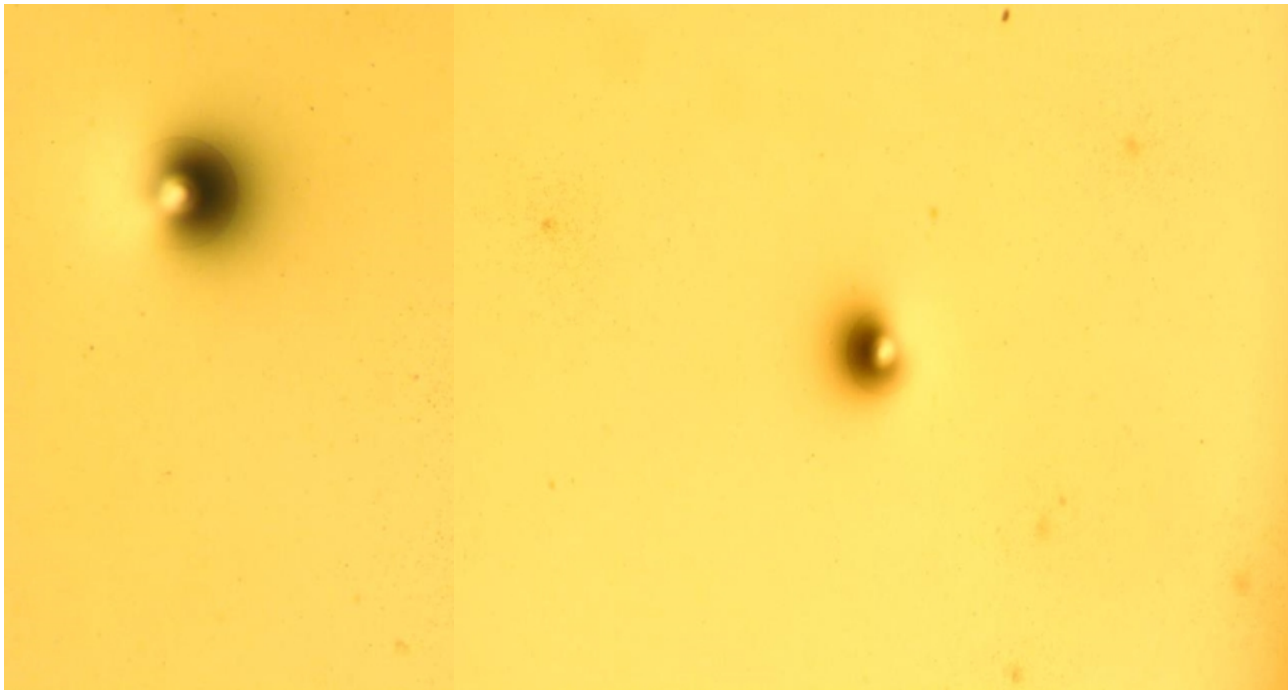
Culture & PCR results



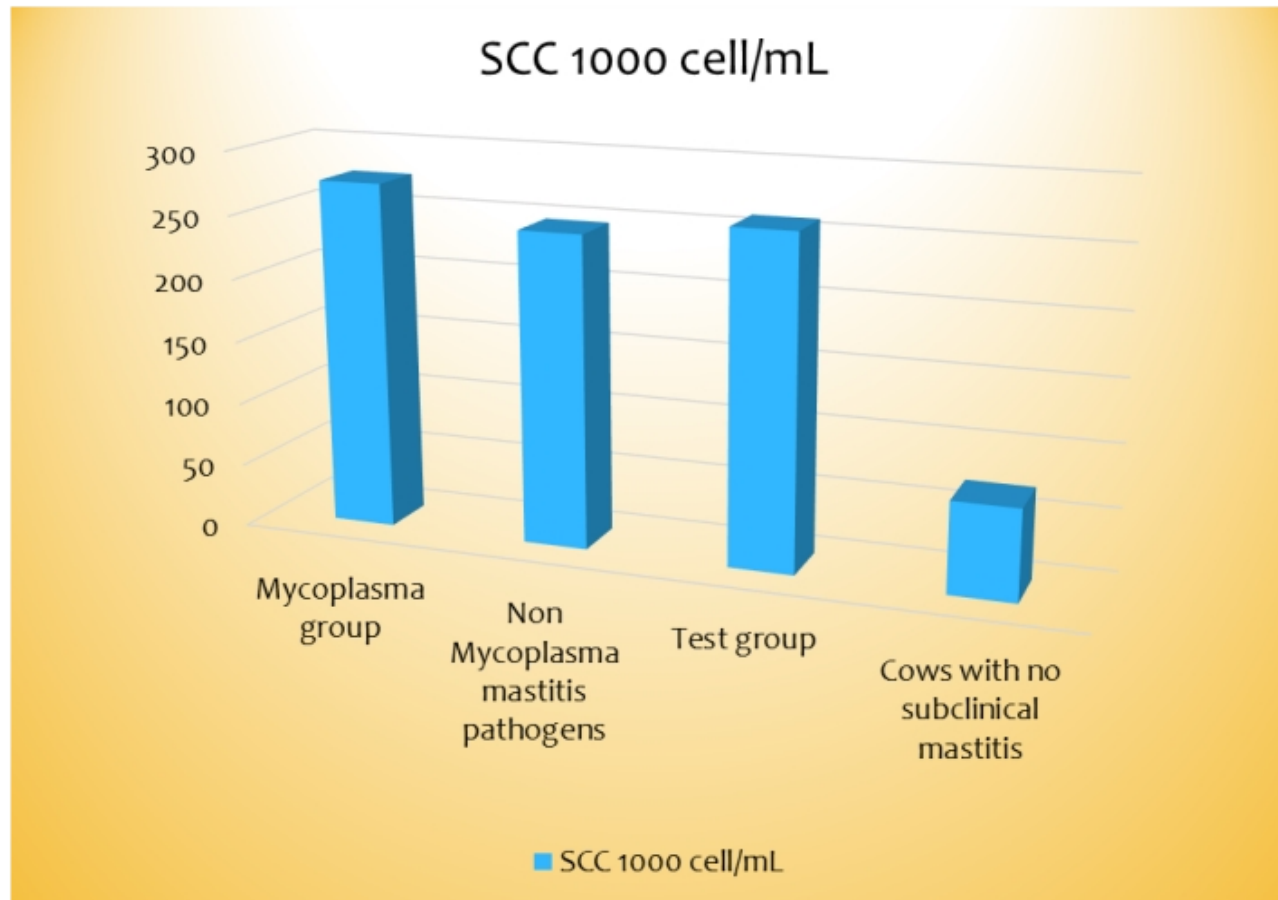


Mycoplasma colonies

