5. Appendix B: Koala research updates

Condition 5(b): include in each Compliance Report a summary of the progress and any findings of each project of the Koala research at least until the end of year 10.

Updates on each research project is provided below.

5.1 Chlamydia Vaccine Trial – University of Sunshine Coast

Coomera Connector Koala vaccine project, Summary report January 2024 - University of the Sunshine Coast (Professor Peter Timms and Dr Samuel Phillips).

Aim 1: Provide further evidence to the koala *Chlamydia* vaccine therapeutic response by vaccinating koalas with mild ocular and urogenital disease without antibiotic intervention and observe infection loads over three – six weeks.

Aim 2: Determine the longevity of protection koalas generate from *Chlamydia* vaccination and understanding immunological boosting effects due to subsequent infections post vaccination.

Measure any boosting effects of humoral immune responses in vaccinated koalas by natural infection post vaccination.

Determine the longevity of the vaccine induced immune response over a four-year period.

Identify risk factors that contribute to changes in vaccine specific immune responses.

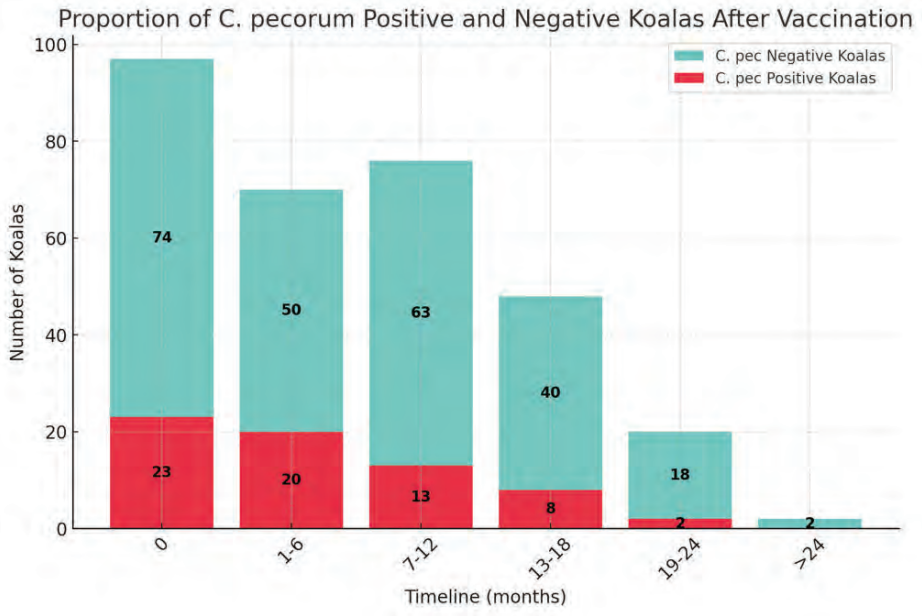
February 2025 Coomera Report

A total of 4,052 samples were received from EVE, 1,727 of them were swab samples and the remaining 2,325 samples were of blood origin. Of the swab samples, 797 ocular swabs were collected, 909 urogenital swabs were collected, 15 urine sediment swab samples, 3 rectal swabs and 4 oropharyngeal swabs.

As of February 2025, 92% of the collected swab samples had been processed. These samples represented 320 individual koalas, with 42.2% (135/320) of the koala's male, 54.1% (173/320) female and 3.7% (12/320) unknown gender.

Screening for *C. pecorum* DNA from swab samples revealed a 21.6% (344/1,589) overall prevalence, 18.3% (63/344) from ocular sites, 80.8% (278/344) from the urogenital site and the remaining 0.9% (3/344) from urine sediment. When assessed for individual koalas the overall prevalence increased to 45% (144/320) prevalence. Of the infected koalas, 43.7% (63/144) were male, 51.4% (74/144) were female and 4.9% (7/144) were of unknown gender.

In total, 42.8% (137/320) of the sampled koalas were vaccinated, of which 43.8% (60/137) were male, and 56.2% (77/137) were female. When *C. pecorum* infection was assessed within the vaccinated koalas a decrease in prevalence was observed every six months for the next two years following vaccination, from 23% at vaccination, 20% at six months, 13% at 12 months, 8% at 18 months and 2% at 24 months. Furthermore, no infections have been observed in koalas vaccinated greater than 24 months (although this only included two koalas).



