

Royal Society of Queensland Research Fund

Project title: Comparing Koala Retrovirus (KoRV) infection between Central and Southeast Queensland koalas

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Project members: Prof. Peter Timms, USC; Dr. Alistair Melzer, Central Queensland University (CQU)

Background and Significance

The survival of the koala is under serious threat, with this iconic marsupial declared 'vulnerable' by the Australian government in 2012. A major contributor to this decline is disease, with *Chlamydia* widely recognised as the major infectious threat and the relatively recently discovered retrovirus, Koala Retrovirus (KoRV), a threat itself and worse when combined with *Chlamydia*. KoRV is from the same family of viruses as HIV and has been detected in all Southeast Queensland koalas tested to date (1). Currently, seven subtypes of KoRV (A-I) are recognised and KoRV-B infection is significantly linked to chlamydial disease and cancer deaths in koalas from Southeast Queensland (1, 2). However, similar testing has not been



Removal of a KoRV-implicated tumour



Biogeographical barriers in Queensland (4) – areas that are more grassland and less forest, preventing koalas from easily moving through that area

conducted in other parts of Queensland. Testing location is important, as genetic analysis of Queensland koalas revealed two different lineages of koalas co-occurring north of Brisbane, with possible biogeographic barriers at the St Lawrence Gap (near Rockhampton) and the Brisbane Valley (at Brisbane) (3, 4). This suggests that it may not be accurate to extrapolate test results from koalas around Brisbane to koalas north of Rockhampton. With strong evidence that KoRV is involved in very serious koala health

conditions, it is time for focused research into KoRV across Queensland.

Objective

The objective of this study is to determine the prevalence and diversity of all seven subtypes of KoRV in two distinct areas of Queensland. We will evaluate whether there are differences in KoRV between Southeast Queensland koalas and Central Queensland koalas that could affect management practices (like relocating koalas) between these areas.

Study Design and Methodology

To determine the KoRV prevalence and diversity in Queensland, 50 koalas will be sampled from 4 sites:



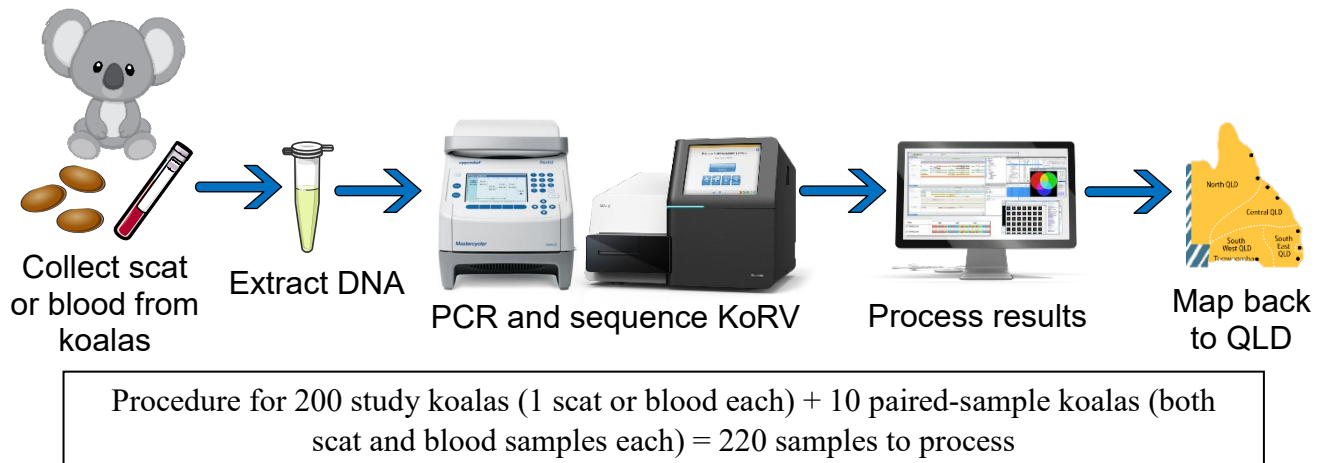
- 1) St Bees Island, northeast of Mackay
- 2) Clarke-Connors Ranges, east of Rockhampton
- 3) Moreton Bay region, north of Brisbane
- 4) Gold Coast region

