An Investigation of the Health of Wild Koala Populations in South-East Queensland

By

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This thesis is submitted to the University of Queensland in partial fulfillment of the requirements for the Degree of Bachelor of Applied Science (Animal Studies) (Honours) in the School of Animal Studies, The University of Queensland.

June 2010

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10th June 2010

Abstract

Disease in koalas, particularly chlamydiosis, has only recently been acknowledged as one of the key threatening processes contributing to the dramatic decline of south-east Queensland (SEQ) populations. Numerous studies have investigated the health of koalas but the true extent and seriousness of its impact on wild koala populations is currently not known and may have been underestimated. This study investigated the prevalence of disease in four wild SEQ koala populations from both the Moreton Bay Local Government Area (LGA) (Brendale and Narangba populations), and the Gold Coast LGA (East Coomera and Clagiraba populations). Using a standardised veterinary protocol, veterinary health examinations were conducted on koalas under general anaesthesia, together with ancillary tests designed to detect most known conditions in koalas. Longitudinal monitoring of koalas in-situ using radio-telemetry allowed follow-up health examinations to be performed, and the incidence of new disease cases to be calculated for the Brendale and Narangba koala populations. Fifty koalas from Kangaroo Island, South Australia, were also examined and sampled for the purposes of comparison, representing a healthy population.

Chlamydiosis was the most common disease detected in every SEQ koala population with 41% (14/34), 36% (8/22), 26% (9/34), and 100% (4/4) of koalas exhibiting low to high-grade chlamydial disease severity from the Brendale,

Narangba, East Coomera and Clagiraba populations, respectively. Interestingly, a large proportion of these koalas had no overt physical signs of illness and it was only by using thorough veterinary investigative techniques that disease was detected. The observation of overt signs of chlamydial disease (detected by usual survey techniques) without capture of the koala, was found to underestimate disease prevalence by a factor of five.

Of the sexually mature females in each population, the proportions that had joeys or were pregnant at their first health examination were 36% (5/14) at Brendale, 55% (6/11) at Narangba and 56% (9/16) at East Coomera. Of the four koalas examined from the Clagiraba population only one was female and she was infertile. Excluding the Clagiraba population, the prevalence of reproductive disease causing infertility in females was highest in the Brendale population (57%; 8/14). Infertility in the female koalas at Narangba was 45.5% (5/11) and at East Coomera was 31% (5/16). The combined annual incidence of newly developed infertility in previously healthy female koalas in the Brendale and Narangba populations was 32%. Reproductive disease was not evident in any of the Kangaroo Island female koalas.

Of the 94 koalas from SEQ examined in this study, 6 (6.4%) were found to have evidence of immune dysfunction, and/or illness consistent with an AIDS-like condition. These koalas were given the diagnosis of “AIDS(?)” and placed into

the category of “KoRV-associated disease” as currently no other clear explanation exists for their syndromes.

There was no significant difference in disease prevalence between the Brendale, Narangba and East Coomera koala populations ($p>0.05$). However, despite the very small sample size of the Clagiraba koala population, the chi-square analysis revealed its disease prevalence to be significantly different from the Narangba and East Coomera populations, but not the Brendale population. When the disease prevalence of each SEQ population was compared with the Kangaroo Island koala population, the differences were significant. No pathological condition was detected in any of the Kangaroo Island koalas.

Overall, the results of this study show that disease is threatening the survival of at least some koala populations in SEQ. If the data on disease prevalence and incidence derived from this study is indicative of the situation for koalas more broadly, the reduction in fecundity and death of koalas caused by chlamydiosis (and other diseases) is significantly contributing to their decline. Further research is required to validate this hypothesis more broadly and determine the critical factors causing high disease prevalence and severity in Queensland and New South Wales koala populations.

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Acknowledgements

Firstly, I sincerely thank my supervisors, Andrew Tribe and Jon Hanger for all of their support, encouragement and guidance. I also thank the following individuals and collaborators:

- Australian Wildlife Hospital- all of the staff and volunteers
- Ecological Services Unit- Ben Nottidge and Tosh Tucker
- Bonnie “Pickles” O’Connell
- Gold Coast City Council- John Callaghan, Alicia Bell, Kellie Pforr, Kim Edwards and Kathy Friebe
- Wildlife Relocation and Management Services- Graeme Lloyd
- Staff at the Kangaroo Island Veterinary Clinic
- Queensland University of Technology- Peter Timms, Ken Beagley, Adam Polkinghorne and Avinash Kollipara
- The University of Queensland (UQ) Koala Retrovirus (KoRV) Research Group- Paul Young, Greg Simmons, Jo Meers, Kiersten Jones and Daniel Clarke.
- UQ (Gatton)- Allan Lisle

Without their graciously provided assistance, this project would have been significantly less meaningful.

It is with much gratitude that I thank Australia Zoo Wildlife Warriors Worldwide Ltd for providing much of the financial support that allowed this project to occur, and also CSR Ltd, for their generous funding and for allowing access to their sites.

Communications

Conference Presentations

Hanger, J. & Loader, J. 2009, Infectious Disease in Koalas: Implications for Conservation, *Koala Conservation Conference,* Friends of the Koala, Southern Cross University, Lismore, 22nd May 2009.

Other Presentations

Loader, J. 2009, The prevalence of disease in two south-east Queensland koala populations, Australian Wildlife Hospital Training Program, Australian Wildlife Hospital Conference Centre, Beerwah, 29th July 2009.

Loader, J. 2010, The prevalence of disease in two south-east Queensland koala populations, Koala Disease Research Meeting, Australian Wildlife Hospital Conference Centre, Beerwah 13th Jan 2010.

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CHAPTER 1: General Introduction

Despite being one of Australia's most charismatic and prominent native species, the koala (*Phascolarctos cinereus)* is nevertheless experiencing ongoing threats to its survival (Melzer *et al.,* 2000; Gordon and Hrdina 2005). Although Queensland harbours a large proportion of Australia's naturally occurring wild populations (EPA 2006), koalas in the south-east Queensland (SEQ), Brigalow Belt and Mulga Lands bioregions are continuing to suffer dramatic declines and contractions in range (Cogger *et al.,* 2003; Sullivan *et al.,* 2004; Gordon *et al.,* 2006; EPA 2007; AKF 2008a; Lane 2008; DERM 2009a; Seabrook *et al.*, 2010, cited in Lawler 2010). These declines have been primarily attributed to the clearing of native forests and woodland for urban and agricultural development. In contrast, some South Australian and Victorian populations are remarkably successful, having exceeded carrying capacity to the point where overbrowsing has created serious food shortages and substantial habitat damage (Masters *et al.,* 2004; Duka and Masters 2005). As a result, intensive management strategies are required in these regions to control koala numbers (Melzer *et al.,* 2000; Masters *et al.,* 2004; Duka and Masters 2005).

Of the threats confronting SEQ koala populations, the clearing of native vegetation and the consequent loss and fragmentation of habitat has been considered to be the most severe (EPA 2006). However, disease, which has been recognised as an important cause of mortality and morbidity in koalas for

at least a century, has only recently been acknowledged as an important factor contributing to the decline of SEQ koala populations (Australian Wildlife Hospital unpublished data; EPA 2006; DERM 2009b; Hanger and Loader 2009; Natural Resource Management Ministerial Council 2009) and its impact, thus far, may have been underestimated. A high prevalence of chlamydial infection has been reported in many free-living koala populations (Jackson *et al.,* 1999; Timms 2000; Devereaux *et al.,* 2003), and it is one of the most significant causes of disease in this species (Brown *et al.,* 1987; Timms 2005; Markey *et al.,* 2007; Higgins 2008). Furthermore, the koala retrovirus (KoRV) with its postulated role in leukaemia and related diseases (Canfield *et al.,* 1988; Hanger 1999; Tarlinton *et al.,* 2006; 2008) has also been suggested as causing an immuno-deficiency state in the koala which may be a contributing factor to the severity of chlamydial disease and opportunistic infections (Hanger 1999; Tarlinton *et al.,* 2005; 2008).

Disease in koalas has been well-studied in individual animals, however little quantitative information is available on the health of wild koala populations. Reports of disease are generally limited to chlamydiosis, and prevalence has mostly been estimated by the presence of overt physical signs (Gordon *et al.,* 1990; White and Kunst 1990; White and Timms 1994; Jackson *et al.* 1999). Clearly, the effective conservation of koalas requires a detailed understanding of the importance and magnitude of all threats to their survival. To date, this information has been neither accurately collected nor evaluated for disease at the koala population level.

This study investigated the prevalence, incidence and nature of disease detected in four separate wild koala populations in SEQ by conducting thorough health examinations using a standardised veterinary protocol. Fifty koalas from Kangaroo Island, South Australia, were also examined and sampled for the purposes of comparison, representing a healthy population. Cross-sectional studies (also known as prevalence studies) and longitudinal studies (giving incidence data), were conducted in this project in order to detect both chronic and acute disease and mortalities.

Literature on the history and status of koalas is reviewed, together with the key threatening processes influencing the decline of koala populations in SEQ. Finally, priorities and recommendations for the conservation of koalas are suggested based on the results of this study.

CHAPTER 2: Review of Literature

2.1 History of Koalas

Although the colonisation of Australia by Europeans occurred only around 220 years ago, koala populations have been subjected to major change during this time (Martin and Handasyde 1999). According to numerous references in the scientific literature, the reduction in hunting practices attributable to the decline of Aboriginal people may be one reason koalas experienced a dramatic increase in numbers following European settlement (Lunney and Leary 1988; Melzer *et al.,* 2000; Penn *et al.,* 2000; Gordon and Hrdina 2005). However the contention that koalas were relatively scarce at the time of settlement was refuted by the Australian Koala Foundation (AKF) in recognition that it had never been substantiated by scientific evidence and was a persistent myth due to its presence in '…literature and government propaganda' (AKF 2004).

The latter part of the nineteenth century marked the onset of an uncontrolled harvest where koala pelts were marketed into a thriving international fur trade (Lee and Carrick 1989; Martin and Handasyde 1999). Unfortunately, hunting pressures were too great in Victoria and South Australia during this time, and by the early 1900s, many populations had been shot to extinction (Robinson 1978; Martin and Handasyde 1999). Widespread concern about the demise of the koala prompted many states to designate it a protected species, and, in 1906, Queensland also adopted this change (Martin and Handasyde 1999; Jackson

2007). Nevertheless, overhunting during a regulated harvest between 1906 and 1927 resulted in the dramatic decline of Queensland koalas (Martin and Handasyde 1999; Gordon and Hrdina 2005).

In addition to hunting, koala population crashes were also assisted by the clearing of native forest, disease, drought and bushfires (ANZECC 1998; Martin and Handasyde 1999). In fact, by the cessation of the fur trade, it was estimated that fewer than 10,000 koalas remained in Queensland (Jackson 2007). A number of populations in southern Australia had reached even lower numbers to the extent that active management measures were taken by the respective state governments to protect and re-establish koalas in their natural range (ANZECC 1998). This included establishing koala colonies on islands upon which they did not naturally occur, in the hope of one day restocking the mainland (ANZECC 1998; Martin and Handasyde 1999).

The first islands to be used for koala introductions were Phillip Island and French Island, off of the Victorian coast (ANZECC 1998). To counteract population losses that had also occurred throughout South Australia, between 1923 and 1925 approximately 18 adult koalas were translocated from French Island to Kangaroo Island in an attempt to safeguard the species (Duka and Masters 2005). Koala numbers flourished on this island habitat, probably due to the absence of chlamydial disease and natural predators (Martin and Handasyde 1999; Duka and Masters 2005). It was estimated that by 2001, the

koala population had grown to as many as 27,000 individuals, a significantly larger number than had been documented in previous years (Masters *et al.,* 2004). Over-browsing by koalas resulted in the severe defoliation of native vegetation and inevitably marked the beginning of a serious food shortage (Martin and Handasyde 1999). Management strategies to combat these high densities were therefore needed to prevent further habitat degradation (Martin and Handasyde 1999; Duka and Masters 2005). To date, this has included surgical sterilisation programs to suppress fertility, and translocation of koalas to the mainland (Melzer *et al.,* 2000; Masters *et al.,* 2004). Although a costly, time consuming and labour intensive procedure, koala numbers in localised areas of the island have significantly reduced, however further measures are necessary for the recovery of habitat and further stabilisation of koala populations (Duka and Masters 2005).

Large fluctuations in koala numbers have been observed since European settlement, and the factors contributing to their present decline, in most regions of Australia, are remarkably similar to those of previous years (ANZECC 1998; Martin and Handasyde 1999). While the hunting of koalas is no longer permitted, land-clearing and urbanisation pressures from the ever-increasing human populace, disease and stochastic events remain the key threatening processes affecting the species today (ANZECC 1998). Furthermore, significant changes in the natural distribution and density of koala populations are a result of anthropogenic influences, hence large-scale conservation efforts to reverse their

decline are necessary to protect and ensure the future of this species (Martin and Handasyde 1999).

2.1.1 Distribution and abundance

Koalas occupy a broad range, from the Atherton Tablelands in northern Queensland through New South Wales and Victoria to regions of South Australia (Lee and Carrick 1989; Martin *et al.,* 2008). They inhabit forested and woodland areas throughout much of eastern and south-eastern Australia (Lee and Carrick 1989). Historically, the distribution of koalas was far more widespread than it is today. However, habitat loss and fragmentation has isolated many remnant populations, separating them from one another by tracts of unsuitable habitat (Martin and Handasyde 1999).

Various methods have been used to estimate population numbers and density of koalas at the local and regional level, including line-transect sampling (Dique *et al.,* 2003b), faecal-pellet sampling (Sullivan *et al.,* 2002), and community surveys (Lunney *et al.,* 1997). Partly due to the cryptic nature of the species, and its wide distribution, accurate estimates of the national population do not exist (Sullivan *et al.,* 2004). Most figures suggested are at best educated guesses, and most koala biologists agree that realistic or meaningful estimates of the Australia-wide population are impossible, based on currently available data.

Queensland densities vary from around 0.005 koalas/ha to 2.5 koalas/ha, with the forested regions of SEQ supporting between 0.2-0.5 koalas/ha (EPA 2006). It has been estimated that only 100,000 to 300,000 koalas remain in Queensland today (EPA 2006; Predavec 2008), but these figures are disputed by the Australian Koala Foundation who contend that there are far fewer (AKF 2009).

2.1.2 Status of the koala

The koala is a protected species in all states and territories in which it naturally occurs, however its conservation status and the perceived threats vary across its range (Melzer *et al.,* 2000). At the federal, state, and regional levels, the conservation status of the koala is defined by separate regulatory frameworks which reflect these differences (ANZECC 1998; Melzer *et al.,* 2000; Predavec 2008). On a national scale, the proposed listing[1] of koalas as '*vulnerable*' under the *Environment Protection and Biodiversity Conservation Act 1999 (*EPBC Act*)* has been a topic of considerable debate (AKF 2004; 2008b; Predavec 2008). Although koalas are declining in many regions of Australia, the atypical response of a few "over-abundant" populations in Victoria and South Australia has been influential in the decision to reject the nomination for this listing (AKF 2004; 2006; Predavec 2008). Proponents of their national listing as vulnerable

[1] At the time of writing the Department of the Environment, Water, Heritage and the Arts (DEWHA) was reconsidering the listing of koalas

have been unable to demonstrate a decrease in numbers equal to or greater than 30% over the past three generations, hence previous applications did not meet the eligibility criteria (AKF 2006; Predavec 2008).