5.2 Chlamydia RAT Development – University of Sunshine Coast

34-36 Ellingworth Parade, Box Hill, VIC,3128, Australia

j.li@radetecdiagnostics.com; +61 430530651

Report on CPEC RAPID Test Development

Prepared by: John Li, Radetec

Date: 18/02/2025

Project Overview:

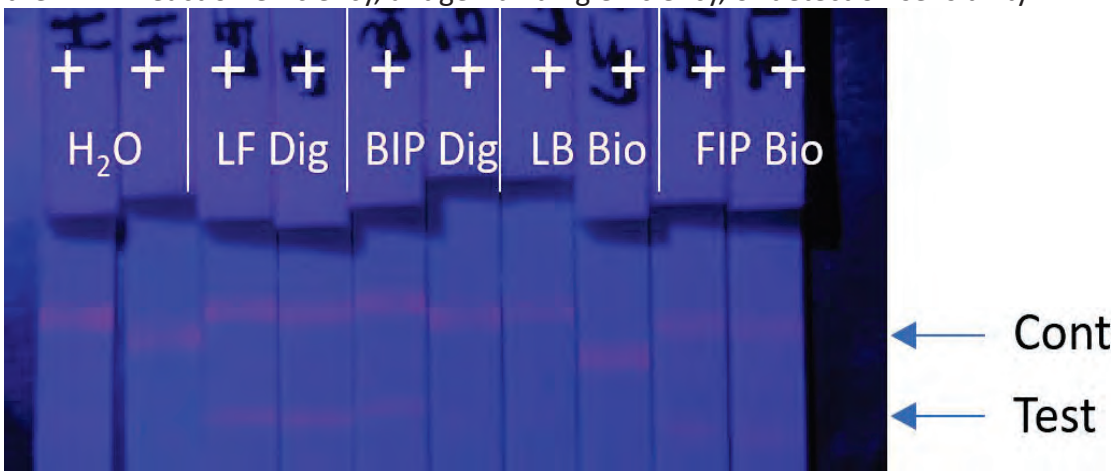
The objective of this project is to develop a highly sensitive and specific lateral flow assay (LFA) for CPEC detection using the Loop-Mediated Isothermal Amplification (LAMP) method. The project progressed through three key stages, with continuous optimisation of reagents, materials, and protocols. Despite various modifications, challenges persist, particularly false positives and weak signals in real positive samples.

Stage 1: Initial Development Using QDs conjugates in Solution

LAMP amplification products were detected using quantum dots (QDs) conjugated to detection amplicons in solution.
The conjugate was directly applied to the sample pad before running the test.
The test was performed on a lateral flow strip, and signals were recorded.

Results:

The assay produced an acceptable signal in some cases. However, false positives were observed intermittently. Weak signals in real positive samples indicated an issue with either the LAMP reaction efficiency, antigen binding efficiency, or detection sensitivity.



Cost by Radetec:
Master mix: 1500 AUD

34-36 Ellingworth Parade, Box Hill, VIC,3128, Australia

j.li@radetecdiagnostics.com; +61 430530651

Issues Identified:

Inconsistent LAMP product detection, possibly due to inefficient hybridization with detection probes. Potential aggregation or instability of QD conjugates in the sample mixture. Non-specific interactions between LAMP amplicons and the detection system.

Stage 2: Drying QD Conjugates onto Conjugate Pad

Modifications and Improvements to the test design:

The QD conjugates were dried onto the conjugate pad rather than being applied in solution in order to make the product more ready. Systematic changes were made to the antibodies (seven different suppliers were tested), in order to find the best one can be dried onto the conjugate pad.
A new supplier for the master mix was selected to optimise the LAMP reaction as well. This supplier also able to dry the primers into the master mix, that allows one step (adding the sample solution into the master mix) to prepare the reaction mixture.

Results:

After contacting a few companies and learning the method to dry the conjugates. The production method finally established. However, it did not significantly improve the signal intensity. And false positives persisted despite changing multiple antibody suppliers.
Positive sample detection remained weak, indicating a fundamental issue in the sensitivity of the LFA-LAMP detection system.

Issues Identified:

Drying the QD conjugates may have affected their stability or bioactivity.
The quality of antibodies and LAMP reaction components remained critical, but changing suppliers did not resolve the issue.