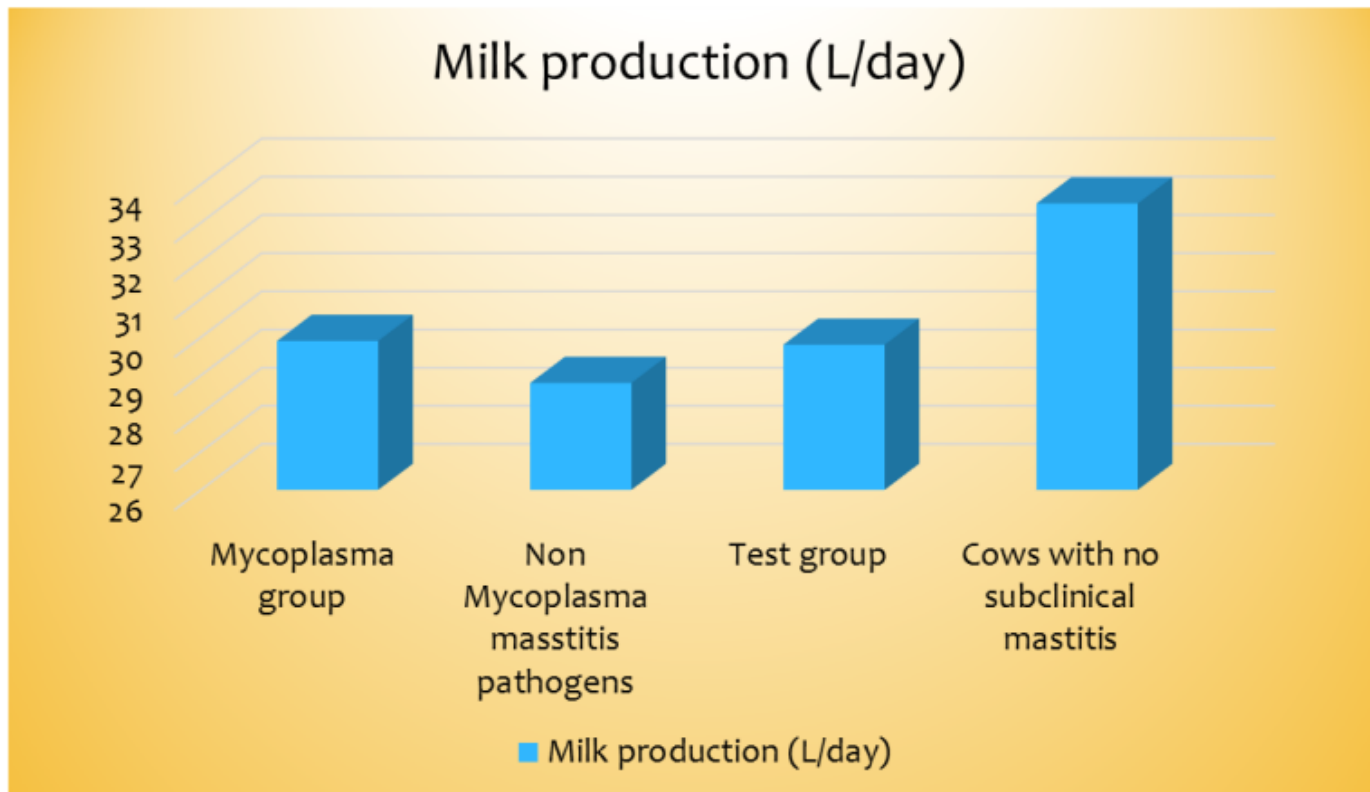
Typical fried egg appearance of colony of
Mycoplasma-like organisms under the
stereomicroscope (10 x magnification)