The conservation status of the koala varies between each state as well as regionally in Queensland (Melzer *et al.,* 2000). Koalas living in the SEQ Bioregion are classified as '*vulnerable to extinction'* under the *Nature Conservation Act 1992,* but in the remainder of state they are considered of '*least concern'* despite the absence of evidence for stable populations (EPA 2006; Queensland Government 2009). In New South Wales the koala is *'vulnerable'* under the *Threatened Species Act 1995;* in South Australia they are *'rare'* under Schedule 9 of the *National Parks and Wildlife Act 1972;* and in Victoria there is no official listing for the koala; they are designated as 'o*ther protected wildlife'* together with all native animals under the *Wildlife Act 1975* (Melzer *et al.,* 2000; EPA 2006; Predavec 2008; Government of South Australia 2009).

2.2 Threatening Processes Affecting Koalas in SEQ

Considering that SEQ has one of the most rapidly expanding human populations in Australia (Australian Bureau of Statistics 2009), the impacts of fragmentation and loss of habitat associated with urban and agricultural expansion pose an immediate and significant threat to the survival of koalas in the region (EPA 2006). In addition to reducing carrying capacity, the diminishment of available

habitat may also cause other deleterious effects including reduction in genetic diversity, reduced dispersal and reproductive success, modified social interactions, as well as increasing exposure to edge effects (Lindenmayer and Burgman 2005). Altered fire regimes post-European settlement and the occurrence of chance events, including drought and bushfires, are also known to cause major impacts on koala populations, particularly in isolated regions, and may increase their susceptibility to, or actually cause, localised extinctions (Fowler *et al.,* 2000; Lunney *et al.,* 2004). In addition to these factors, the high prevalence of disease is accountable for a large proportion of mortality and morbidity in the species (Australian Wildlife Hospital unpublished data; Obendorf 1983; Brown *et al.,* 1987; Weigler *et al.,* 1987; Stalder *et al.,* 2008).

2.2.1 Implications of land-clearing on koala populations in SEQ

Throughout Australia, the broad-scale clearing of remnant vegetation for urban and agricultural development significantly impacts native wildlife and has resulted in the loss of hundreds of millions of vertebrates every year (McAlpine *et al.,* 2002; Cogger *et al.,* 2003; Johnson *et al.,* 2007). It is recognised as the leading threat to biodiversity and one of the major causes of faunal decline (McAlpine *et al.,* 2002; 2006b; Johnson *et al.,* 2007; Brown *et al.,* 2008). Cogger *et al. (*2003) estimated that in Queensland alone, 19,000 koalas died annually between 1997 and 1999 due to the clearing of remnant vegetation. By inference, this alarming figure excludes those killed by illegal clearing and clearing of non-remnant vegetation (regrowth) which is significant and not regulated (Hanger

and Nottidge 2008). Therefore, the number of actual mortalities is presumably much higher. In the report, the authors estimated that during this same period, approximately 1.7% of the total number of trees in the SEQ bioregion had been cleared compared to a startling 58.8% in the Brigalow Belt bioregion, with over 112 million trees lost to land-clearing each year (Cogger *et al.,* 2003).

Land-clearing disrupts ecosystems by modifying the landscape composition and fragmenting habitat, leading koala populations and individuals to become more vulnerable to predation, misadventure, edge effects, stochastic events, and inbreeding, amongst other damaging effects (Gibbons and Lindenmayer 2007; Hanger and Nottidge 2008). Although land-clearing can pose an immediate risk to individual animals and populations, others may be affected more gradually (Cogger *et al.,* 2003; Hanger and Nottidge 2008). Cogger *et al.* (2003) reported that in some cases, the full effects of land-clearing practices on affected wildlife populations and ecosystems can take decades to occur.

Aside from instantaneous mortalities that occur during clearing operations, many individuals sustain traumatic injuries likely to cause pain, suffering and a prolonged death (EPA 2006; Johnson *et al.,* 2007; Hanger and Nottidge 2008). Others that survive the initial clearing process may succumb to misadventure, starvation from the loss of their food source, or become more vulnerable to predators due to increased exposure (Cogger *et al.,* 2003; Hanger and Nottidge 2008). Generally, displaced koalas encounter these problems due to their

inability to reach adequate and intact habitat (Cogger *et al.,* 2003). The opportunity for, or ability of koalas to disperse and interact with conspecifics is also compromised as connectivity and corridors are often lost after land has been cleared (Hanger and Nottidge 2008). This has severe implications for the maintenance of genetic diversity because isolated populations may be subject to inbreeding and significant decreases in fecundity (Cocciolone and Timms 1992; McAlpine *et al.,* 2002; Tucker *et al.,* 2007).

In order to protect remaining koala habitat and prevent the ongoing decline of koala populations, Melzer *et al.* (2000) and McAlpine *et al.* (2002) similarly remarked that the clearing of native vegetation needed to stop. Gibbons and Lindenmayer (2007) stated that significantly reducing land-clearing in Australia is a complicated task, as land-clearing facilitates human population expansion and boosts productivity, which are primary drivers of economic growth in the country. Consequently, local and regionalised extinctions will become more likely if substantial measures are not taken to monitor and cut back on land-clearing practices (McAlpine *et al.,* 2002; Cogger *et al.,* 2003).

2.2.2 Animal welfare issues associated with land-clearing

Apart from the devastating environmental consequences of land-clearing, it is also responsible for a significant number of koala injuries and deaths (Cogger *et al.,* 2003). As a semi-arboreal species, the koala is particularly prone to injuries associated with tree felling, which can result in bone fractures, head injuries and

severe internal organ damage. Unfortunately injured koalas often remain undiscovered during clearing operations, so those not immediately killed may be subject to delayed and distressing deaths (Hanger and Nottidge 2008). According to records from the Australian Wildlife Hospital, a wildlife rehabilitation facility in Beerwah, SEQ; less than 2% of hospital admissions pertain to land-clearing injuries sustained by koalas each year (Australian Wildlife Hospital unpublished data). This proportion is remarkably low, when considering the many thousands of koala deaths Cogger *et al.* (2003) estimated to occur in Queensland over the two year period (mentioned in section 2.2.1) in the late 1990s. This tends to support the contention of Hanger and Nottidge (2008) that most animals killed or injured during land-clearing are not found.

Most of the research conducted on land-clearing and its effect on wildlife has largely focused on the ecological outcomes and long-term consequences for wildlife populations (McAlpine *et al.,* 2002; Hobbs 2005; Brown *et al.,* 2008), but direct impacts on individual animals, in particular animal welfare issues, have essentially been ignored. This paucity of information and data on the topic makes it difficult to obtain realistic estimates of the scale of the welfare impacts of land-clearing. Further research in this area is certainly warranted to guide the development of protocols and strategies to minimise the risk of harm to animals during land-clearing operations.

2.2.3 Habitat fragmentation and edge effects

Although habitat loss and degradation through land-clearing practices has been identified as the primary threat to koala populations, fragmentation of the remaining habitat has also been responsible for drastically influencing their decline (McAlpine *et al.,* 2006b). Fragmentation occurs when habitat that was once continuous is broken up into isolated regions, generally as a result of agriculture and urban development (McAlpine *et al.,* 2006a). Often these fragments are too small or detached from other regions of habitat to support some animal populations (McAlpine *et al.,* 2002). The size of habitat remnants is often directly correlated with the time before extirpation (localised extinction), with extirpation occurring earlier in smaller fragments (Hanski and Ovaskainen 2002; Malanson 2002).

According to the EPA (2006), koalas successfully utilise highly modified habitat fragments that contain a small proportion of the original vegetation. In contrast, much of the scientific literature suggests that koalas are in fact sensitive to fragmentation (Dique *et al.,* 2003a; 2003d; McAlpine *et al.,* 2004; 2006b). McAlpine *et al.* (2004) considered fragmentation to be devastating to koala populations as it reduces or prevents the occurrence of natural ecological processes, including immigration and emigration. These processes are required for genetic fitness and generally benefit population fecundity; hence their impairment may threaten the viability of future populations. McAlpine *et al.* (2006b) also reported that although koalas are able to move across non-forested

gaps, where cleared land has been replaced by roads, fences and urban development, edge effects resulting in trauma-associated mortalities and other misadventure are more likely, and increase the likelihood of local extinction.

In contrast, edge effects in rural regions tend to be less serious and affect koala populations to a lesser degree than in urban areas, for example, by lower exposure to vehicular activity, domestic dog attacks and other misadventure (White 1999; Jones 2008). Nevertheless, koalas are more exposed to wild dog predation when traversing areas of fragmented habitat in rural regions (White 1999; McAlpine *et al.,* 2007). This may include movement through cleared areas such as paddocks, to isolated trees (White 1999).

The fragmentation of koala habitat increases the susceptibility of populations to stochastic events (Lunney *et al.,* 2004; Ewers and Didham 2006). Stochastic events are chance events such as bushfire, which are more likely to cause local extinctions in small habitat remnants (Lindenmayer and Burgman 2005). Habitat fragments in urban areas are likely to suffer increased frequency of bushfire events due to the higher frequency of deliberate/malicious fire-lighting, increased frequency of hazard or fuel-load reduction burning, and accidental fires from the irresponsible discarding of cigarette butts. Fire events have caused the extirpation of koalas from a number of habitat remnants on the Gold Coast in which recolonisation by koalas has not since occurred (J. Hanger pers. comm., 25th May 2009).

2.2.4 Inbreeding

Koala populations have experienced serious population bottlenecks throughout the past century as a consequence of the clearing of native vegetation, overhunting during the fur trade, disease epidemics and bushfires (Worthington Wilmer *et al.,* 1993; Houlden *et al.,* 1996). In reaction to local extinctions and significant decreases in koala numbers during the early 1900s, particularly in southern Australia, large-scale manipulations were implemented to combat these declines (Houlden *et al.,* 1996). These involved the translocation of a substantial number of koalas from French Island in Victoria to their former range on the mainland, in addition to the establishment of some island populations (Taylor *et al.,* 1997). Considering that the French Island koala colony was derived from as few as two individuals, it is understandable that these koalas and their descendent populations possess low levels of genetic variability (Houlden *et al.,* 1996; Taylor *et al.,* 1997). Furthermore, Seymour *et al.* (2001) and Critescu *et al.* (2009) found that poor genetic diversity in South Australian koalas is associated with testicular aplasia and other morphological abnormalities, and has the potential to adversely impact the long-term viability of these populations by increased susceptibility to a variety of threats.

In contrast, Queensland koalas have maintained higher levels of genetic diversity than their southern counterparts, despite in the past being subjected to population crashes, habitat loss and fragmentation, and vast reductions in range (Houlden *et al.,* 1996; Seymour *et al.,* 2001). This is likely due to their ability to

have recovered to current numbers without significant anthropogenic intervention (Houlden *et al.,* 1996). Unfortunately koalas in these regions are still being subjected to further fragmentation of their habitat, which has the potential to compromise the genetic diversity and fitness of these populations to a far greater degree than is apparent presently (Sherwin *et al.,* 2000).

2.3 Effects of Urbanisation

Significant changes have occurred in SEQ as a result of rapid human population growth in both coastal and inland regions (McAlpine *et al.,* 2006a). For example, Moreton Bay Regional Council (2009) estimated that in the Moreton Bay region, a renowned koala 'hotspot', almost 350,000 people were known to populate the area in 2007. With a growth rate of around 3.3% per year, it has been recognised as one of the fastest growing regions in Australia (Lindenmayer and Burgman 2005; MBRC 2009). In such circumstances, the extent of the human expanse, has, and will continue to severely impact koala populations, not only by further fragmentation and degradation of forest and woodland habitat remnants, but also due to the uniquely severe 'edge effects' associated with urbanisation (Dique *et al.,* 2003b; Rhodes *et al.,* 2006).

The progressive expansion of anthropogenic development has forced more and more koala populations to subsist in isolated pockets of remnant habitat (McAlpine *et al.,* 2006b). Dique *et al.* (2003d) reported that koalas in heavily urbanised environments are more likely to suffer collisions with vehicles, a

common threat accounting for a large proportion of mortalities. Domestic and wild dog attacks are an additional danger, particularly throughout the breeding season and when koalas are undergoing dispersal (EPA 2006). Collectively, the high frequency of deaths caused by road trauma and dog attacks result from increased urbanisation and are major factors accelerating koala population declines in urban and peri-urban areas (McAlpine *et al.,* 2005).

2.3.1 Dog attacks and motor vehicle incidents

As koala habitat is cleared and replaced by fenced yards, roads and residential developments, the incidence of dog attacks and vehicle collisions drastically increases (McAlpine *et al.,* 2004; Predavec 2008). Between 1997 and April 2008, 1,281 dog attack and 3,792 motor vehicle koala trauma cases were collectively presented to two koala hospitals in SEQ (DERM 2009b). Given that these figures represent only koalas presented for care (rather than actual numbers of koalas suffering from trauma, many of which are probably not found), the numbers are significant, especially in regions of highly fragmented habitat where high mortality rates threaten the viability of populations (McAlpine *et al.,* 2006a).

During, and leading up to the breeding season, koalas move around more and there is an increase in motor vehicle and dog attack trauma, particularly in males (Weigler *et al.,* 1987; Canfield 1991; Dique *et al.,* 2003a; 2003c). A radio-telemetry study by Dique *et al.* (2003c) investigated the dispersal of 40 koalas in

SEQ and found that 10 koalas died during dispersal events, with domestic dog attack and road trauma accounting for 80% of deaths. The remaining 20% were due to misadventure, such as drowning. This high mortality rate indicates that individuals dispersing in fragmented and heavily urbanised regions are more likely to die and therefore less likely to contribute to the maintenance of populations (Dique *et al.,* 2003a).

The home ranges of many koalas living in urban habitats are divided by roads and other linear infrastructure (EPA 2006). In an attempt to reduce the high number of road-related deaths, measures have included reducing speed limits, improving road design, installation of designated crossing points such as underpasses, and the use of koala exclusion fencing in known koala 'hotspots' (Dique *et al.,* 2003d; Taylor and Goldingay 2003; EPA 2006). A study by Dique *et al.* (2003d) found that vehicle speed alone was not responsible for the high number of koala collisions on the Koala Coast in SEQ. It was suggested that a high incidence of road mortalities was also related to habitat loss associated with urban development, traffic volume and density of koalas (Dique *et al.,* 2003d; Preece 2007).

A koala mortality survey conducted in northern New South Wales over a 20 year period indicated that trauma was accountable for almost 30% of cases presented for necropsy examination (Stalder *et al.,* 2008). Weigler *et al.* (1987) reported a much higher figure, with 60% of koalas succumbing to trauma. It is

important to note, however, that such mortality surveys are heavily biased in favour of conditions or circumstances likely to result in koalas being presented for treatment or post-mortem examination (Stalder *et al.,* 2008). In other words, koalas with overt disease, or those found injured or dying close to human habitation (for example, on roads) are more likely to be represented in mortality surveys. These should not be interpreted as indicative of the relative proportions of causes of death or illness in the general koala population (Hanger 1999; Stalder *et al.,* 2008).