34-36 Ellingworth Parade, Box Hill, VIC,3128, Australia

j.li@radetecdiagnostics.com; +61 430530651

The master mix composition was modified, but the core problem of weak positive signals remained.

Cost by Radetec:
Master mix: [redacted] AUD
Antibodies: [redacted] AUD

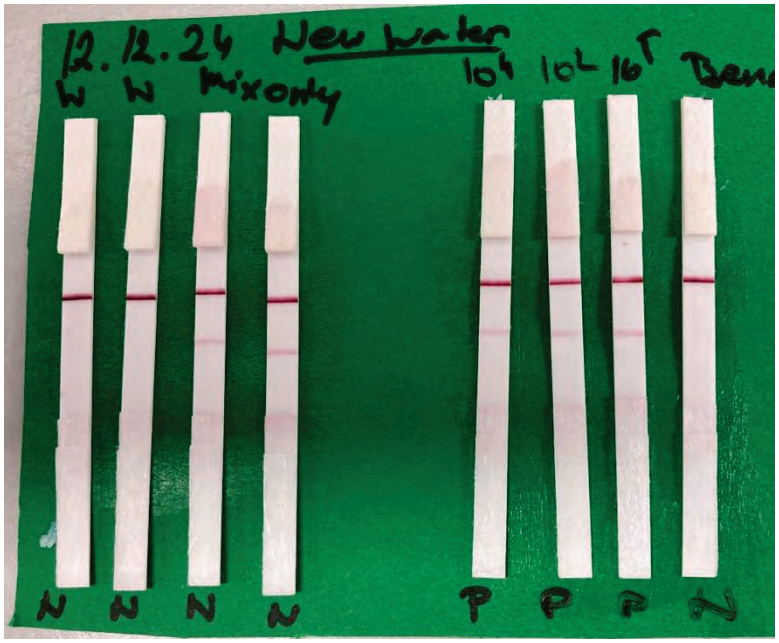
Stage 3: Transition to Gold Nanoparticle (AuNP) LFA

Modifications:

To address potential QD-related issues may cause all the issues, a manufacturer was engaged to produce gold nanoparticle (AuNP)-based LFA strips for LAMP product detection. In this study, it will eliminate all the production error by Radetec as well.
The AuNP-based system was tested using the same LAMP reaction conditions, antibody pairs, and test setup. The master mix composition was finalised and remained constant.

Results:

The AuNP-based assay produced similar results to the QD-based assay. Low signal in positive samples persisted. False positives continued to appear in some cases, suggesting non-specific interactions.



Conclusion:

34-36 Ellingworth Parade, Box Hill, VIC,3128, Australia

j.li@radetecdiagnostics.com; +61 430530651

Since the issue persisted even after switching to gold nanoparticles, the problem likely does not lie with QDs but rather with the LAMP-LFA integration. Non-specific binding of LAMP products to the test line. Interference from the sample matrix, affecting specificity and signal intensity.

Cost by Radetec:
Master mix: [redacted] AUD
Strips production: [redacted] AUD

Next Steps

The development of the CPEC RAPID Test using the LAMP method integrated with lateral flow detection has undergone multiple refinements across three stages, with persistent challenges in sensitivity and specificity. The results suggest that the root cause may not be the type of nanoparticles used (QDs vs. AuNPs), but rather LAMP reaction efficiency, non-specific binding, or matrix effects.

We decided to use LAMP method only, but make the test much easy to do compare to current LAMP test and much cheaper. A new reader will be made cheaper than current device. Much cheaper to the consumables total less than [redacted] dollars, and able to run 1 test per run. The testing protocol also need to be simpler, such as: Swap the sample > dissolve into lysis buffer > add to the dry mix > put it into the device and run.

5.3 Refinement of Mitigation – Koala Egress – Endeavour Veterinary Ecology/University of Queensland

Koala Egress Trials- April 2025 Update

Urban koala populations in Queensland face significant risks from drownings in swimming pools, domestic dog attack and vehicle collisions as they navigate increasingly fragmented habitats. These barriers not only threaten an individual koala's survival but compromise long-term population viability by restricting gene flow and safe movement through the landscape to other areas of habitat.