Somatic cell counts SCC

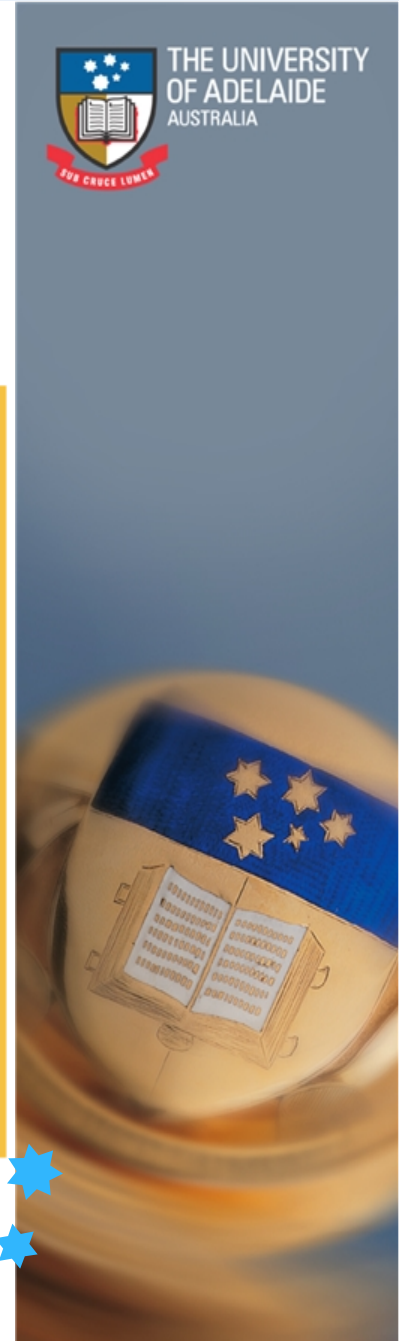
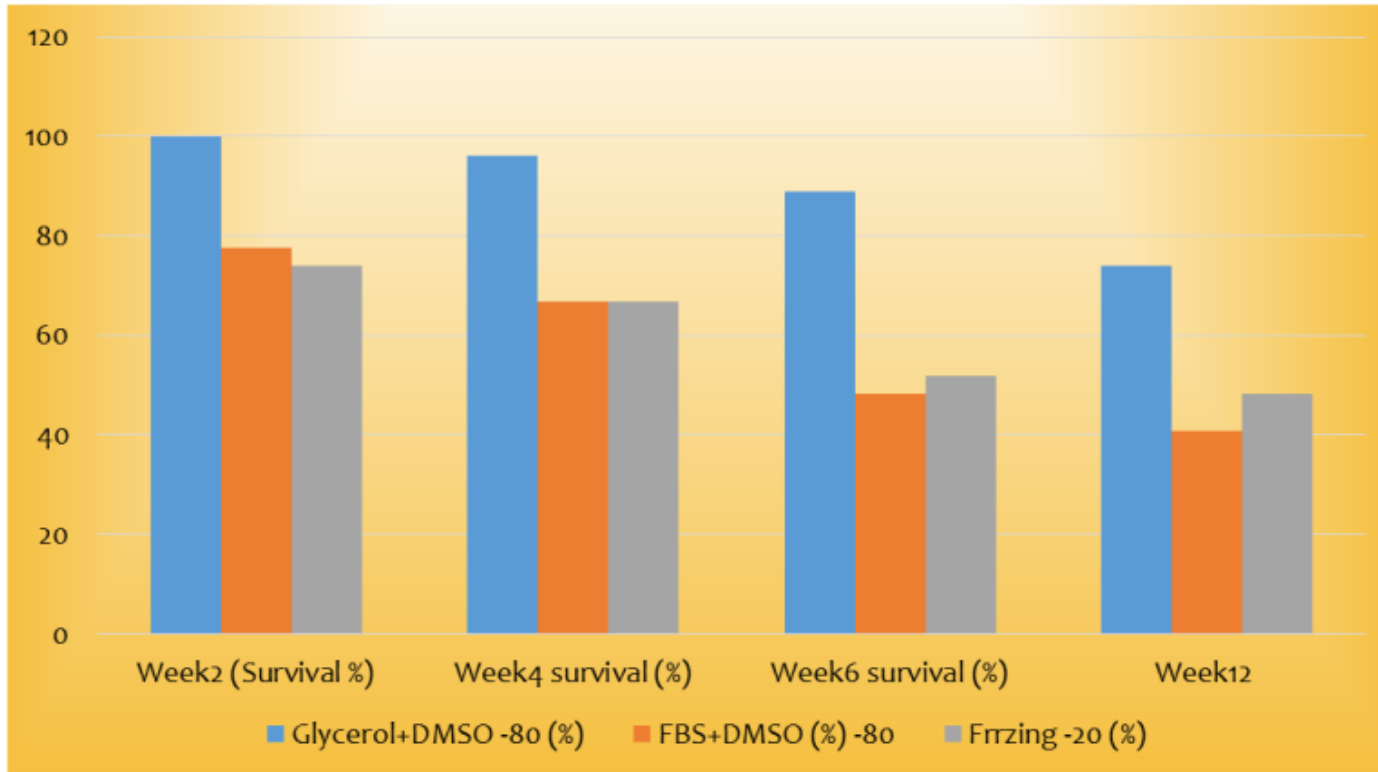