2.4 Disease

Two of the most widely recognised disease agents impacting the koala are *Chlamydia* and the koala retrovirus (KoRV) (Timms 2005; Hanger 2008a; 2008b; Higgins 2008; Predavec 2008). Although habitat loss and fragmentation represent the most serious threats to koala conservation, disease is only just starting to be acknowledged as an important factor contributing to their decline (EPA 2006; Hanger 2008a). As KoRV has only been characterised relatively recently (Hanger 1999), the impact of this retrovirus and associated diseases on koala populations is not well known. The high incidence of KoRV-associated disease has been suggested as evidence for its relatively recent incursion into the species via an undetermined host-jump event (Hanger *et al.,* 2000). By comparison, some workers suggest that koalas have had a long association with *Chlamydia* (Martin and Handasyde 1990), although this has not been scientifically proven. In fact, Timms *et al*. (1996) demonstrated that koala strains

of Chlamydiae are genetically most similar to strains infecting domestic livestock, suggesting that koala infection may have resulted from a recent host jump.

Only one study (Jones 2008) has reported thorough investigations into the prevalence of overt and subclinical chlamydial disease in koalas, making it difficult to understand the real consequences of *Chlamydia* on wild populations. Instead, most ecological and health studies have largely focused on the prevalence of chlamydial infection (not prevalence of disease) in populations (Mitchell *et al.,* 1988; White and Timms 1994; Jackson *et al.,* 1999; Santamaria 2002).

2.4.1 Chlamydial disease

Chlamydial infection is one of the most important and common causes of disease in koalas, and affects almost all of Australia's wild koala populations (Devereaux *et al.,* 2003). The exceptions are a few *Chlamydia*-free populations, such as French Island and Kangaroo Island (Martin and Handasyde 1990). Chlamydial disease has long been recognised as a significant cause of infertility, poor health and death in the species (Masters *et al.,* 2004; Timms 2005; Markey *et al.,* 2007). Both *Chlamydia pecorum* and *Chlamydia pneumoniae* are responsible for a number of disease syndromes in koalas including ophthalmic disease such as kerato-conjunctivitis, respiratory disease, and urogenital tract

infections resulting in urinary incontinence and often leading to infertility in females (Devereaux *et al.,* 2003; Timms 2005).

In 1999, a study conducted by Devereaux *et al.* (2003) found that chlamydial infection was present in 72% of the Pine Creek State Forest koala population. Similarly, Jackson *et al.* (1999) sampled two free-ranging koala populations in SEQ and reported the level of chlamydial infection to be 85% and 10% in the Mutdapilly and Coombabah populations, respectively. Chlamydial prevalence rates have been known to vary between regions, although the majority of studies tend to report medium to high levels of infection (Ueno *et al.,* 1991; White and Timms 1994; Devereaux *et al.,* 2003; Timms 2005).

Jackson *et al.* (1999) indicated that the interpretation of studies investigating the level of chlamydial infection in wild koala populations is a complex task, especially considering that some studies have involved the random sampling of individuals within a similar geographical range, rather than being representative of the population. The outcome of these studies is also dependent on the method of detection used (Markey *et al.,* 2007), with some relying on the observation of overt clinical signs to estimate prevalence of disease (without actually measuring or estimating prevalence of *infection*) (Dique *et al.,* 2003c; Lane 2008), while others take advantage of more investigative techniques including serological assessment (Mitchell *et al.,* 1988; Ueno *et al.,* 1991) and PCR (Devereaux *et al.,* 2003) (to estimate prevalence of *infection* rather than

disease). It has been suggested by Timms (2005) that around 10-20% of chlamydial infections manifest as disease, however it was not clear from which studies these figures were based. In any case, these data were consistent with findings by Jackson *et al.* (1999) who reported that in the Mutdapilly koala population (previously mentioned), overt signs of chlamydial disease were evident in only 17% of infected animals. According to the EPA (2006), for a population to be considered healthy, less than 20% of the koalas should be affected by chlamydiosis. This figure seems somewhat arbitrary and of little use: it is not suggested to have any predictive value, in terms of population viability analysis, for example; neither do the author(s) suggest in what context such a figure may have value.

Although the exact origin of koala chlamydial strains is unknown (Timms 2005), it is generally accepted that *Chlamydia* has been present in koala populations at least since European settlement (Gordon and Hrdina 2005). As chlamydial infection is widespread in many populations (White and Timms 1994; Devereaux *et al.,* 2003), some studies have claimed that environmental stress, particularly in the urban expanse, may be responsible for inducing an immune response, or suppression, in infected koalas leading to the expression of clinical disease (Canfield *et al.,* 1991; Ellis *et al.,* 1993; White and Timms 1994; McCallum and Dobson 2002; Timms 2005). However, none of the literature has substantiated this theory with strong scientific evidence. McDonald *et al.* (1990) and Booth *et*

al. (1990) indicated that there is still a great deal to learn about the koala's ability to deal with stress.

The severity of chlamydiosis has dramatic implications for koala conservation and is a major factor influencing their decline (Girjes *et al.,* 1993; Devereaux *et al.,* 2003). Apart from being the cause of significant morbidity and mortality in the species, a serious consequence of chlamydial disease is its effect on reproductive potential (Brown *et al.,* 1984; Timms 2005). In Oakey, Queensland, 54% of female koalas sampled were affected by reproductive tract disease, rendering them infertile (Brown *et al.,* 1984). These results demonstrate that chlamydial disease can lower fecundity and negatively impact on population growth (Brown *et al.,* 1984; Gordon *et al.,* 1990). Nevertheless, Augustine (1998) predicted that in the absence of factors that are likely to contribute to changes in birth and mortality rates, such as increased transmission rates, disturbance by humans, and fragmentation, chlamydial disease is unlikely to result in extinction of the species. Higgins (2008) questioned the accuracy of these predictions in present times, especially as new findings into the cause, transmission and control of chlamydial disease emerge. These predictions were also made without consideration of other important koala diseases, and before the koala retrovirus was of known significance.

2.4.2 Koala retrovirus (KoRV) – associated disease

The koala retrovirus (KoRV) has been associated with neoplastic disease, including lymphoma and leukaemia, in both captive and wild koalas (Hanger 1999; Hanger *et al.,* 2000; Tarlinton *et al.,* 2005). It has been suggested that it may cause an immunosuppressive state in koalas and be responsible for the manifestation of severe chlamydiosis and opportunistic infections (Hanger 1999; Tarlinton *et al.,* 2008). In some other species, *Chlamydia* have been considered to be opportunistic pathogens commonly linked to retroviral infections such as feline immunodeficiency virus (FIV) in domestic cats (O'Dair *et al.,* 1994; Fiebig *et al.,* 2006). For this reason, Fiebig *et al.* (2006) suggested that it is possible that KoRV can promote immunosuppression in koalas. However, in humans, chlamydial infections are *not* significantly more common in HIV/AIDS patients; in other words, HIV/AIDS is not considered a risk factor for human genital chlamydiosis (P. Timms and J. Debattista pers. comm., 19th May 2009).

According to Tarlinton *et al.* (2006), evidence suggests that KoRV has entered the koala population only within the last 100-200 years. Recent studies suggest that KoRV is present in all SEQ koalas, however there is a mixed prevalence of KoRV in Victorian populations, and infection was absent from a limited number of samples from Kangaroo Island koalas (Tarlinton *et al.,* 2006; 2008). More recent work has demonstrated KoRV presence in some Kangaroo Island koalas (G. Simmons and P. Young pers. comm., 20th May 2009). It has been suggested that poor genetic diversity in some koala populations, such as that on Kangaroo

Island, could render them more susceptible to the effects of a genetic parasite, such as KoRV (J. Hanger pers. comm., 15th May 2009).

2.5 Evidence for Decline of the Koala in SEQ

Until recently, there have been few scientific studies supporting the notion that koala populations in Queensland (particularly SEQ), are in rapid decline (EPA 2007; Hanger 2008c; DERM 2009a). Most of the evidence for their decline has been anecdotal, although compelling, to say the least. Crude estimates (or wild guesses) of koala numbers throughout the State have caused much controversy due to conflicting views in the scientific community (Sullivan *et al.,* 2004; Predavec 2008). Sullivan *et al.* (2004) indicated that until a standardised survey method is adopted across all states to estimate koala numbers, the wide variation in these estimates tends to confound conservation efforts for the species.

Marked population declines have been observed in the Koala Coast koala population (in SEQ), with recent studies indicating a 64% decline in numbers over the past decade, and, even more alarmingly, a 51% decline in the past 3 years (EPA 2007; DERM 2009a). The Koala Coast is a rapidly urbanising region covering an area of 375km^2, located 20km south of Brisbane (DERM 2009a). It is bordered by Manly Road and Lota Creek to the north; Moreton Bay to the east; Logan River to the south; and arterial roads including the Pacific and Gateway Motorways to the west (EPA 2007; DERM 2009a).

According to the first Koala Coast koala surveys (1996-1999), population numbers were estimated at around 6246 individuals (Dique *et al.,* 2004). Using the same methods as in previous years, the 2005-2006 koala survey estimated the population had decreased to around 4611 individuals, representing a 26% decline in abundance (EPA 2007). In May 2009, the Department of Environment and Resource Management (DERM) released the most recent figures from the 2008 koala survey, which indicated that the population had declined to around 2279 animals (Figure 2.1) (DERM 2009a).

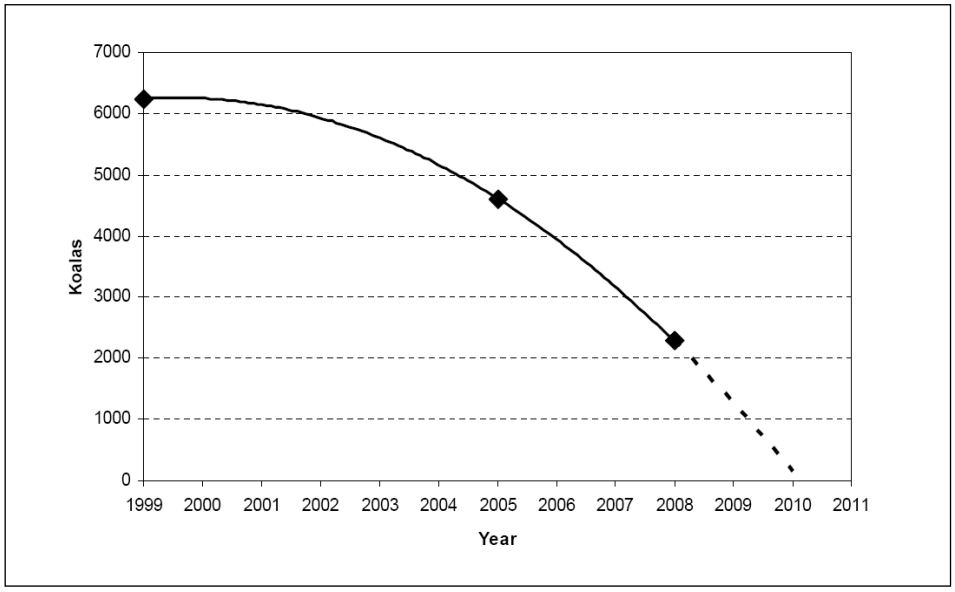

Figure 2.1: The observed and predicted decline of the Koala Coast koala population (DERM 2009a)

This report is potentially the single most powerful document in terms of effecting a change in government awareness of the magnitude of the problem facing koalas in SEQ. This is for two main reasons: 1. It was written by DERM; and 2. It demonstrates, unequivocally, the catastrophic decline and imminent extinction of a SEQ koala population previously presumed to be sustainable. Whether it does, in fact, induce meaningful change in statute and policy remains to be seen.

2.6 Priorities for Conservation of Koalas in SEQ

Over the past 200 years, approximately one quarter of global mammalian extinctions have occurred in Australia (Lindenmayer and Burgman 2005; Johnson *et al.,* 2007). Considering Australia's poor track record for species loss, it is important that significantly greater efforts are taken to preserve existing levels of biodiversity (Lindenmayer and Burgman 2005). Koalas are one of many species heading down the path of decline and are considered a flagship species for forest ecosystem conservation: "If we can't save koalas, what hope have other species got?" (D. Tabart pers. comm., 16th May 2009).

Koalas are greatly affected by a range of threatening processes, and consequently, a variety of different strategies must be implemented to address them (EPA 2006; Predavec 2008). Given the rate of decline and limitations on resources likely to be available to mitigate threats, it is necessary to prioritise actions for the conservation of this species. Clearly, the most important priority

would be a moratorium on the clearing of koala habitat; however this will only be achieved through statutory changes and enforcement (EPA 2006; Hanger 2008a). The restoration and de-fragmentation of habitat must also occur (Scott *et al.,* 2001; Cogger *et al.,* 2003), in addition to addressing the significant causes of death in these animals (Dique *et al.,* 2003d; Hanger 2008a).

2.6.1 Disease research

Despite the large number of studies that have been conducted on the koala, there is still insufficient knowledge regarding the real impacts of disease in wild populations. Some priorities for future disease research may include:

- further investigation into the pathogenesis and epidemiology of chlamydiosis and KoRV-associated disease (Hanger 2008b);
- determining the prevalence of chlamydiosis and KoRV-associated disease in rural and urban Queensland populations (Hanger 2008a; 2008b);
- establishing whether a relationship exists between KoRV infection and the manifestation of chlamydial disease (Hanger 2008a); and
- the development of a vaccine for the prevention and control of chlamydial disease (Brown *et al.,* 1987; Carey *et al.,* 2010).

2.6.2 Conservation-based research

In order to protect and conserve koalas throughout SEQ, research efforts need to be largely focused on ways of minimising anthropogenic impacts (EPA 2006; 2008). Thompson (2006) reported that there is no urgent need to carry out basic ecological studies such as utilisation of habitat, tree preferences, home-range and dispersal patterns. Instead, conservation research priorities for the koala should include:

- extensive survey and monitoring programs to establish more accurate koala population estimates (EPA 2008);
- the development of new and innovative mitigation measures, such as wildlife crossings, to address road-associated mortality, in addition to monitoring the effectiveness of such measures (Dique *et al.,* 2003d; Taylor and Goldingay 2003; Corlatti *et al.,* 2009); and
- identification of all koala habitat, including habitat currently not protected from urban development, such as privately owned land (EPA 2008).

2.6.3 Rescue and rehabilitation process

Each year in SEQ, hundreds of sick, injured and orphaned koalas are rescued from the wild and taken to wildlife rehabilitation facilities for specialised veterinary care (Australian Wildlife Hospital unpublished data; EPA 2006; Hanger 2008a). Although rehabilitation of wildlife is considered insignificant to many scientists for conservation of a species, it is suggested by Dique *et al.*

(2004) and the EPA (2006) that due to the extensive network of wildlife rehabilitation facilities, veterinary personnel and volunteer organisations, the number of koalas released back to the wild that would otherwise have died without intervention, contribute greatly to koala sustainability. Hanger (2008a) also indicated that the general community has expectations that sick and injured wildlife will be rehabilitated and humanely managed.

In order to achieve the best possible outcomes for critically injured animals, it is essential that rescue calls are responded to rapidly, and that veterinary assessment and treatment of patients reflect current best practice (Hanger 2008a; DPI and F 2009). Hanger (2008a) stated that 24-hour attendance of a wildlife facility by suitably qualified or experienced staff is necessary to adequately monitor and respond to pain and distress of in-patients. At the time of writing, standards and guidelines to improve the rescue and rehabilitation process of koalas were being formulated to ensure the application of best practice standards by all koala rehabilitation centres in Queensland (Hanger 2008a; DPI and F 2009).