Since 2021, comprehensive trials at Endeavour Veterinary Ecology's Toorbul facilities have evaluated the effectiveness of structures designed to facilitate safe koala movement across roadways. Three structures were trialled: the koala escape pole, Koala Valve and a push-under Fauna Escape Hatch (FEH). The Phase 1 findings released in mid-2024 revealed that while koalas showed no device preference, the Fauna Escape Hatch) had a 100% success rate use whenever encountered by koalas. Notably, these studies confirmed that the standard FEH successfully accommodate female koalas carrying back-riding joeys (Figure 1).

Figure 1. Female and back rider joey koalas demonstrating movement through the traditional fauna escape hatch (360mm x 1200mm)

These findings demonstrated that the device is a promising addition to existing koala road egress solutions across our road networks. Based on these findings, the devices have been manufactured for deployment in the field by local councils, initially undertaking further in-field trials in conjunction with motion-activated camera monitoring. Camera footage demonstrated the effectiveness of the 'one-way' design, with koalas approaching the FEH from the bushland but failing to manoeuvre through the structure onto the road, preventing koalas and other medium sized animals from accessing the road corridor.

The research team continues to expand this work, with additional trials investigating complementary technologies such as virtual fencing, with results scheduled for 2025. These ongoing studies aim to develop an integrated system of wildlife movement solutions to mitigate the impacts of habitat fragmentation on vulnerable koala populations.

These findings provide evidence-based guidance for wildlife management authorities implementing koala conservation measures. The demonstrated effectiveness of the FEH design supports its integration into comprehensive wildlife movement solutions across urban and peri-urban landscapes.

5.4 Chlamydia Vaccine Trial (Queensland University of Technology)

Kenneth W Beagley

Professor of Immunology

Side by side comparison of 1-shot versus 2-shot chlamydial vaccine in a wild population.

The study includes samples from 18 animals in the single-shot vaccine group and 20 animals in the 2-shot vaccine group. Most animals have been sampled at the 6 and 12-month post vaccination time points (see table 1). All animals remain LAMP-negative at 12 months.

Samples were analysed by ELISA for IgG anti-MOMP antibodies against the 3 MOMP genotypes (G/A/F) in the vaccines (See figure 1) at 6 and 12-months post-vaccination. At the 12-month time-point, the levels of antibody against all MOMP types appear to be dropping off in the single shot vaccine group. The 18 and 24-month samples are being collected and will be analysed shortly.

Table 1: Chlamydia status of koalas at 6 and 12-months post-vaccination. Urogenital swabs were collected from Koalas vaccinated with Single shot (Triadj) and Double shot (Iscomatrix) vaccines. *Chlamydia* status determined by LAMP assay (EVE labs).

Name	Sex	LAMP UGT Result		
Single shot		Pre-Vaccine	6-month	12-month
Aphrodite	Female	Negative	Negative	Negative
Ariel	Female	Negative	Negative	Negative
Barbie	Female	Negative	Negative	Negative
Beckenham	Female	Negative	Negative	Negative
Cambridge	Male	Negative		
Cannon	Male	Negative	Negative	Negative
Cedar	Male	Negative	Negative	Negative
Dartford	Female	Negative	Negative	Negative
Flower	Female	Negative	Negative	Negative
Gospel	Female	Negative	Negative	Negative
Gunnersbury	Male	Negative	Negative	
Hatton	Male	Negative	Negative	Negative
Jack-Jack	Male	Negative		
Lucius	Male	Negative	Negative	Negative
Purfleet	Female	Negative	Negative	Negative
Shoreditch	Male	Negative	Negative	Negative
Whitechapel	Female	Negative	Negative	Negative
Zazu	Male	Negative	Negative	Negative

Name	Sex	LAMP UGT Result		
Double shot				
Abuela	Female	Negative	Negative	Negative
Anerley	Female	Negative	Negative	Negative
Athena	Female	Negative	Negative	Negative
Bonnie	Female	Negative	Negative	Negative
Catford	Male	Negative	Negative	Negative
Chalford	Female	Negative		
Cheam	Female	Negative	Negative	Negative
Duke	Male	Negative	Negative	Negative
Elmer	Female	Negative	Negative	Negative
Epsom	Male	Negative	Negative	Negative
Farringdon	Female	Negative	Negative	Negative
Finsbury	Female	Negative	Negative	Negative
Hamm	Male	Negative	Negative	Negative
Harold	Male	Negative	Negative	
Notting	Male	Negative	Negative	Negative
Penny	Female	Negative	Negative	Negative
Squishy	Male	Negative	Negative	Negative
Sutton	Female	Negative	Negative	Negative
Swanley	Female	Negative	Negative	Negative
Wallington	Male	Negative	Negative	Negative

