Chlamydia pecorum and arthropod-associated Chlamydiae identified in an expanded range of marsupials and their parasitising tick species

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Co-Supervisor: Professor Peter Timms
Bacteria of the *Chlamydiales* are ubiquitous wildlife pathogens

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Chlamydia pecorum infections in koalas

The biggest infectious disease threat to koalas are caused by C. pecorum

- Prevalence of 10 - 90% in mainland koala populations
- Routes of transmission are not confirmed, strong support for sexual transmission
- Early stage infections are treatable with antibiotics
- If left untreated infections can progress to blindness and infertility often resulting in death

(Polkinghorne et al., 2013)
Can *C. pecorum* infect other marsupials?

There is limited evidence of chlamydial infections in non-koala marsupials

- Mountain brushtail possums
- Western barred bandicoots
- Greater bilby’s
- Gilberts potoroo
- Greater gliders

- Ocular chlamydiosis was observed (~40%) however disease presentation was not dependent on *Chlamydiales* or *C. pecorum* PCR positivity (~30%)

(Bodetti et al., 2003; Warren et al., 2005; Kumar et al., 2007)

Few studies which lack depth and breadth, while larger marsupials such as kangaroos, wallabies and quolls remain unscreened
Koala and Australian livestock *Chlamydia pecorum*

*C. pecorum* infections are also prevalent in livestock globally

Genotyping has revealed distinct Australian *C. pecorum* strains:
- Koala strains
- Livestock strains
- Koala and sheep strains
- Australian strains identical to those found in European and American livestock (Jelocnik et al., 2013, 2014, 2015; Bachman et al., 2014, 2015)

(Walker et al., 2015)
Presence of *Chlamydiales* in ticks suggests vector transmission

*Chlamydia* species were first isolated from ticks in 1969 (Eddie et al., 1969)

- Recent studies are detecting a range of *Chlamydiales* prevalence and diversity

- Switzerland = 28.1% and
- Algeria = 45% (Croxatto et al., 2014)
- Switzerland = 6.4% (Pilloux et al., 2015)

➢ This evidence confirms that’s ticks harbour *Chlamydiales*, however, such high biodiversity suggests ticks may serve not only as a reservoir but also a vector of *Chlamydiales*
To fill in the knowledge gaps surrounding the origin, genetic relationships, transmission and prevalence of *C. pecorum* and other *Chlamydiales* infecting native Australian marsupials and livestock, I aimed to:

1) Identify the prevalence and diversity of chlamydial agents in tick species parasitising Australian wildlife

2) Identify the prevalence, diversity and host range of chlamydial infections in non-koala marsupials
Tick collection and processing

Ticks are removed from marsupials presenting at:

- Port Macquarie Koala Hospital
- Endeavour Veterinary Ecology
- Australia Zoo Wildlife Hospital

- Cleaned
- Identified to the species level
- Separated based on species, sex and developmental stage
- Pooled
- Stored in 80% ethanol
- Modified quadrisection
- DNA extraction QIAmp DNA mini kit

<table>
<thead>
<tr>
<th>Wildlife tick host</th>
<th>Ixodid species, (number), state collected</th>
</tr>
</thead>
</table>
| Koala (Phascolarctos cinereus)           | Ixodes tasmani (300) QLD, NSW  
I. holocyclus (100) QLD, NSW               |
| Bare nosed wombat (Vombatus ursinus)     | Bothriocroton auruginans (2) TAS  
I. tasmani (3) TAS                         |
| Eastern grey kangaroo (Macropus giganteus) | Haemaphysalis bancrofti (9) QLD                                               |
| Red necked wallaby (Macropus rufogriseus) | Ixodes holocyclus (1) QLD  
Haemaphysalis bancrofti (1) QLD  
Haemaphysalis longicornis (1) QLD          |
| Spotted tail quoll (Dasyurus maculatus)   | Ixodes holocyclus (4) NSW                                                     |
| Long nosed bandicoot (Perameles nasuta)   | Ixodes tasmani (1) QLD  
Ixodes holocyclus (1) QLD  
Haemaphysalis humerosa (1) QLD             |
| Platypus (Ornithorhynchus anatinus)       | Ixodes ornithorhynchi (2) QLD                                                 |
| Squirrel glider (Petaurus norfolcensis)   | Ixodes tasmani (2) QLD                                                        |
| Short eared possum (Trichosurus caninus)  | Ixodes tasmani (1) QLD                                                        |
| Brush tail possum (Trichosurus vulpecula) | Ixodes tasmani (1) QLD  
Ixodes holocyclus (6) QLD                   |
| Ring tailed possum (Pseudocheirus peregrinus) | Ixodes tasmani (2) QLD                             |
| Totals:                                  | n = 438, species = 7                                                          |

Marsupial swab collection and processing

Prospective sampling:
Ocular, urogenital and rectal swabs collected from each marsupial (AE# ANS1539)
- Australia Zoo Wildlife Hospital
- Eumundi Wildlife Rehabilitation Centre
- North Queensland Wildlife Care Inc.
- Port Macquarie Koala Hospital
- University of Tasmania

Retrospective sampling
- 217 DNA extracts from the Northern Territory that were collected as part of a large-scale study of small mammal health
- 126 DNA extracts from the Northern Territory that were collected as part of a re-introduction program of the northern quoll