2.7 Conclusions

Koala populations in Queensland, particularly in SEQ (but also other southern bioregions), are facing numerous threats to their survival, and as a consequence, are experiencing dramatic population declines. Although habitat loss and fragmentation are logically the most critical threatening processes

affecting the species, disease (in particular chlamydiosis and KoRV-associated disease), and the impacts of urbanisation such as motor vehicles and domestic dogs, are also reported as significant factors contributing to their decline.

Future directions and research for the conservation of koalas should also include:

- Protection of all remaining koala habitat from further land-clearing and degradation;
- Restoration of habitat connectivity (de-fragmentation);
- Mitigation of human impacts (eg facilitating the safe movement of koalas across roads);
- Addressing the significant causes of death in koalas (eg motor vehicles, dogs, disease);
- An investigation of animal welfare issues associated with land-clearing;
- Rehabilitation of sick, injured and orphaned koalas;
- Disease and conservation-based research; and
- Lobbying of current government to promote a strong and motivated political will for the development and implementation of effective statutory mechanisms.

CHAPTER 3: Hypotheses and Objectives

According to records from wildlife hospitals around south-east Queensland (SEQ), it is evident that koala populations across SEQ are severely affected by disease (Australian Wildlife Hospital unpublished data; DERM 2009b). Although chlamydiosis is the most common disease diagnosed in koalas admitted to wildlife hospitals (with approximately 40% of koalas found to have detectable pathology), conditions including neoplastic diseases and an AIDS-like syndrome (Australian Wildlife Hospital unpublished data) which have been putatively associated with KoRV (Hanger 1999; Hanger *et al.,* 2003) are also regularly encountered (Australian Wildlife Hospital unpublished data; J. Hanger pers. comm., 28th January 2010).

It is important to note that data derived from wildlife hospitals are likely to be somewhat biased, considering that sick animals are more frequently found by the public and brought into care than healthy animals (Stalder *et al.,* 2008). In other words, the subset of the wild koala population admitted to the hospital may not be representative of the whole population, because sick animals will be over-represented (as for human hospital patients). Certainly the frequency of sick koala admissions gives a subjective impression that the wider population is seriously affected by disease, but does not allow accurate measurement of the true prevalence and incidence of disease. Consequently, the true extent and seriousness of the effect of disease on wild koala populations is currently not

known. This study investigated disease in four wild koala populations in SEQ (with health data from the Kangaroo Island koala population included for comparison). The aim was to determine the true prevalence of disease in SEQ populations by conducting thorough veterinary health examinations on most/or all of the resident koalas from each population (with the exception of the East Coomera population which included a sample of koalas from various proposed development sites within this region). It also aimed to provide an indication of the incidence of new disease in the population (acquired since their initial health examination) throughout the period of the study.

The hypotheses proposed for this study were that:

1. Chlamydiosis is the most common disease affecting wild koalas, while many KoRV-associated diseases will be common but more likely to be encountered in longitudinal studies which will be reflected in the incidence data (as many KoRV-associated diseases are often acute);

2. The prevalence of disease is higher in wild SEQ koala populations than has been estimated previously;

3. The incidence of new serious disease (determined by longitudinal monitoring) in wild koala populations is also high when compared with humans;

4. A large proportion of koalas have chlamydiosis, despite showing no overt signs of disease; and

5. Infertility in female koalas is high when compared with humans.

The objectives of this study are:

1. To conduct cross-sectional and longitudinal population health assessments to determine the most common diseases causing morbidity in koalas;

2. To determine the prevalence of disease in four geographically separate wild koala populations in SEQ by conducting thorough veterinary health examinations on most/or all of the koalas in each population;

3. To monitor the health of wild koala populations by conducting follow-up health examinations to determine the incidence of new disease;

4. To conduct veterinary health assessments using a range of diagnostic tests, including ultrasound imaging and urinalysis, to enable the detection of chlamydial disease that may not be overtly apparent; and

5. To ascertain the fecundity of female koalas by recording the presence of young and by conducting ultrasound examinations for the detection of pregnancy or reproductive disease.

CHAPTER 4: General Materials and Methods

4.1 Permits and Approvals

This study formed part of a number of research collaborations with various organisations throughout Queensland. These included the Ecological Services Unit (ESU) (a division of Australia Zoo Wildlife Warriors Worldwide Ltd.), Gold Coast City Council, The University of Queensland (koala retrovirus research) and Queensland University of Technology (*Chlamydia* research). Animal ethics approvals and Scientific Purposes Permits (SPP) were required and obtained for each of these projects prior to the commencement of this study.

4.1.1 Brendale and Narangba- Veterinary health examinations

Animal ethics approval for disease and ecological research being conducted by the ESU at the Brendale and Narangba study sites had originally been sought through the Department of Primary Industries and Fisheries (DPI and F), now known as the Department of Employment, Economic Development and Innovation (DEEDI) (Qld DPI and F Community Access AEC approval numbers: CA 2008/04/258 and CA 2008/05/271). For the purpose of this study, these permits were later ratified by the University of Queensland (UQ) Animal Ethics Committee (AEC) (SAS/QDPI/675/08 and SAS/QDPI/674/08, respectively).

Scientific Purposes Permits for the capture, health assessments and radio-telemetry of Brendale and Narangba koalas were also obtained (Permit number: WISP05234408 and WISP05694009).

4.1.2 Gold Coast City Council- Veterinary health examinations

As part of the East Coomera Koala Conservation Project, a koala translocation and monitoring program being conducted by the Gold Coast City Council, an animal ethics approval and an SPP were required to conduct veterinary health examinations on resident koalas. The veterinary health examinations of koalas in the East Coomera and Clagiraba regions, which are reported in this study, were conducted in accordance with DPI and F Community Access AEC approval number CA 2008/06/273. A SPP was also obtained by the Gold Coast City Council (WISP05591008).

4.1.3 The University of Queensland/Queensland University of Technology- koala retrovirus/*Chlamydia* sample collection

Blood samples taken from koalas for koala retrovirus research were collected in accordance with UQ AEC approval number MICRO/PARA/612/08/ARC.

Swab samples taken from koalas for *Chlamydia* research were collected in accordance with QUT University Animal Research Ethics Committee approval number 0900000267.

4.1.4 Kangaroo Island koala sample collection

Blood, bone marrow and cloacal swab samples taken from Kangaroo Island koalas in South Australia were collected in accordance with UQ AEC approval number SVS/488/09/ARC/WWW and South Australian Wildlife Ethics Committee approval number 51/2009. A Scientific Permit to conduct scientific research in South Australia was also required (U25790 1), in addition to a Queensland Ecoaccess movement permit for the import of biological samples (WIWM06555009).

4.2 Study Sites

Two of the study populations were located in the Moreton Bay Regional local government area (LGA), (situated to the north of Brisbane City), and two in the Gold Coast City LGA (situated to the south of Brisbane City) in south-east Queensland. Those in the Moreton Bay Region were in the suburbs of Brendale and Narangba, and the Gold Coast koala populations were in the suburbs of Clagiraba and East Coomera (Figure 4.1).

The Moreton Bay and Gold Coast koala populations were chosen for this study as they formed part of existing research projects being conducted by the Ecological Services Unit and the Gold Coast City Council, respectively. Each study site, with the exception of Clagiraba, was proposed for urban and/or industrial development and will have a major impact on resident koala populations. In order to provide a more scientific approach during the

development process and to achieve better animal welfare outcomes, the koala populations at each site were being actively managed by the respective organisations. At the time of writing, koalas were being monitored by radio-telemetry prior to, and during the land-clearing process. In some cases, koalas were to be translocated to areas of secure habitat considering that minimal habitat would be available to support all of the resident koalas once clearing commenced. One of the recipient sites for translocation of the East Coomera koalas was the Clagiraba site (Lower Beechmont Conservation Area).

Some advantages of these study populations included:

- A large number of koalas were available through various research collaborations to conduct thorough investigations of population health throughout SEQ;
- An opportunity to gather comprehensive health data from koalas in four geographically distinct regions of SEQ, especially considering no previous studies had been done in this regard;
- The health of each koala population could be monitored over a prolonged period because individual animals were tracked using radio-telemetry over a number of years.

The health of resident koalas from all of these sites is reported in this study. A brief summary of the overall health of each koala is outlined in Appendix 1.

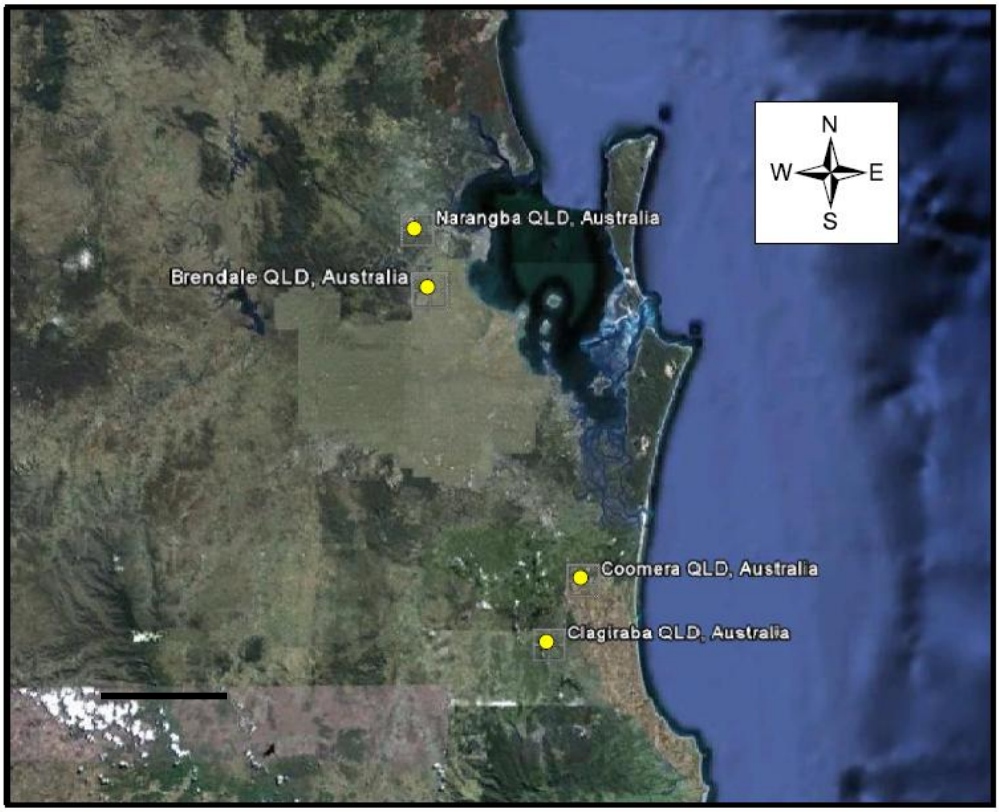

Figure 4.1: Composite aerial view of southeast Queensland showing koala population study sites (Bar = 20km) (Google Earth)

4.2.1 Brendale (Moreton Bay LGA)

The Brendale study site covered an area of approximately 122 hectares and was intersected by two main roads, Kremzow Road and Old North Road (Figure 4.2). A clay mining quarry made up a large portion of the north-western region of the site. To the immediate north of the quarry there was an ecotone of open woodland and grassland. This area was bordered by residential housing and future housing developments.

The south-western boundaries were adjacent to private land used for sand-blasting and clay mining. The south-eastern boundary adjoined land cleared for cattle grazing.

Along the drainage line, which occurred in the north eastern corner of the site, the vegetation was made up of extensive stands of *Melaleuca, Lophostemon*, and *Glochidion*. The remainder of the site was vegetated with mixed Eucalypt open woodland, in addition to species of *Corymbia* and *Angophora,* predominantly of regrowth status.

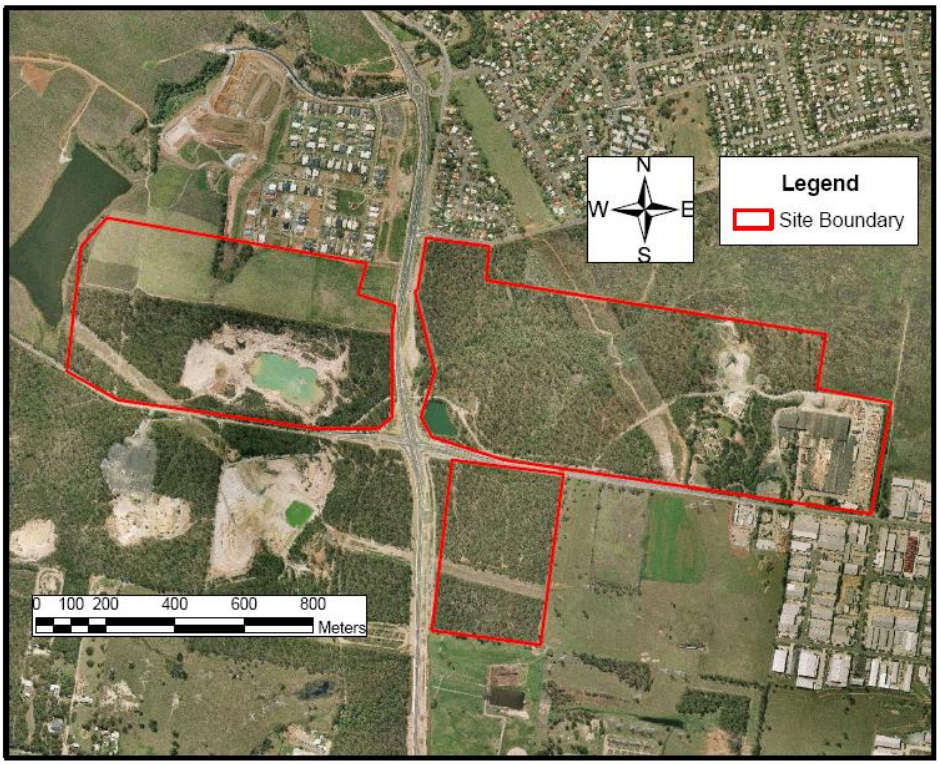

Figure 4.2: Aerial view of the Brendale study site

4.2.2 Narangba (Moreton Bay LGA)

The Narangba study site covered an area of approximately 44.2 hectares (Figure 4.3). It was bordered by residential properties to the north and west, acreage properties to the south, and a railroad to the east running parallel to O'Mara Road. The majority of vegetation was characterised by mixed Eucalypt open woodland, predominantly of regrowth status. The site contained a number of disturbed areas including a clay mining quarry, which covered a significant portion of the eastern section of the site. To the west of the quarry, a drainage line comprising *Melaleuca* swampland ran from the northern to southern boundary.