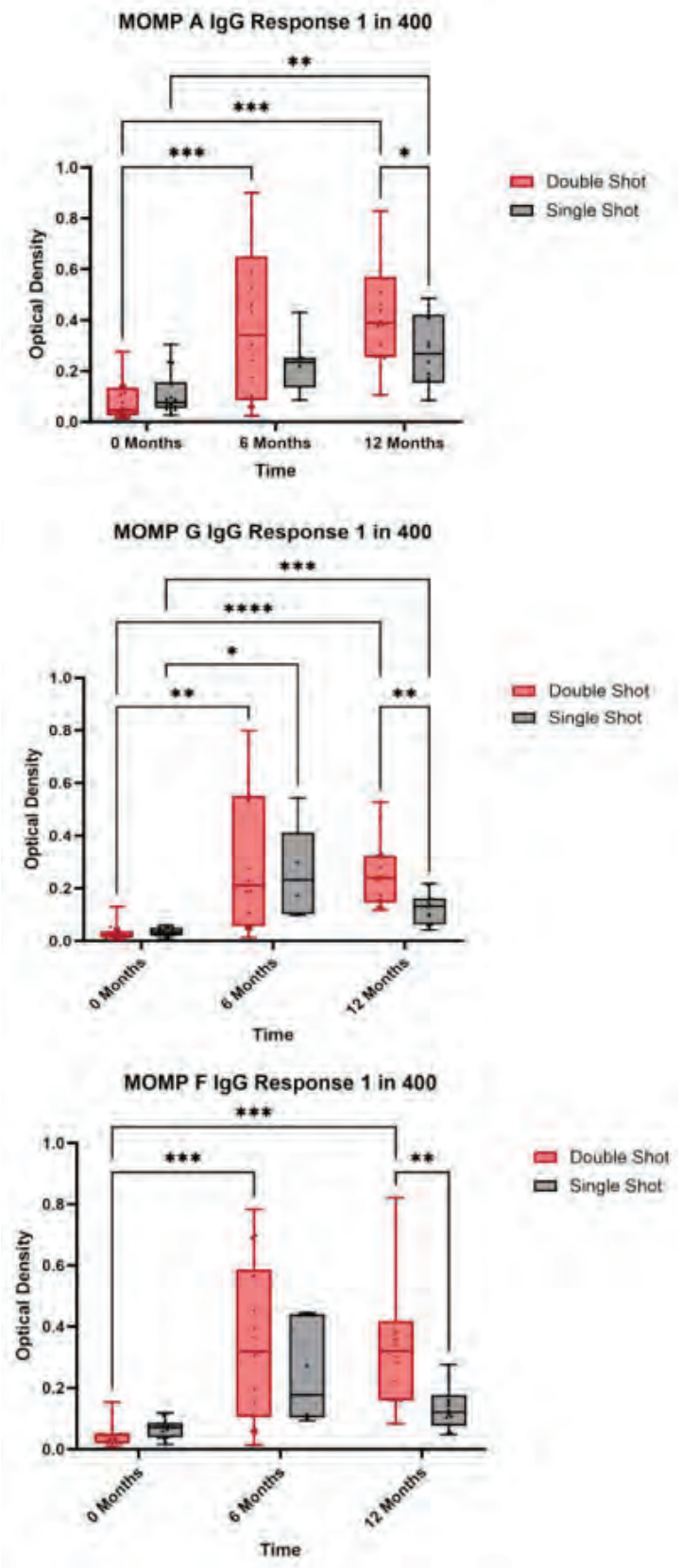


Figure 1. The figures to the left depict detection of IgG using an indirect ELISA. ELISA performed using *C. pecorum* MOMP A, G and F as coating antigens. Differences between groups were determined by assessing MOMP specific IgG titres using koala serum from wild koalas as samples. Significant differences were determined using Mixed-effects model with the Geisser-Greenhouse correction as sphericity was not assumed. Tukey's multiple comparisons tests was also performed ($p < 0.05$). Graph and statistical analysis were generated using GraphPad Prism (v10). $\alpha = 0.05$ (* $p < 0.05$, ** $p < 0.025$, *** $p < 0.001$, **** $p < 0.0001$).