<table>
<thead>
<tr>
<th>Marsupial Species</th>
<th>Total # of Individuals</th>
<th>Swab sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ocular</td>
</tr>
<tr>
<td><strong>Northern Territory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern quoll (Dasyurus hallucatus)</td>
<td>103</td>
<td>5/62</td>
</tr>
<tr>
<td>Northern brown bandicoot (Isoodon macrourus)</td>
<td>37</td>
<td>n/a</td>
</tr>
<tr>
<td>Common brushtail possum (Trichosurus vulpecula)</td>
<td>13</td>
<td>n/a</td>
</tr>
<tr>
<td>Fawn antechinus (Antechinus bellus)</td>
<td>1</td>
<td>0/1</td>
</tr>
<tr>
<td>Total number of marsupial species = 4</td>
<td>154</td>
<td>5/63</td>
</tr>
<tr>
<td><strong>East Coast</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ring tailed possum (Pseudocheirus peregrinus)</td>
<td>13</td>
<td>4/13</td>
</tr>
<tr>
<td>Common brushtail possum (Trichosaurus vulpecula)</td>
<td>26</td>
<td>22/28</td>
</tr>
<tr>
<td>Short eared possum (Trichosurus cinereus)</td>
<td>3</td>
<td>3/3</td>
</tr>
<tr>
<td>Spotted tail quoll (Dasyurus maculatus)</td>
<td>5</td>
<td>7/10</td>
</tr>
<tr>
<td>Eastern grey kangaroo (Macropus giganteus)</td>
<td>4</td>
<td>1/4</td>
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<tr>
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<td>1/1</td>
</tr>
<tr>
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<td>1</td>
<td>1/1</td>
</tr>
<tr>
<td>Squirrel glider (Petaurus norfolcensis)</td>
<td>2</td>
<td>1/2</td>
</tr>
<tr>
<td>Total number of marsupial species = 8</td>
<td>55</td>
<td>40/62</td>
</tr>
<tr>
<td><strong>Tasmania</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common brushtail possum (Trichosaurus vulpecula)</td>
<td>22</td>
<td>44/44</td>
</tr>
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<tr>
<td><strong>Totals</strong></td>
<td></td>
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</tr>
<tr>
<td>Overall total number of marsupial species = 11</td>
<td>231</td>
<td>169</td>
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</table>

Order specific 16S Chlamydiales PCR

To screen marsupials and their parasitising ticks for *Chlamydiales*:

- 800 bp fragment of the 16S rRNA gene partially covering the *Chlamydiales* signature sequence

16SIGF (5'-CGG CGT GGA TGA GGC AT-3')

806R (5'-GGA CTA CCA GGG TAT CTA AT-3')

- Roche High Pure PCR Product Purification Kit
- Macrogen Inc. (Seoul, Korea) di-deoxy sequencing in both directions
- Chromatograms aligned in Geneious R9.1.3 = consensus sequence
- BLAST
- GenBank representatives of the order *Chlamydiales*
- Bayesian phylogenies
Tick *Chlamydiales* abundant and diverse

Actual tick *Chlamydiales* PCR positivity
- *I. tasmani* 73.9%
- *I. holocyclus* 82.9%
- 5 other tick species all 100%
- Koala only tick species 70.9%

Six genotypes were identified from two families within the order *Chlamydiales* from 80% of the samples sequenced
- *Simkaniaceae*
- *Ca.* Rhabdochlamydiaceae

➢ No *Chlamydiaceae*, including *C. pecorum*

Phylogenetic relationships of the novel Chlamydiales genotypes identified in Australian ticks

Tick *Chlamydiales* but no *C. pecorum*?

- Australian ticks highly positive for *Chlamydiales* – higher than other global studies

- Only novel *Chlamydiales* genotypes were identified – other studies did not identify down to species level

- No *C. pecorum* was identified – despite majority of ticks being removed from koalas that were in a geographical region where *C. pecorum* infection rates are somewhat high
Marsupial *Chlamydiales* abundant and diverse

<table>
<thead>
<tr>
<th>Marsupial Species</th>
<th># of <em>Chlamydiales</em> PCR Positive Individuals</th>
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<td>0</td>
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<td><strong>89</strong></td>
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Seven genotypes were identified from three families within the order *Chlamydiales* from 20% of the samples sequenced:

- *Chlamydiaceae* – including *C. pecorum*
- *Ca. Rhabdochlamydiaceae*
- *Ca. Parilichlamydiaceae*
Non-koala marsupials carry *Chlamydia pecorum* and arthropod-associated Chlamydiae.

Non-koala marsupials carry *C. pecorum* and tick *Chlamydiales*

- Non-koala marsupials carry *C. pecorum* – congruent with pilot studies

- A novel and previously described *C. pecorum* genotype was identified – pilot studies not comparable

- No clinical signs of *C. pecorum* present – pilot studies reported both asymptomatic and symptomatic cases

- *C. pecorum* found in Tasmania - outside koala natural range

- A *Ca. Rhabdochlamydia porcellionis* genotype found in four marsupials was identical to a genotype identified in five marsupial parasitising ticks
Summary of non-koala marsupial and tick *Chlamydiales*

- Ticks that parasitise Australian wildlife carry novel *Chlamydiales* – no *C. pecorum*

- Non-koala marsupials carry *C. pecorum*

- Ticks are probably not a vector for *C. pecorum* in Australia

- Marsupials and their parasitising ticks share a *Ca. Rhabdochlamydia* genotype – transmission route?

**Further Research**

- Whole genome sequencing of *C. pecorum* and *Ca. Rhabdochlamydia* genotypes
  - Origin/Genetic relationships
  - Transmission events
  - Pathogenesis

Resolution of evolutionary models will aid in the potential management and control of chlamydial infections in Australian iconic host species such as the koala and determine the risk associated with previously undescribed chlamydial species
Acknowledgements

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