Tree species commonly found on both the Narangba and Brendale sites included *E. microcorys, E. propinqua, E. resinifera, E. tindaliae, E. siderophloia, E. fibrosa, E. tereticornis, E. racemosa, Corymbia intermedia, C. citriodora, Angophora woodsiana, Lophostemon suaveolens, L. confertus, Glochidian sumatranum* and *Melaleuca quinquenervia.*

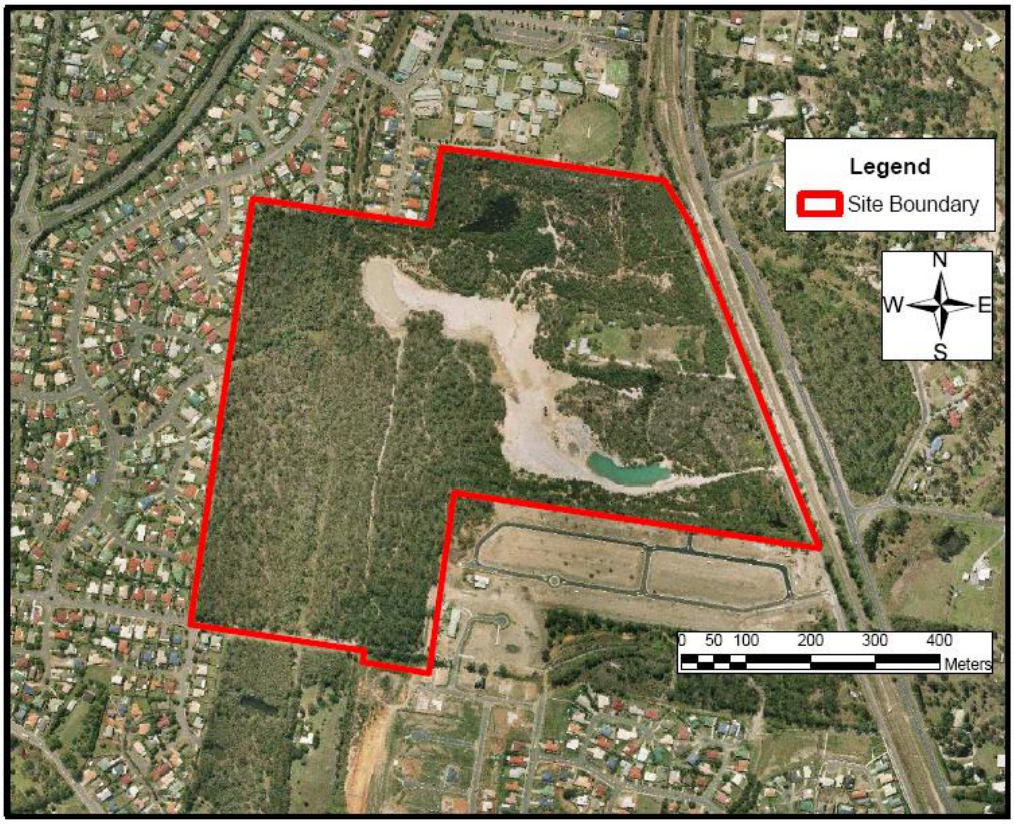

Figure 4.3: Aerial view of the Narangba study site

4.2.3 East Coomera (Gold Coast City LGA)

The East Coomera region in the Gold Coast LGA is bounded on its south by the Coomera River, its west by the M1 motorway, to its east by the waterways of the southern extent of Moreton Bay and to its north by the cane fields of Jacobs Well (Figure 4.4). The region is under significant development pressure due to a State government imperative to develop a new town centre and development approvals which stretch back over 20 years. Much of the area is high-quality

koala habitat, and supports a significant population of koalas, estimated by Biolink Ecological Consultants (2007) to be in excess of 500. Koalas used in this study were from a number of sites earmarked for development throughout the East Coomera region.

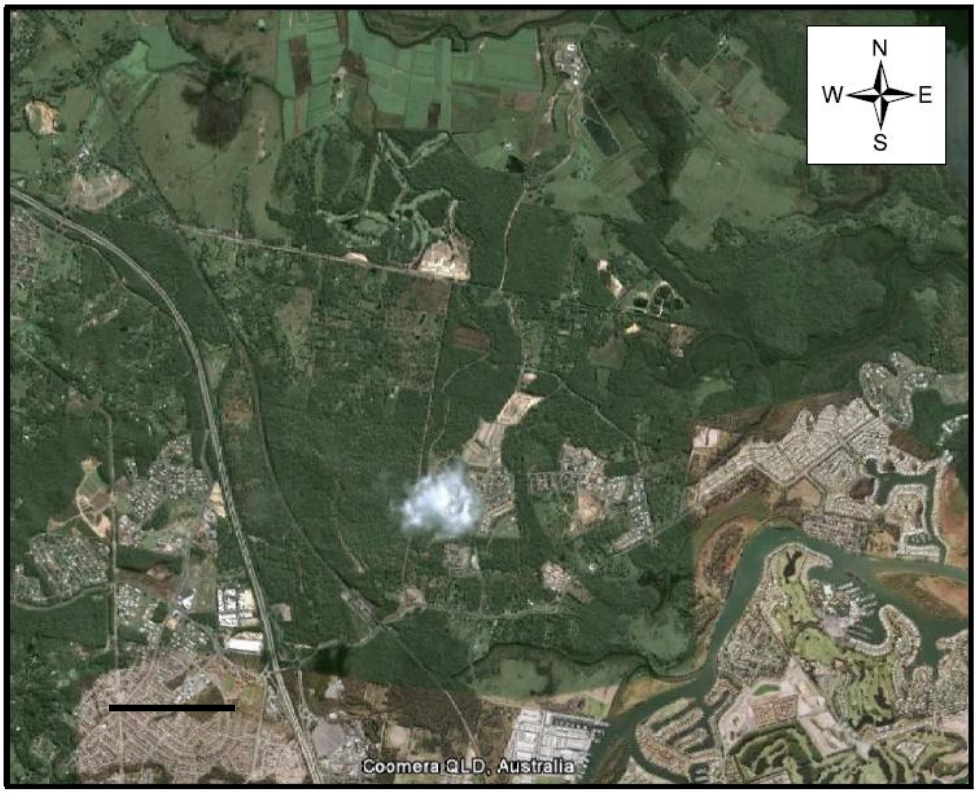

Figure 4.4: Aerial view of the East Coomera study region (Bar = 1km) (Google Earth)

4.2.4 Clagiraba (Gold Coast City LGA)

The Clagiraba study population was located in the Lower Beechmont Conservation Area (LBCA), a 470 hectare site, designated for nature conservation (Figure 4.5). The LBCA is an area of relatively secure and intact habitat away from urban pressures. The undulating terrain is characterised by mixed Eucalypt open forest and areas of rainforest. Tree species commonly found on this site include *E. propinqua, E. microcorys, E. tereticornis, E. carnea. E. tindaliae, E. siderophloia*, *C. citriodora* and *Lophostemon confertus.*

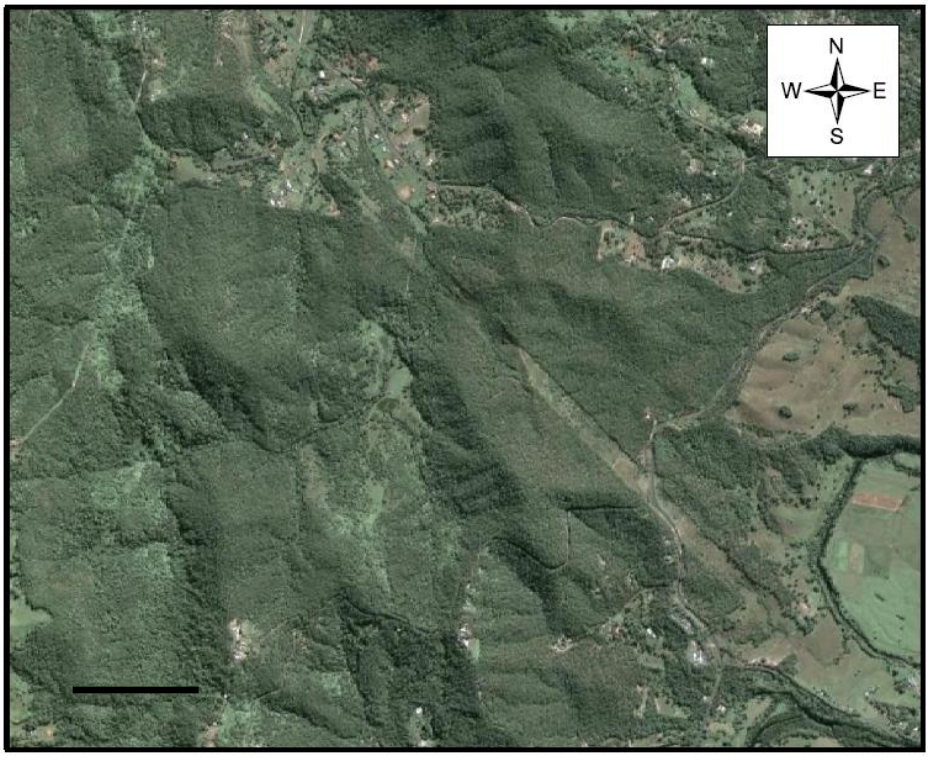

Figure 4.5: Aerial view of the Clagiraba study region (Lower Beechmont Conservation Area) (Bar = 550m) (Google Earth)

4.3 Koala Location and Capture

At each study site, extensive searches were conducted over a period of weeks by experienced koala spotters in an attempt to locate and capture most or all of the resident koalas. Koala captures at the Brendale and Narangba sites began in July 2008 and August 2008, respectively. For the purposes of this study, final captures for veterinary health examination were conducted in April 2010 for the Brendale population and February 2010 for the Narangba population, however research conducted on these populations will be continued for an extended period by the Ecological Services Unit. Capture of the Gold Coast City koalas commenced in October 2009. Similar to the Brendale and Narangba koala populations, research will be continued by the Gold Coast City Council over an extended period, however captures for the purposes of this study concluded in May 2010.

Various koala capture techniques were employed throughout the study. The capture technique was dependent on the nature of the tree in which the koala was residing, and the proximity of the tree to other vegetation. A number of other factors were also taken into consideration including the presence of a joey, weather conditions, and potential hazards and risks to personnel and the koalas. After each capture, a range of data including the GPS location was recorded so the koala could be released back to this point if deemed healthy at their

veterinary health examination (unless the koala was to be translocated as part of the East Coomera Koala Conservation Project).

Koalas were captured using either the flagging method or a koala trap (described in Jones (2008)). A modification to the trap included a remote alert device (Titley Scientific™, Australia) which sent a text message to a designated mobile phone when the trapdoor was triggered. This reduced the amount of time spent monitoring the traps. In the absence of the remote alert device, traps were checked every few hours to prevent overheating and/or stress to koalas that may have been captured.

Koala traps have a number of advantages in that there is minimal stress and virtually no risk to the koala, and hence were employed when there was significant risk of injury or harm to the koala and/or capture team. However, they are not suitable for immediate captures and may take up to a few days to catch an animal. In addition, koalas that have been previously caught by the trap method may also become 'trap-shy', making it harder to catch them during subsequent attempts.

Once captured, koalas were placed into a 60 cm long x 50 cm high x 40 cm wide weld-mesh (top opening) carry cage with a plastic bottom for transportation to the veterinary team. A towel was placed at the bottom of the cage and a cage cover was used to reduce the koala's exposure to visual stimuli.

After capture, koalas from the Moreton Bay Region study sites were immediately transported in an air-conditioned vehicle to the Australian Wildlife Hospital for veterinary health examination and sampling. Those from the Gold Coast City study sites were examined in a mobile veterinary unit set up on site.

4.4 Koala Veterinary Health Examinations and Sampling

Veterinary health examinations of adult koalas and independent joeys (approximately 11 months and older) were performed under general anaesthesia to minimise stress to the animal and to facilitate examination and sampling. To ensure consistency, all clinical assessments were carried out by the same wildlife veterinarian (Dr Jon Hanger) and veterinary nurse (Jo Loader).

Prior to general anaesthesia, koalas were observed for overt physical signs of disease. This included signs of disease that were indicative of chlamydiosis including:

1. Urine staining and/or wetness of the rump (Plates 4.1 and 4.2);
2. Kerato-conjunctivitis affecting the eye(s) and/or ocular discharge (Plate 4.3);
3. Nasal discharge (Plate 4.4); and
4. Cloacal exudate (Plates 4.5 and 4.6).

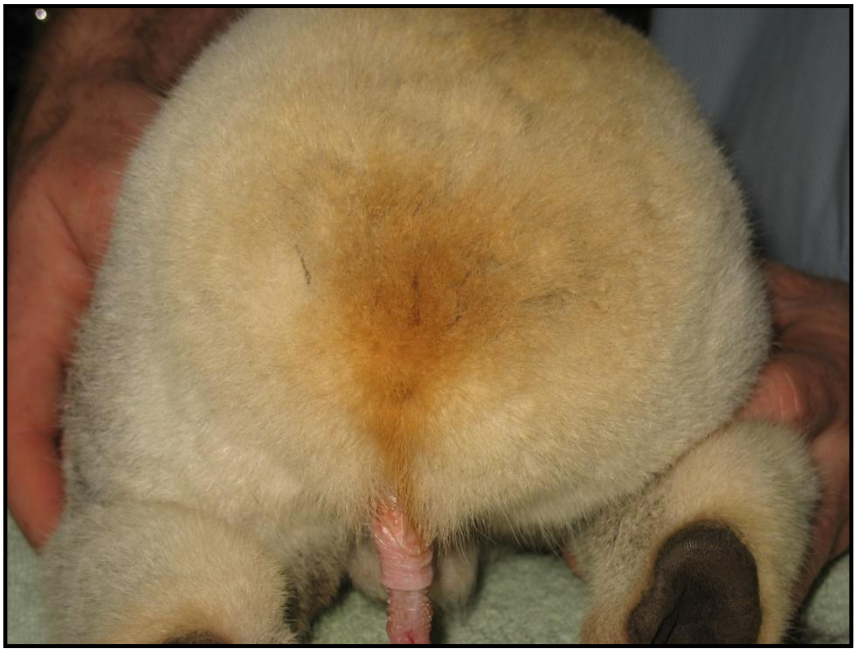

Plate 4.1: Mild staining of the rump- koala with cystitis (Koala ‘Indie’)

Plate 4.2: Marked staining and wetness of the rump- koala with cystitis (Koala ‘Natashi’)

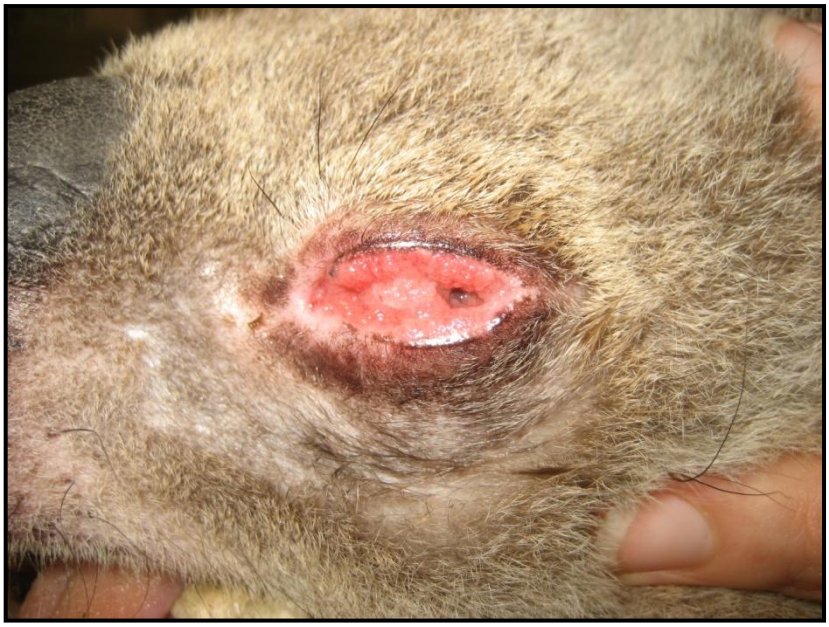

Plate 4.3: Kerato-conjunctivitis- marked proliferative change

Plate 4.4: Muco-purulent nasal discharge (Koala ‘Renee’)