Study sample sites across Queensland. Stars indicate sample sites, dashed lines indicate proposed biogeographical barriers in Queensland

These sites have been specifically chosen to span hypothesised biogeographical barriers, in an attempt to maximize the likelihood of sampling koala populations that differ in genetics and KoRV infection characteristics.

From sites 1 and 2, scat samples will be collected from monitored koalas by members of the Koala Research – CQU team and shipped to the USC. From sites 3 and 4, blood samples have been collected from monitored koalas by experienced wildlife veterinarians in accordance with USC Animal Ethics (AN/A/13/80) and Queensland Government (WISP11532912). Ten additional paired scat and blood samples from the same koala have also been collected from site 3 to allow for comparison of KoRV test results between the two sample types. Preliminary testing done in-house indicates that KoRV test results are consistent within a koala regardless of sample type analysed.

DNA will be extracted using a QIAamp DNA Mini Kit (Qiagen). To determine KoRV subtypes, a region of the KoRV envelope gene known to distinguish the seven subtypes (positions 22-514 bp) (2) will be amplified and sent for sequencing at the Ramaciotti Centre for Genomics (Sydney). Sequence analysis using established software (cutadapt, FLASH, primseq, QIIME) will be used to generate a profile of KoRV diversity within each koala tested (1). These profiles will then be assessed for correlation with site (using a Chi-squared test) to determine patterns of KoRV infection across Queensland.



We expect all Queensland koalas to be infected with at least one KoRV subtype, however, we anticipate different patterns in virus diversity between the biogeographical barriers. Learning how these patterns differ between koalas will be important for KoRV vaccine development (underway by the USC research team) and will inform *Chlamydia* treatment and koala translocation strategies across Queensland.

Timeline and budget

To complete this project, we anticipate the following timelines and costs:

Dates	Activity	Cost
Nov/Dec 2019	Collect samples from koalas in Central Queensland (n = 100) and ship to USC	\$100
Jan/Feb 2020	DNA extract samples	\$300
Mar 2020	KoRV testing on samples	\$280
Apr 2020	Sequence KoRV products on MiSeq sequencer	\$4290
May/June 2019	Analyse KoRV results and share results	\$0

Total anticipated timeline for project: 7 months

Total anticipated cost for project: \$4970

Capacity of the applicant to achieve the outcome

Dr. Bonnie Quigley is a post-doctoral research fellow with 12 years' experience in microbiology research. She has been studying infectious diseases of koalas for the past two and a half years, having already generated five high-impact publications in the field (1, 5-8). Her work on understanding KoRV in Queensland koalas is recognised by virologists as outstanding in the field, with her last KoRV publication highlighted by the Journal of Virology as a “Spotlight – articles of significant interest in this issue” and being placed on the journal cover (1). The methodologies Dr. Quigley uses in her research, such as deep amplicon sequencing and bioinformatics processing, are state-of-the-art, making her a leading researcher in Australia of this virus.



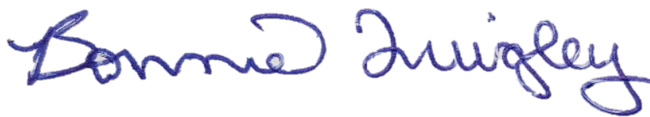
Based at USC, Dr. Quigley currently works with Prof. Peter Timms, a world-leading expert on chlamydial disease and vaccination in koalas. Together with established koala ecologist Dr. Alistair Melzer, research program leader of the Koala Research Centre of Central Queensland at CQU, this team has access to important Queensland koala populations and the research experience to successfully characterise KoRV and apply these findings for better koala management across Queensland.

References

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Signature of applicant



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