Milk production





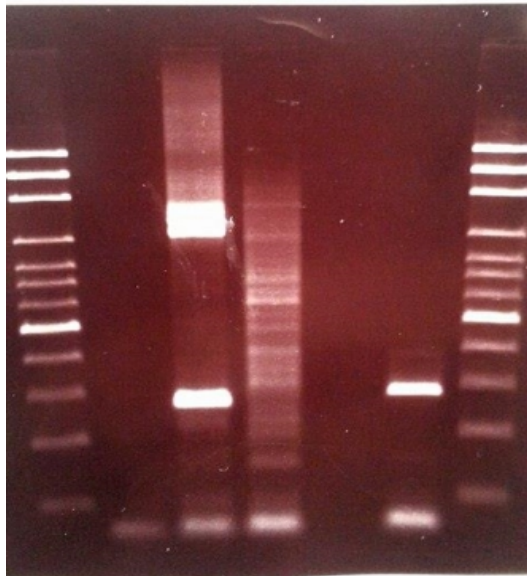
Survivability Results



Molecular results

Conventional PCR
Universal 16S rRNA

Chemidoc 2015-04-17 15hr 14rr



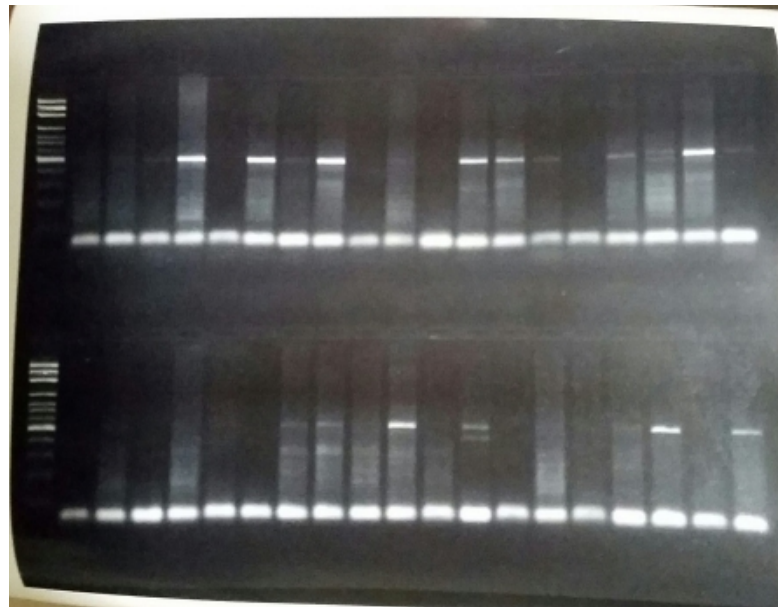
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Positive
sample