Plate 4.5: Cloacal discharge (seminal plug -post-mating) (Koala ‘Lisa’)

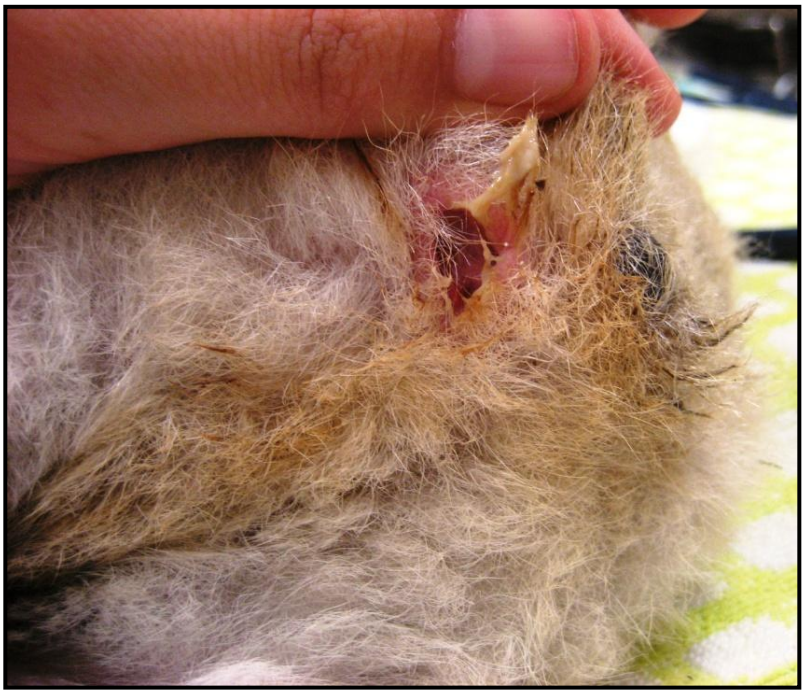

Plate 4.6: Suppurative cloacal discharge- severe reproductive disease (Koala ‘Maggie’)

Overt signs of disease (apparently unrelated to chlamydial infection) that may be indicative of a sick koala included:

1. Poor body and/or coat condition;
2. Abnormal growth/tumour/asymmetry;
3. Skin condition (eg alopecia, dermatitis); and
4. Any other obvious abnormality.

Anaesthesia was induced using alfaxalone 10 mg/ml (Alfaxan CD-RTU®, Jurox Pty Ltd) injected intramuscularly (quadriceps muscle) at a dose rate of 3 mg/kg, and maintained with isoflurane (Isoflo™, Abbott)/medical oxygen administered via a face mask (Plate 4.7). Intubation was only performed if the koala experienced apnoea and respiratory support was required.

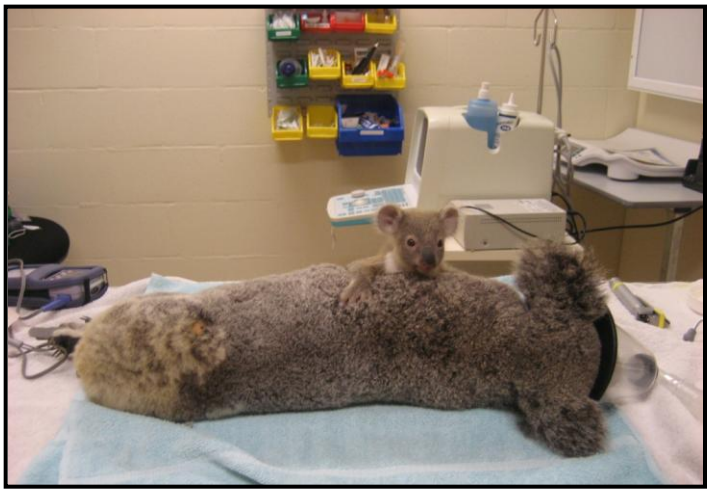

Plate 4.7: Veterinary health examination of a female koala (with a joey) under general anaesthesia (Koala 'Jacquie')

Following a standardised veterinary examination protocol (*Koala Examination Data Sheet* - Appendix 2), each individual was subjected to a complete physical examination and a range of ancillary diagnostic tests aimed at detecting most known conditions in koalas. These included:

- Routine diagnostic techniques including blood and bone marrow assessment;
- Abdominal paracentesis;
- Ultrasound examination of the bladder, female reproductive tract, prostate, kidneys and ureters;
- Urinalysis and cytology using ultrasound-guided cystocentesis;
- Swabbing of eyes, nares, urogenital sinus and urine sediment for Clearview® *Chlamydia* MF testing and real-time quantitative PCR (polymerase chain reaction); and
- A KoRV viraemia titre estimation on blood collected from the some of the koalas.

Other diagnostic aids, including radiographs, were only utilised if clinically indicated. In addition to *Koala Examination Data Sheets*, clinical examinations of koalas were documented using digital photographs, photomicrographs, and sonograms. Evidence of overt disease that was apparent during capture or handling (prior to veterinary examination), such as dirty tail or kerato-conjunctivitis, was recorded.

Note: as *Chlamydia* is recognised as the most common aetiological agent in koalas with cystitis, kerato-conjunctivitis and reproductive disease, it was assumed that manifestations of these conditions in koalas examined for this study were the result of chlamydial infection.

Koalas considered to be healthy by the wildlife veterinarian were ear tagged, microchipped and fitted with a VHF transmitter collar and released at their point of capture. Those found to have a significant pathological condition were either admitted to the Australian Wildlife Hospital for treatment until they were healthy enough for release, or humanely killed if the severity of disease or prognosis warranted euthanasia. Post-mortem examinations were performed on the majority of koalas that were euthanased.

4.4.1 General physical examination

A distant examination was first performed on each koala to develop a sense of its general condition prior to anaesthesia. Some components of the distant examination could be completed while the koala remained in the transport cage. This included an assessment of their general demeanour and neurological state, symmetry and breathing patterns. However, if there was any history of lameness, major traumatic injury or neurological dysfunction, the koala was removed from the cage for further observations of behaviour, gait and posture. The presence or absence of a joey was also noted.

Once anaesthetised, a complete physical and clinical assessment was performed and the findings recorded on a standardised data record sheet (*Koala Examination Data Sheet)* (Appendix 2). This included:

- Sex, weight, tooth wear (according to Gordon 1991) and body condition score. The body condition score was determined by palpating the suprascapularis and infrascapularis muscle masses associated with the scapular ridge while also considering the overall condition of the animal (eg muscle masses covering the cranium, spine, rump, biceps and quadriceps). The score was based on a scale of 1 (emaciated) to 10 (excellent);
- Auscultation of the heart and lungs;
- Examination of the head (head symmetry, eyes, ear, nose) and mouth (lips, teeth, gingiva, cheek pouches, tongue, larynx, pharynx, palate and fauces);
- Palpation of the peripheral lymph nodes (facial, rostral mandibular, mandibular, superficial cervical, axillary, and inguinal lymph nodes);
- Examination of the skin and coat condition (also making note of any ectoparasites);
- Musculoskeletal examination (clavicles, ribs, limbs and joints);
- Examination of paws, claws and digits;
- Abdominal palpation to assess stomach and abdominal fill and consistency (in females, palpation of the abdomen adjacent to the

epipubic bones was often useful for the detection of reproductive tract cysts or abscesses, although ultrasound examination was used to confirm any suspicion);

- Inspection of the scrotum, pouch, mammary glands, scent gland, cloaca, penis and clitoris; and
- Examination of the rump for wetness, staining, cloacal inflammation, ulceration or protrusion, and ulceration of the rump. Each koala was given a dirty tail score of 0 (no signs of cystitis) to 10 (marked physical signs of cystitis) (outlined in Appendix 2).

4.4.2 Blood collection

A 2ml blood sample was collected from the cephalic vein using a 23G (19mm) or 22G (25mm) needle and placed into two 0.5ml EDTA tubes, a 0.5ml serum tube and the remainder used for in-house blood tests including blood smear examination and measurement of packed cell volume and total plasma solids. Blood smears were dried immediately after smearing (using a hair dryer), fixed and stained with Diff Quik (Lab Aids, Narrabeen) and examined by Dr. Jon Hanger.

A 200µl aliquot of plasma was separated from EDTA blood samples from the majority of koalas and added to 300µl of RNA-Later (QIAGEN, Hilden, Germany) to determine the level of koala retrovirus (KoRV) viraemia (see Chapter 6). These analyses were performed by the Koala Retrovirus Research

Group at the University of Queensland. A detailed description of their methods is beyond the scope of this thesis.

4.4.3 Bone marrow collection

As bone marrow disorders are common in koalas (Hanger 1999), bone marrow collection formed part of the routine veterinary assessment. Approximately 10-15µl of bone marrow was aspirated from the iliac crest using an 18G (38mm) needle attached to a 2.5ml syringe. This method was effective and less expensive than using specialised bone marrow collection needles (as described in Spencer and Canfield 1995).

Under general anesthesia, koalas were placed in lateral recumbency and the iliac crest was clipped and prepared with a pre-operative skin disinfectant (alcohol-cetridine solution). By applying moderate pressure, the needle was then inserted perpendicularly into the cancellous bone of the iliac crest using a gentle twisting motion (to a depth of around 3-5mm). A vacuum of 0.3-0.5ml was applied to the syringe by pulling back on the plunger until a flash of marrow could be seen in the needle hub (Plate 4.8). The vacuum was then released before removing the syringe and needle from the site. A drop of marrow was immediately placed onto the centre of a glass microscope slide. A second slide was delicately placed on top of the first slide, the marrow allowed to spread for around 1 second, and then the slides were drawn apart. The slides were dried,

fixed and stained with Diff Quik (Lab Aids, Narrabeen) and then examined by Dr. Jon Hanger.

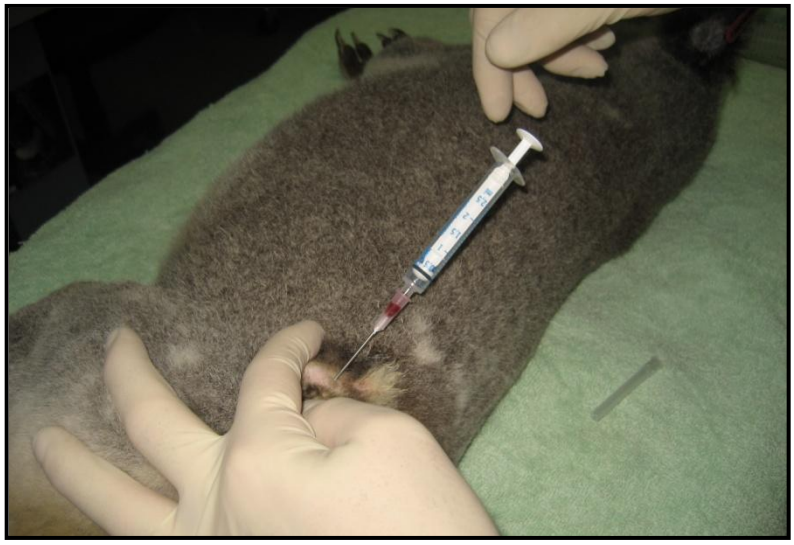

Plate 4.8: Bone marrow aspirate from the iliac crest of a koala

4.4.4 Abdominal aspirate

Paracentesis was used to collect fluid from the abdominal cavity by disinfecting a small region of the flank. A 25g butterfly catheter and 2.5ml syringe were used to aspirate the fluid. The needle was slowly inserted perpendicularly to the skin and abdominal wall, and a small vacuum applied. As the caecum and proximal colon take up a large proportion of the abdominal cavity, they are at risk of being punctured and contamination of the sample occasionally occurred. Contamination was minimised by reducing the depth at which the needle was advanced. If contamination did occur, a new needle and syringe were used and the collection technique was repeated at a slightly different site. Only a small

amount of fluid was required for analysis. One drop was placed onto a microscope slide and then smeared, fixed and stained in the same manner as the bone marrow aspirate (described in 4.4.3).

In koalas, abdominal aspirates can be used to diagnose some neoplastic diseases in koalas (e.g. mesothelioma, lymphoma), to detect blood in the peritoneal cavity (often the result of trauma), or to diagnose conditions such as peritonitis. Abdominal paracentesis forms part of the routine clinical assessment for trauma koalas at the Australian Wildlife Hospital (J. Hanger pers. comm., 11th Jan 2009).

4.4.5 Urinalysis

Urogenital tract pathology, particularly cystitis, is often characterised by staining and wetness of the rump (also known as ‘dirty tail’ or ‘wet bottom’), however in many cases it is not apparent as overt disease (Blanshard and Bodley 2008; Hanger and Loader 2009). Examination of urine is therefore necessary for the rapid diagnosis of diseases such as cystitis, and formed part of the routine veterinary assessment used in this study.

A urine specimen was collected from each koala using ultrasound-guided cystocentesis (Plate 4.9). With the koala in dorsal recumbency, a 25g (38 mm) needle was used to aspirate urine from the urinary bladder. The bladder in females was generally imaged through the pouch using sonographic gel, however females with small pouch joeys were placed in lateral recumbency and

the bladder was imaged via the caudal flank. Occasionally a koala may have emptied their bladder prior to general anaesthesia and in these cases urine was unable to be collected and analysed.

The following tests were conducted on each urine sample:

- Dipstick urinalysis using the Combur[9] Test® (Roche Diagnostics Australia)
- Urine specific gravity (measured with a clinical refractometer)
- Urine sediment: examined microscopically for the detection of crystals, bacteria, blood, and inflammatory cells.

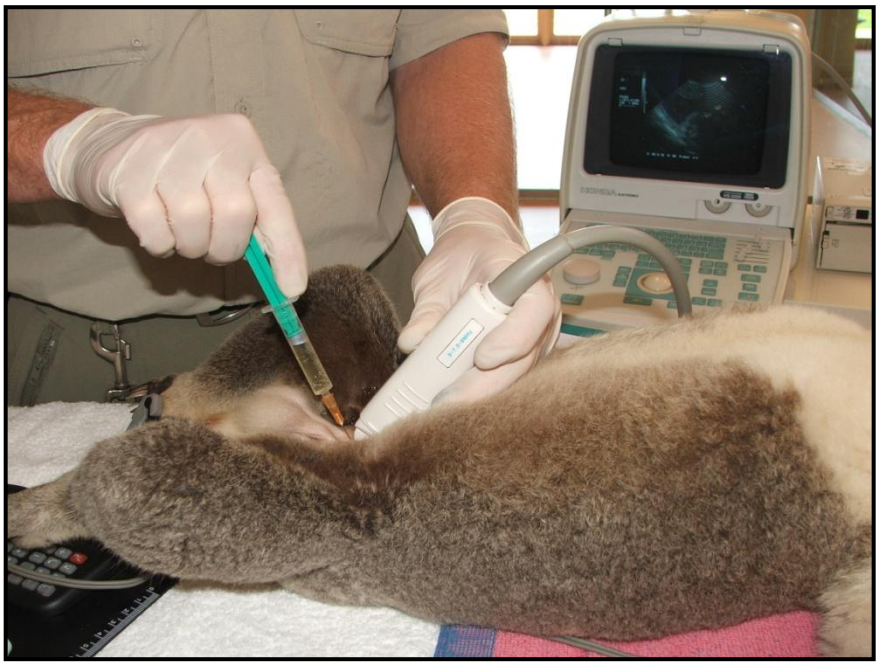

Plate 4.9: Ultrasound-guided cystocentesis of a male koala

4.4.6 Clearview® *Chlamydia* MF test and PCR swabbing

Duplicate swab samples were collected using a cotton-tipped aluminium-shafted swab from the left eye, right eye, nares, and urogenital sinus (Plate 4.10). The tip of the swab was moistened with sterile sodium chloride (NaCl 0.9%) solution to prevent irritation during collection and to facilitate entrance of the swab into the prostatic urethra. If urine could be collected from the koala by cystocentesis (as described in 4.4.5), a swab was also taken of the urine sediment. This was done by brief centrifugation of the 1ml of urine in a microtube, removing the supernatant, and then swabbing the pellet (urine sediment) at the bottom of the tube.