5.4.1 Drivers and biomarkers of disease and success in wild koalas

University of Sydney – Endeavour Veterinary Ecology

Researcher: Yasmine Muir

The following key findings comprise elements of a manuscript under preparation for publication in a PhD thesis and a peer reviewed journal.

Drivers and biomarkers of disease and success in wild koalas

University of Sydney – Endeavour Veterinary Ecology

The long-term impacts and outcomes of koala rehabilitation are not well understood nor are the population-specific factors that might influence these outcomes. It is important that we determine this to optimise treatment and rehabilitation methods and guide the development of new protocols. Chlamydiosis is the main infectious disease affecting koala welfare and conservation but advances in its treatment and management are held back by our limited understanding of the role played by several co-infecting agents and the many host, pathogen, and environmental factors with potential to affect outcomes. Research on this has been limited due to the difficulties in acquiring long-term monitoring data on rehabilitated and released koalas and conducting multivariate analyses on small sample sizes. The koala monitoring project led by Endeavour Veterinary Ecology (EVE) and supported by the Coomera Connector project [Queensland Transport and Main Roads] provided the opportunity to bridge these gaps in the current knowledge.

To meet the need for long-term, post-treatment monitoring studies, this study monitored 221 koalas originating from two neighbouring populations over a 2-year period. This study investigated the relationships between survival, frequency of disease, and treatment outcomes in two populations with differences in general morbidity and mortality; based on preliminary analyses, koalas located north of the Coomera River were categorized as the “high morbidity” population, and those to the south were classified as the “low morbidity” population. Using multivariate analysis, this study showed that immunological variation among koalas may be an important indicator of individual and population health. The research identified a range of adaptive immune markers associated with better outcomes and longer survival and innate immune and retroviral markers associated with mortality. Cohort specific co-infection status and morbidity rates effected the relationship between detection of circulating *Chlamydia* and survival in koalas. Collectively, this study demonstrates the importance of population specific co-infection and immunological variation on long-term health and survival in koalas. Findings here suggest that targeted conservation strategies should be tailored to regional variations, and infectious and immunological markers should be further developed to aid individual and whole population health monitoring in both wildlife management and clinical settings.

5.5 TMR and City of Gold Coast - Data sharing collaboration

Note: This research collaboration is addition to research proposed in the PER.

TMR received a data sharing request on Friday 7th February from the City of Gold Coast (CoGC) to support their ongoing koala management in the east-Coomera area. The Coomera Connector North package (Foxwell Road to Helensvale Road) interfaces with the CoCG study area. As a result, TMR are progressing a data sharing agreement to support CoGC on their study to ensure strategic planning of koala habitat in this area of the Gold Coast.

Specifics of the study area and data request are as follows:

The **PROJECT**:

CoGC are undertaking a project to gain a better understanding of the koalas remaining in isolated patches of vegetation surrounded by road, rail, river or new development in the East Coomera area. Refer to *Figure 1.*

The primary purpose of this project is as follows:

Stage 1 – Scoping study: Undertake a review and analysis of the status of the koala population within the project area (see attached) using existing data and knowledge, desktop assessments, and ground truthing where necessary. Guided by this review and analysis, prepare high-level advice and recommendations for the management of koalas in the project area to ensure their safety now and into the future.

Stage 2 – Koala Management Plan: Develop a comprehensive Koala Management Plan (KMP) for this specific koala sub-population.

CoGC information supplied:

Figure 1 Project area – Proposed Locations For High Risk Koalas: Coomera-Pimpama

The **DATA**:

Koala movement/location data - derived from in-field tracking events and GPS data from Endeavour Veterinary Ecology's (EVE) K-Tracker biotelemetry tags utilised on Coomera Connector Stage 1.

This data provides information relating to habitat use, dispersal and movement paths in that landscape.

The extent of this dataset shared as part of this agreement is limited to all koalas within the ***Proposed Locations For High Risk Koalas: Coomera-Pimpama.*** Refer to as *Attachment B* of this agreement.

Koala population data (age/sex, disease status/health, reproductive status, causes of mortality) – derived from veterinary exams and in-field observations.

This data provides information on population demographics, population viability (all diseased and not viable population will gradually reduce and become locally extinct, or healthy breeding population requiring dispersal/movement options, age of koalas to determine why individuals moved e.g. SA dispersal event, when looking at the movement/location data).