-ve sample

+ control

cPCR using *Acholeplasma* specific
primer



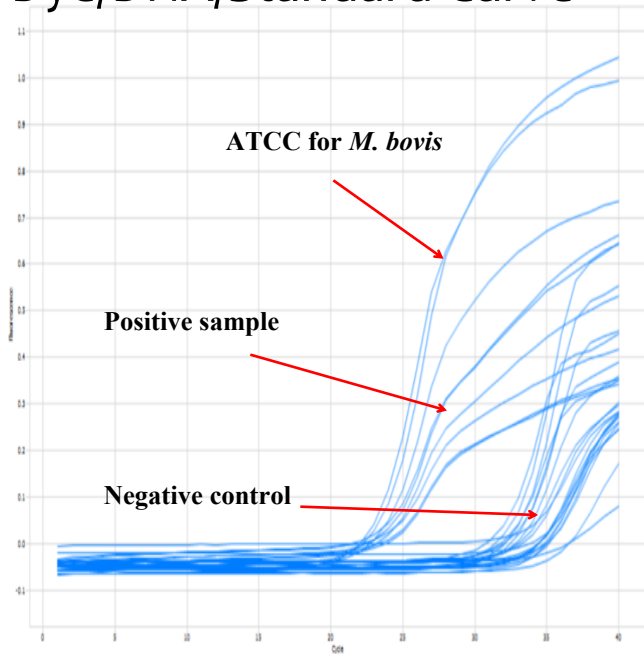
Life Impact The University of Adelaide



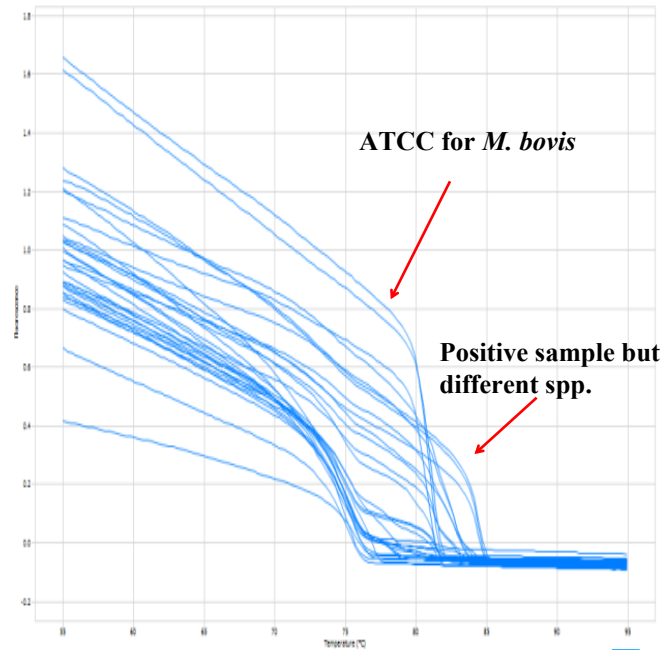


Real time PCR

Real Time PCR
Quantification/DNA Binding
Dye/DNA/Standard Curve



Component Melt Data



Sequencing

n=16

9/16 → *M. bovis*

7/16 → *Acholeplasma laidlawii*

	score	score	cover	value	
Acholeplasma laidlawii strain NEG175 16S ribosomal RNA gene, partial sequence	793	793	100%	0.0	100% KP742977.1
Acholeplasma laidlawii strain PG-8A 16S ribosomal RNA gene, complete sequence	793	793	100%	0.0	100% NR_074448.1
Uncultured bacterium clone Acl.a2_M01 16S ribosomal RNA gene, partial sequence	793	793	100%	0.0	100% JX146014.1
Acholeplasma laidlawii strain Algen 16S ribosomal RNA gene, partial sequence, 16S-23S ribosomal RNA intergenic spacer, complete sequence, and 23S ribosomal RNA gene, partial sequence	793	793	100%	0.0	100% JN935890.1
Acholeplasma laidlawii strain BN1-JA1 16S ribosomal RNA gene, partial sequence, 16S-23S ribosomal RNA intergenic spacer, complete sequence, and 23S ribosomal RNA gene, partial sequence	793	793	100%	0.0	100% JN935888.1
Acholeplasma laidlawii strain KHS 16S ribosomal RNA gene, partial sequence, 16S-23S ribosomal RNA intergenic spacer, complete sequence, and 23S ribosomal RNA gene, partial sequence	793	793	100%	0.0	100% JN935887.1
Acholeplasma laidlawii strain Concha-2 16S ribosomal RNA gene, partial sequence, 16S-23S ribosomal RNA intergenic spacer, complete sequence, and 23S ribosomal RNA gene, partial sequence	793	793	100%	0.0	100% JN935875.1
Acholeplasma laidlawii strain NBRC 14400 16S ribosomal RNA gene, partial sequence	793	793	100%	0.0	100% NR_113658.1
Acholeplasma laidlawii strain KSR01 16S ribosomal RNA gene, partial sequence	793	793	100%	0.0	100% HQ661833.1