One swab from each anatomical site was tested using the Clearview® *Chlamydia* MF test kit (Inverness Medical, Unipath Ltd). Duplicate swabs from each site were given to the *Chlamydia* Research Group at QUT for PCR (methods and results are beyond the scope of this thesis). The Clearview® *Chlamydia* MF test is an ELISA test designed to detect *Chlamydia trachomatis* antigens in humans, however it has been used for the diagnosis of chlamydial infection in koalas for a number of years (Woods and Timms 1992; Blanshard and Bodley 2008).

A positive test result is indicated by a line in the results window of the test device. The line of positivity often varied in intensity, so a scoring system was

devised to quantify this variation (Plate 4.11). The following scoring system was adopted:

- **Score 0:** A negative result which was indicated by the absence of a line forming in the results window of the test device after 15 minutes.
- **Score 1:** The formation of a line that was barely perceptible in the results window, evident after 15 minutes.
- **Score 2:** The formation of a weak, but easily perceptible line in the results window, evident after 15 minutes.
- **Score 3:** The formation of a strong line in the results window that was less intense than the line in the control window, evident after 15 minutes.
- **Score 4:** The formation of a strong line that was greater than or equal to the intensity of the line in the control window, evident after 15 minutes.

Any koala with a Clearview® *Chlamydia* MF test score of 2^+ to 4^+ on their urogenital swab (even if they had no detectable chlamydial disease), was treated at the Australian Wildlife Hospital (described in section 5.4).

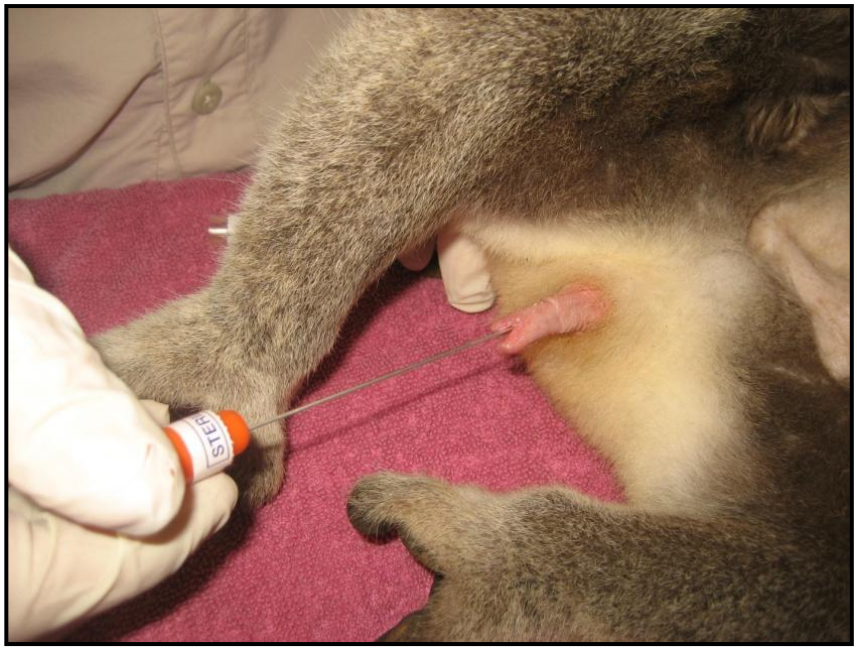

Plate 4.10: Taking a swab of the urogenital tract (prostatic urethra in male koalas)

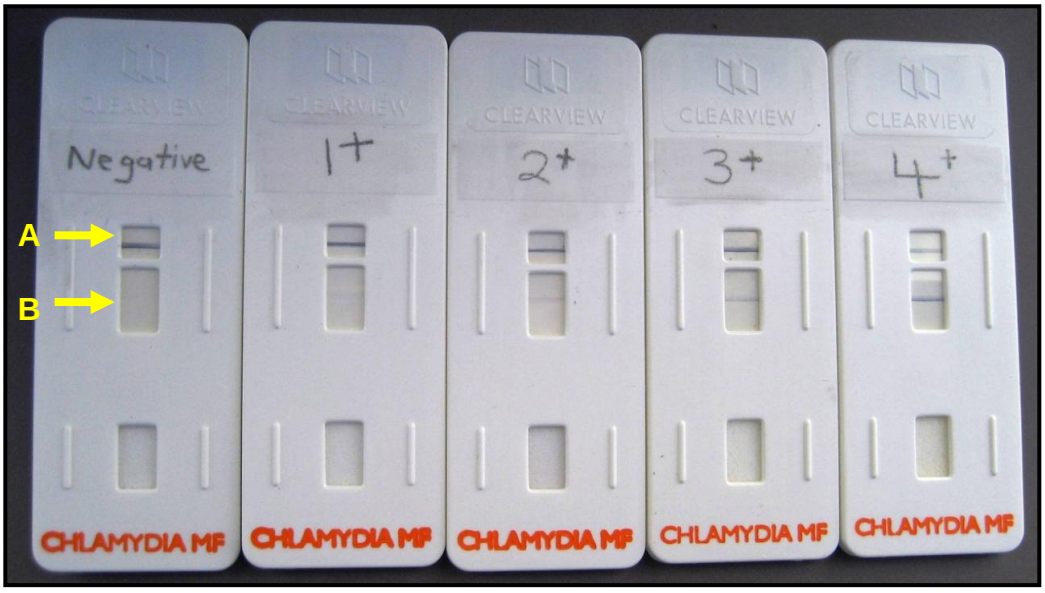

Plate 4.11: Clearview® *Chlamydia* MF test scoring system (A- control window, B- results window)

4.4.7 Ultrasound imaging

Ultrasound imaging is an important diagnostic tool used for the assessment of internal organs, and for the detection of pathological lesions (Blanshard and Bodley 2008). The bladder, kidneys, ureters, female reproductive tract and prostate of each koala were routinely examined using an HS-2100 ultrasound scanner (Honda Electronics, Japan) with a 9MHz convex transducer.

Bladder

As mentioned in section 4.4.5, the bladder in female koalas was imaged through the pouch using sonographic gel (except when a small joey (<5 mths) was present) or alternatively from the caudal flank using methylated spirits. In male koalas, the bladder was imaged by soaking the fur with methylated spirits just cranial to the scrotum.

As diseases of the urinary tract are extremely common in Queensland koalas (Australian Wildlife Hospital unpublished data; Brown and Grice 1984; DERM 2009b), ultrasound examination was useful for detecting an increase in bladder wall thickness, a consequence of cystitis (Plates 4.12 and 4.13). The luminal contents (urine) of the bladder were examined for flocculence, which may be indicative of crystalluria, and any other abnormalities.

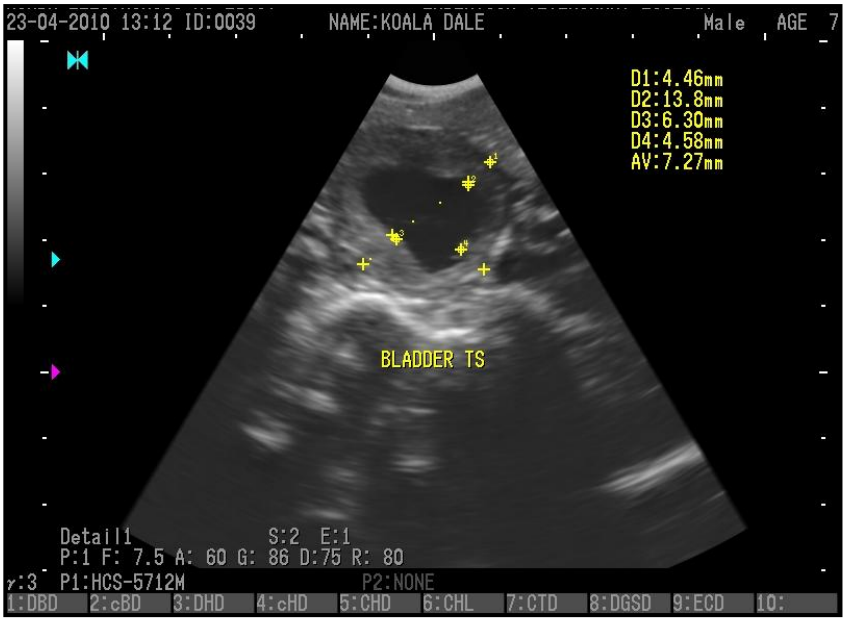

Plate 4.12: Sonogram of a moderately thickened bladder wall- koala with cystitis (Koala 'Dale')

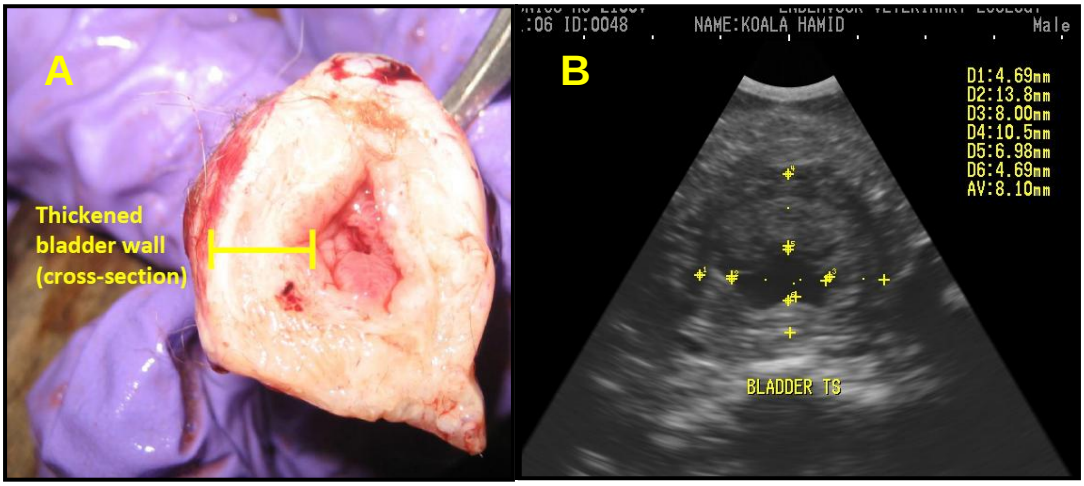

Plate 4.13: Bladder- cystitis **A**, Photo taken at post-mortem examination (cross-section). **B**, Sonogram of a markedly thickened bladder wall (Koala 'Hamid')

Kidneys and Ureters

The kidneys and ureters were imaged by placing the koala in lateral recumbency. To enable imaging of the opposite kidney and ureters, the koala was rolled over to the opposite side. Each kidney was examined for overall structure, parenchyma echo, and abnormalities. Some of the changes detectable by ultrasound include inflammation, dilated ureters and renal pelvis, cysts/abscesses, renal calculi and scarring (Nyland *et al.,* 2002).

Female Reproductive Tract

Ultrasound provides a greater sensitivity for the detection of reproductive disease than palpation, although this is dependent on the experience of the operator (Mattoon and Nyland 2002; J. Hanger pers. comm., 14th September 2009). Uterine, oviductal and ovarian bursal cysts and/or abscesses and metritis are commonly detected by ultrasound examination (Mathews *et al.* 1995; Markey *et al.* 2007). Care should be taken, however, as pregnancies may be confused with cystic structures and *vice versa* (J. Hanger pers. comm., 14th September 2009) (Plates 4.14 and 4.15).

The reproductive tract was imaged by applying sonographic gel to the pouch, except when a pouch joey was present. By scanning in both a transverse and longitudinal plane, both uteri could be imaged. The uteri are located in the epipubic region lying dorsal to the bladder.

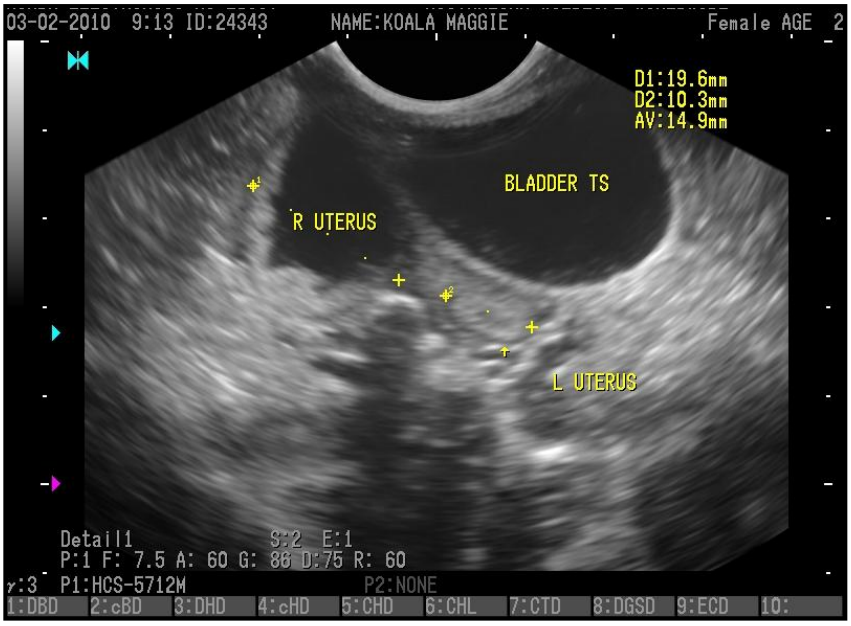

Plate 4.14: Cystic reproductive disease (Koala 'Maggie')

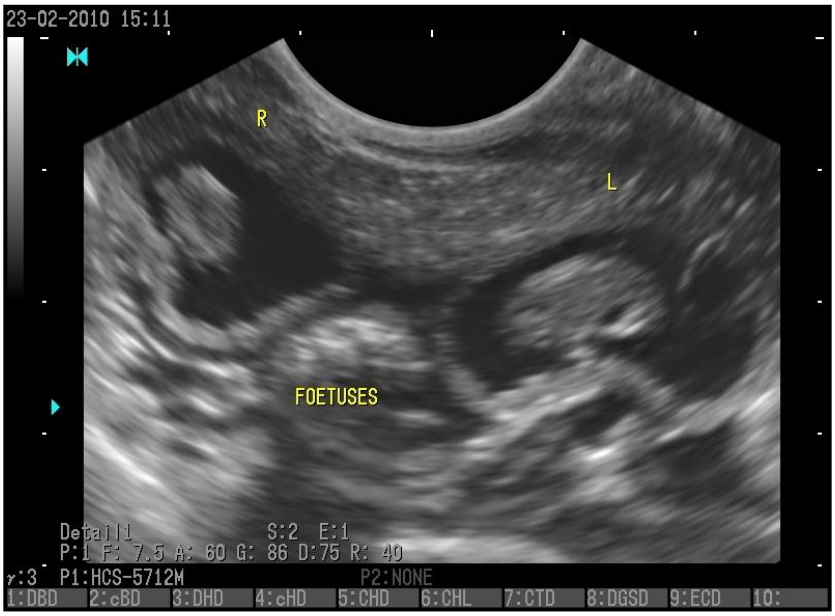

Plate 4.15: Advanced pregnancy- foetuses in both uteri (Koala 'Bec')

Male Reproductive Tract

Ultrasound imaging can also be useful in examining the male reproductive tract, and prostatic abscesses and inflammation are occasionally detected by this method (J. Hanger pers. comm., 14th September 2009).

