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

- 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
Total herd
2500

Sampled cows
368

Mycoplasma mastitis

Other pathogens
Materials and methods

- **Enrichment**
  - Anaerobically for 5 days

- **Culture**
  - Anaerobically 7-10 days
Mycoplasma selective media
• Development of PCR for *Mycoplasma* in milk & culture samples
• Run all samples

2.

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

3.

• Development of RT-PCR
Freezing Techniques

- Milk 50%
- DMSO 10%
- FBS 40%
- Glycerol 40%

Milk -20°C
Results
Culture & PCR results

- Samples collected
- PCR Positive
- Culture Positive
Mycoplasma colonies

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

SCC 1000 cell/mL

- Mycoplasma group
- Non Mycoplasma mastitis pathogens
- Test group
- Cows with no subclinical mastitis

Life Impact  The University of Adelaide
Milk production

Milk production (L/day)

Mycoplasma group
Non Mycoplasma masstis pathogens
Test group
Cows with no subclinical mastitis

Milk production (L/day)
Survivability Results

- Week 2 (Survival %)
- Week 4 survival (%)
- Week 6 survival (%)
- Week 12

Glycerol + DMSO -80 (%)  
FBS + DMSO (%) -80  
Freezing -20 (%)
Molecular results

Conventional PCR
Universal 16S rRNA

$cPCR$ using $Acholeplasma$ specific primer

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Real time PCR

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

Component Melt Data

ATCC for *M. bovis*

Positive sample

Negative control

ATCC for *M. bovis*

Positive sample but different spp.
Sequencing

n=16

9/16 → *M. bovis*

7/16 → *Acholeplasma laidlawii*
Conclusion

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


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