Koala survey data – derived from initial surveys carried out by EVE along the Coomera Connector project corridor and any thermal drone koala surveys in the ***Proposed Locations For High Risk Koalas: Coomera Pimpama***.

This data will add to the dataset in the areas within and adjacent to the study area to provide information on population health, distribution, and density.

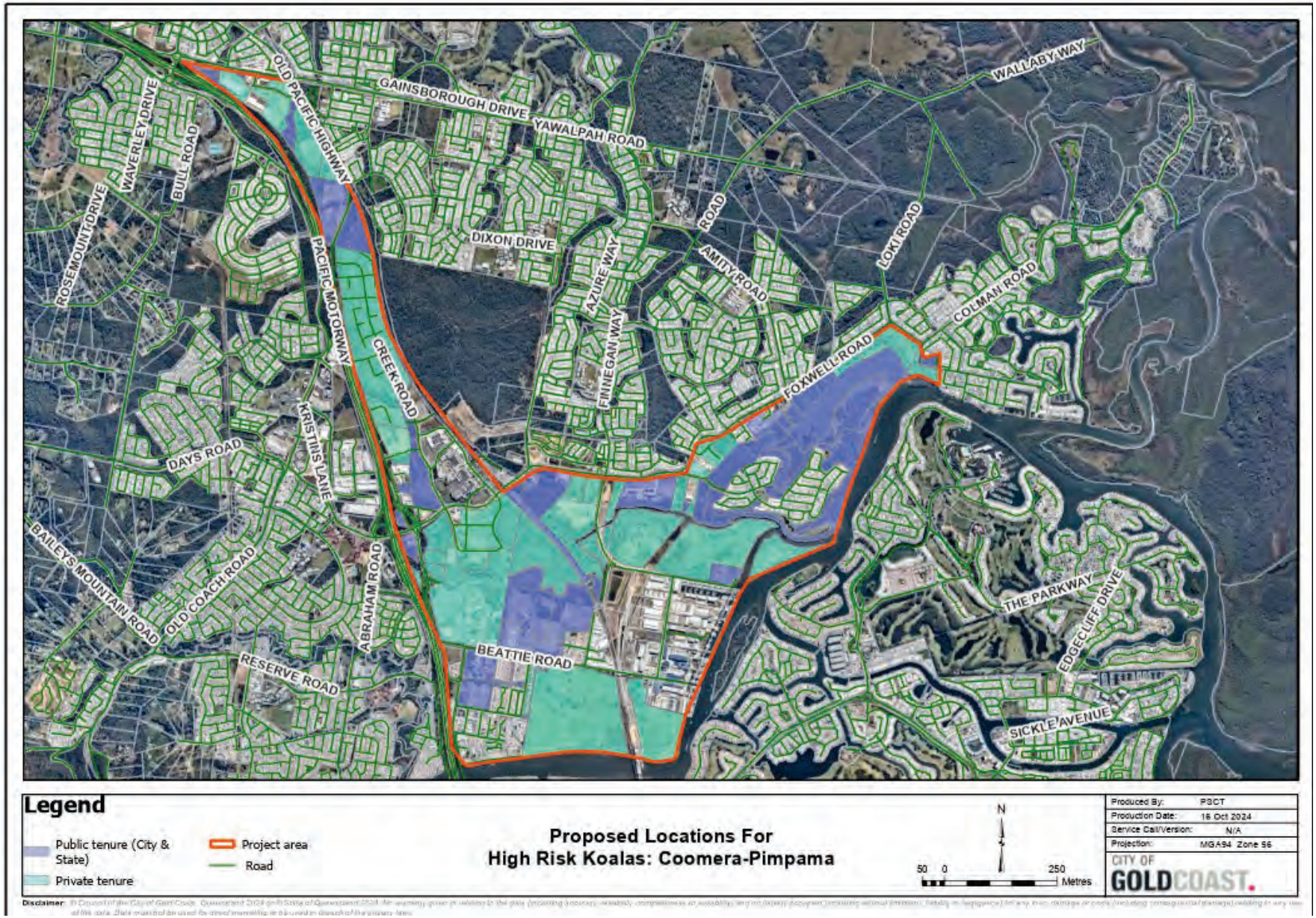


Figure 1: City of Gold Coast Proposed Study Area