C. pneumoniae strains in snakes; genome sequencing underway

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**Summary**

- **Genome of novel uncultivable Chlamydia sp. characterised - first reptilian host**
  - Shotgun deep sequencing approach
  - 1.1Mbp chromosome & 7.5Kbp plasmid
  - Expanded pmp & omp repertoire
  - Lack of “traditional” plasticity zone

- **Chlamydia virulence factors**
  - Inclusion membrane proteins (IncS)
    - 41 predicted
  - Polymorphic membrane proteins (Pmps)
  - Highly variable
  - Frameshift mutations
  - May promote antigenic diversity
  - T3SS effectors

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**Results**

**The genome of Chlamydia sp. 2742-308 does not appear to contain a “traditional” plasticity zone**

- **Region of extensive variation between chlamydial genomes**
  - Described in all species of Chlamydia
  - ~12 kbp to ~86 kbp; 11 to 48 genes
  - Only features present in Chlamydia sp. 2742-308 - accB & accC (Figure 3)
  - Incomplete trp operon
  - No guaA+add operon for purine biosynthesis

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**Results: Genomic characterisation of a novel Chlamydia sp.**

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<td>297</td>
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<td>Plasmid length (Kbp) (No. ORFs)</td>
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**Initial case results: Chlamyrial diversity & genotype distribution**

**Aims:** Characterise the chlamydial diversity of captive snakes in Switzerland; expand our knowledge of chlamydial biology

**Initial case background & methods**

- **5 mortalities in one owner’s snake collection**
- **Additional case:** Chlamydial septicaemia (Ruegg et al., 2015)
- **6 owner’s collections**
- **Choana and cloaca swabs**
- **16S rRNA gene sequencing**
- **Bioinformatics analysis**

**Further sampling (87 animals):**

- **Choana and cloaca swabs**
- **6 owner’s collections**

**Screened for Chlamydialles:**

- **AmyTube, qPCR**
- **1 snake – Chlamydial septicemia (Ruegg et al., 2015)**
- **41 predicted strains**
- **1 snake – Chlamydial septicaemia (Ruegg et al., 2015)**

**Genomic study: methods**

- **DNA extracted from a choana swab (genotype 4; 2742-308)**
- **Host methylated DNA depletion**
- **Multiple displacement amplification**
- **Whole genome sequencing**
- **Illumina MiSeq: 150 bp PE reads**
- **De novo assembly (SPAdes)**
- **Bioinformatic analysis**

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**Results: Comparative analysis of chlamydial genome features.**

1. **Genome of novel uncultivable Chlamydia sp.**
2. **Uncultured C. pneumonia**
3. **C. pneumonia**
4. **C. pneumonia**
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34. **C. pneumonia**
35. **C. pneumonia**
36. **C. pneumonia**
37. **C. pneumonia**
38. **C. pneumonia**
39. **C. pneumonia**
40. **C. pneumonia**
41. **C. pneumonia**

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**Table 2: Comparative analysis of chlamydial genome features. One strain representative for each species was analysed (modified from Taylor-Brown et al., 2016, BMC Genomics).**

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession number</th>
<th>Chromosome length (Mbp)</th>
<th>GC content (%)</th>
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<td>C. pneumonia</td>
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**Figure 1: Phylogenetic tree depicting relationships between novel 16S rRNA genotypes (depicted by green boxes) and related chlamydial lineages.**

**Figure 2a: Cytoskeleton representation of the Chlamydia sp. 2742-308 plasmid.**

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**Figure 3: Region of the genome encoding the plasticity zone in C. pecorum and C. pneumoniae were compared to that of Chlamydia sp. 2742-308 via tBLASTn analysis and their arrangement plotted in EasyFig. Black arrows represent proteins, coded by colour and grey shading represents sequence homology.**

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**Table 1: Genotype designation and distribution for 16S rRNA gene sequences obtained from choana and cloaca swabs from captive snakes in Switzerland (Taylor-Brown et al., 2015, Vet Rec: 178). Green box depicts the novel Chlamydia sp. selected for genome sequencing.**

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<thead>
<tr>
<th>Owner Sample</th>
<th>Anatomical site</th>
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<td>2</td>
<td>Cloaca</td>
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<tr>
<td>6</td>
<td>Choana</td>
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