Acholeplasma laidlawii strain R3.6 16S ribosomal RNA gene, partial sequence	793	793	100%	0.0	100% HQ661832.1
Acholeplasma laidlawii strain CIRG/Alw-1 16S ribosomal RNA gene, partial sequence	793	793	100%	0.0	100% FJ655561.1
Acholeplasma laidlawii strain TTB 103 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer, partial sequence	793	793	100%	0.0	100% FJ590758.1
Acholeplasma laidlawii strain Haij 179L 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer, partial sequence	793	793	100%	0.0	100% FJ226570.1
Acholeplasma laidlawii strain SRCD 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer, partial sequence	793	793	100%	0.0	100% FJ226569.1
Acholeplasma laidlawii strain REP 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer, partial sequence	793	793	100%	0.0	100% EU925161.1
Acholeplasma laidlawii PG-8A, complete genome	793	1586	100%	0.0	100% CP000896.1
A laidlawii 16S ribosomal RNA small subunit gene	793	793	100%	0.0	100% M23932.1
Acholeplasma laidlawii strain FJ-NP 16S ribosomal RNA gene, partial sequence	787	787	100%	0.0	99% KJ870649.1
Uncultured bacterium clone NTS002Powerb6_13634 16S ribosomal RNA gene, partial sequence	787	787	100%	0.0	99% JQ379532.2
Acholeplasma sp. ZJ2005 16S ribosomal RNA gene, partial sequence	787	787	100%	0.0	99% GU985440.1
Acholeplasma laidlawii strain PG8 16S ribosomal RNA gene, partial sequence	771	771	100%	0.0	99% NR_025961.1
Acholeplasma laidlawii 16S rRNA gene	767	767	98%	0.0	99% AM073014.1



Conclusion

- Awareness for the importance of *Mycoplasma* mastitis
- Cornerstone for further research



Acknowledgments

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 - Dr Kiro Petrovski
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- **South East Vets**
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References

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Thanks