The prostate was imaged by applying methylated spirits to the region just cranial to the scrotum. The transducer head was angled into the pelvic canal to give a transverse/frontal section image of the prostate gland.

4.5 Radio-Telemetry

Healthy koalas were fitted with VHF radio-collars (Sirtrack, New Zealand or Titley Scientific™, Australia) and monitored for a minimum of five days per week for the first 2-4 weeks after their release, and then 2-3 times weekly thereafter. Any additional koalas opportunistically located at the study sites during the monitoring period were also captured for a veterinary clinical examination.

When possible, koalas from the Moreton Bay study sites were re-captured every six months for a follow-up health check. In some instances, this was not possible for a variety of reasons such as, radio-collar signal failure, or death of the koala. The Gold Coast koalas had only been monitored for a short duration by the end of this study, so follow-up captures or health examinations were not conducted unless an animal was obviously sick or injured.

CHAPTER 5: Summary of Clinical Findings from Wild Koala Populations in South-East Queensland

5.1 Introduction

Chlamydiosis is the most common disease affecting koalas in Queensland and New South Wales (Brown *et al.,* 1987; Canfield 1990; Higgins 2008). Overt presentations commonly reported include cystitis (resulting in urine staining and wetness of the rump), kerato-conjunctivitis and rhinitis (overtly evident by the presence of nasal discharge) (Cockram and Jackson 1974; Cockram and Jackson 1981; Brown and Grice 1984; Brown *et al.* 1987). However, chlamydiosis has other manifestations which are less apparent and often only detectable by veterinary examination, or at post-mortem. These include pathology of the reproductive tract, prostatitis and renal disease (Brown and Grice 1984; Brown *et al.,* 1984; 1987; Hemsley and Canfield 1996).

Although chlamydiosis is arguably the most recognised disease of koalas, there are other diseases that are also common, and worthy of mention. These diseases include cancers such as leukaemia and lymphoma (Canfield 1987; Canfield 1990; Connolly *et al.,* 1998; Hanger 1999), opportunistic infections, and an AIDS-like syndrome, characterised by a variety of clinical signs suggestive of immune dysfunction (Hanger 1999; Hanger *et al.,* 2003). It has been suggested that infection with the koala retrovirus (KoRV) may have a significant role in the

pathogenesis of these diseases, as well as increasing the severity of chlamydiosis (Hanger 1999; Hanger *et al.,* 2003).

Many diseases suspected to have a causal association with KoRV are not apparent as overt disease; hence thorough veterinary health examinations are required for their detection (Hanger *et al.,* 2003; Hanger and Loader 2009). Often these diseases are not represented in disease surveys as they require a range of specialised diagnostic techniques to diagnose (Hanger and Loader 2009). Furthermore, many of the studies describing the health of wild populations are limited by the lack of specialised veterinary expertise and hence solely report on the observation of overt physical signs of disease (Gordon *et al.,* 1990; White and Kunst 1990; White and Timms 1994; Jackson *et al.,* 1999; Dique *et al.,* 2003a; Lane 2008).

This chapter summarises the main clinical findings and pathology from veterinary health examinations and necropsy investigations conducted on koalas from four wild populations in south-east Queensland. Applying a robust and systematic approach to each koala examination enabled accurate diagnoses and a valid dataset to be collected. This chapter deals mainly with qualitative descriptions of the clinical presentations, findings and pathology observed in the koalas; quantitative analysis of data is discussed in the following chapter.

5.2 Materials and Methods

5.2.1 Koala veterinary examination

Comprehensive health examination were conducted on 94 koalas (45 male, 49 female) from four wild SEQ populations (Brendale, Narangba, East Coomera and Clagiraba) using a standardised veterinary protocol (outlined in detail in Chapter 4). In brief, each koala was subjected to a complete physical examination and diagnostic work-up under general anaesthesia. Koalas found to be healthy were released back into the wild, those requiring veterinary treatment were admitted to the Australian Wildlife Hospital, and those requiring euthanasia on humane grounds (due to severity of illness) were euthanased prior to recovery from anaesthesia. The findings of veterinary examinations were recorded at the time of the assessment on a *Koala Examination Data Sheet* (Appendix 2).

5.2.2 *Overall disease score*

In order to classify each koala according to disease severity, an *overall disease score* was assigned at the completion of the veterinary health examination. The score ranged from 0 (no abnormalities detected) to 5 (severe disease requiring euthanasia or likely to result in the death of the koala). The criteria for each disease score were devised with the assistance of Dr. Jon Hanger and are outlined in Table 5.1. This scoring system was useful as it provided an objective classification of the overall health of the koala and could later be used to

correlate with KoRV titre and/or immune status (as a component of other concurrent studies).

- **Score 0:** No clinical abnormalities detected PLUS no Clearview® test score >1.

- **Score 1:** No clinical abnormalities detected PLUS at least one Clearview® test score >1.

- **Score 2:** Mild clinical disease, likely to respond to drug or surgical therapy with complete remission. Eg: mild or acute conjunctivitis, mild cystitis or prostatitis, mild reproductive tract disease (eg just para-ovarian/bursal cysts). DO NOT include severe reproductive tract disease, eg severe metritis, abscesses etc. or multifocal chlamydial disease, or other concurrent disease. BCS must be 6 or greater.

- **Score 3:** Any of the following:
- Category 2 disease with BCS of <6, OR
- Chronic cystitis BCS 6 or greater, OR
- Chronic severe kerato-conjunctivitis with BCS of 6 or greater, OR
- Multifocal chlamydial disease with BCS 6 or greater, OR
- Disease of moderate severity that could be expected to resolve with treatment.

- **Score 4:** Any of the following:
- Category 3 disease with BCS of less than 6, OR
- Chronic poor doer
- Refractive Chlamydiosis with BCS <6
- Suspect myelodysplasia (dysplastic changes subtle/not definitive)
- Suspect AIDS (eg one of: generalised or multifocal dermatitis, stomatitis, mouth ulceration, chronic typhlocolitis/caeco-colic dysbiosis syndrome, cryptococcosis)
- Severe trypanosomiasis (organisms present in smears PLUS severe clinical signs of anaemia, pain etc)
- Severe/end stage chlamydial disease with poor body condition.

- **Score 5:** Any of the following:
- Cancer (other than benign growth), OR
- Definitive myelodysplasia (overt or severe dysplastic changes)
- Leukaemia
- Definitive AIDS (two or more of the following: stomatitis, severe gingivitis, mouth or lip ulceration, chronic or severe typhlocolitis/caeco-colic dysbiosis syndrome, marginal anaemia with lymphopaenia, severe or generalised dermatitis, severe or life-threatening fungal infection, disseminated cryptococcosis, chronic ill-thrift, unexplained poor body condition).
- Very severe disease requiring immediate euthanasia, or likely to result in imminent death of the koala (eg renal abscess).

Table 5.1: Criteria for *overall disease score* in koalas

5.2.3 *Overall Chlamydia disease score*

Chlamydial pathology in koalas ranges from mild/resolved disease likely to have minimal impacts on the health of the animal, to severe disease causing debilitating illness (J. Hanger pers. comm., 3rd November 2009). To account for the variation in chlamydial disease severity, a chlamydial disease score was devised with the assistance of Dr. Jon Hanger. This score was originally designed to correlate chlamydial disease severity with KoRV titre and immune status (as part of another study), similar to the purpose of the *overall disease score* described in section 5.2.2. The criteria for *overall Chlamydia disease score* are listed in Table 5.2.

- **Score 0:** No detectable disease
- **Score 1:** Mild/resolved chlamydial disease
- **Score 2** Serious/severe chlamydial disease *without* other criteria for AIDS
- **Score 3:** Serious/severe chlamydial disease *with* other criteria for AIDS

Table 5.2: Criteria for *overall Chlamydia disease score* in koalas

Note: Results from studies correlating the severity of disease versus immune status are pending because, at the time of writing, immune function analyses had not yet been conducted. Analyses of *overall disease score* and *overall Chlamydia disease score* versus KoRV titre were, at the time of writing, being evaluated by Greg Simmons from the Koala Retrovirus Research Group at UQ.

5.2.4 *Anatomical Chlamydia disease score*

To correlate chronicity and severity of disease at a particular anatomical site (eyes, and urogenital tract) with *Chlamydia* qPCR results from the corresponding anatomical site, an *anatomical Chlamydia disease score* was also developed. The score for ocular disease ranged from 0 (no apparent disease) to 3 (chronic active disease), and the score for the urogenital tract ranged from 0 to 4. (Note: An analysis of the *anatomical Chlamydia disease score* vs. qPCR results was being conducted by QUT researchers at the time of writing). Criteria for the *anatomical Chlamydia disease score* are listed in Table 5.3.

Anatomical site	Score	Description
Eyes	0	No ocular disease
	1	Mild conjunctivitis with no proliferative change, serous or purulent discharge, consistent with acute/sub-acute inflammation (Plate 5.1)
	2	Chronic kerato-conjunctivitis with minimal or no discharge, and little evidence of active inflammation. May be some corneal opacity (Plate 5.2)
	3	Chronic active inflammation with exudation, proliferative conjunctivitis and keratitis (Plate 5.3)
Bladder/urinary tract/ reproductive tract	0	No detectable disease by overt signs, cystocentesis or ultrasound at either anatomical site
	1	Bladder: Subclinical disease detected by urinalysis and/or urine sediment examination, with mild bladder thickening. No overt signs or urine staining OR bursal or oviductal cysts (irrespective of size) present without marked thickening or luminal exudation of uteri. No significant cloacal discharge, cloacitis or cloacal/clitoral protrusion. No overt signs. BUT NOT BOTH. IF BOTH THEN SCORE IS “2” (Plate 5.4)
	2	Overt signs of cystitis (dirty tail) with confirmation by cystocentesis and/or ultrasound. Inflammatory exudates apparent on sediment examination but no significant haemorrhage, or complications such as ureteral dilatation or nephritis. OR bladder AND reproductive tract disease described in score “1” above OR Uterine disease apparent ultrasonographically, including thickened uterine wall, uterine exudation, uterine cysts, with or without bursal and oviductal disease. No overt signs of cloacal/reproductive tract disease.
	3	Severe chronic/sub-acute cystitis with exudation and significant haemorrhage apparent grossly or on sediment examination. Ureters may or may not be dilated, nephritis may or may not be apparent OR Severe reproductive tract disease with extensive involvement of both uteri and with cloacitis and cloacal exudation OR one or other with a lesser score for the alternative tract BUT NOT BOTH
	4	BOTH urinary and reproductive tract disease as described in “3” above (Plate 5.5 and Plate 5.6)

Table 5.3: Criteria for *anatomical Chlamydia disease score* in koalas

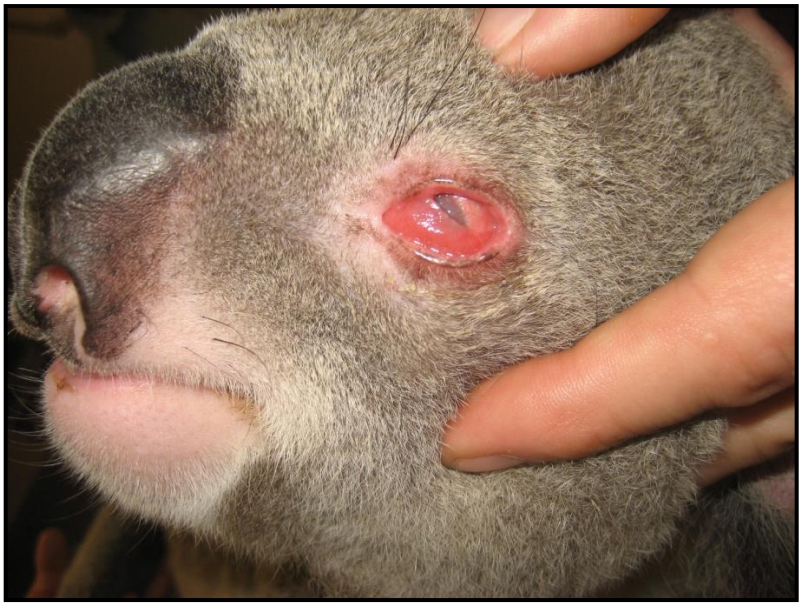

Plate 5.1: ***Anatomical Chlamydia Disease Score*** **(Eyes) = 1** Acute kerato-conjunctivitis- inflammation of nictitating membrane but no proliferative change

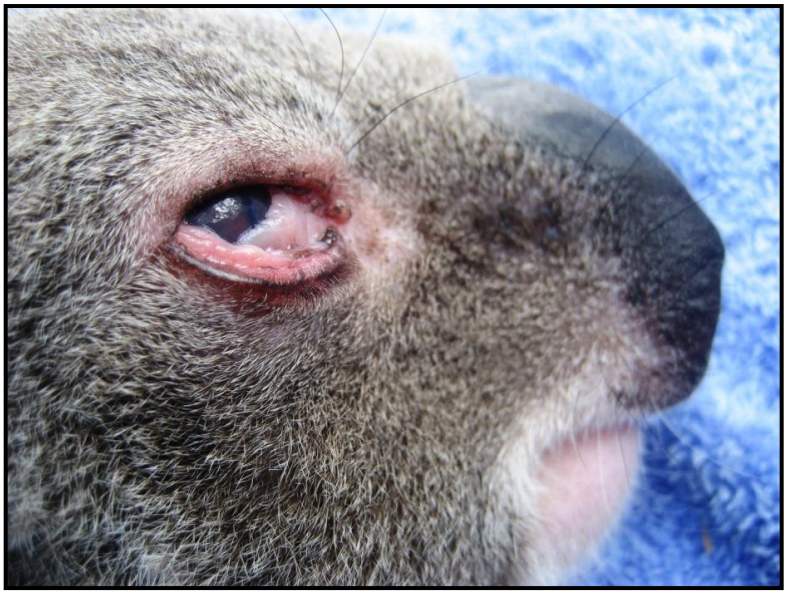

Plate 5.2: ***Anatomical Chlamydia Disease Score*** **(Eyes) = 2** Mild proliferation of conjunctiva and nictitating membrane in a koala with chlamydial kerato-conjunctivitis (Koala 'Althena')

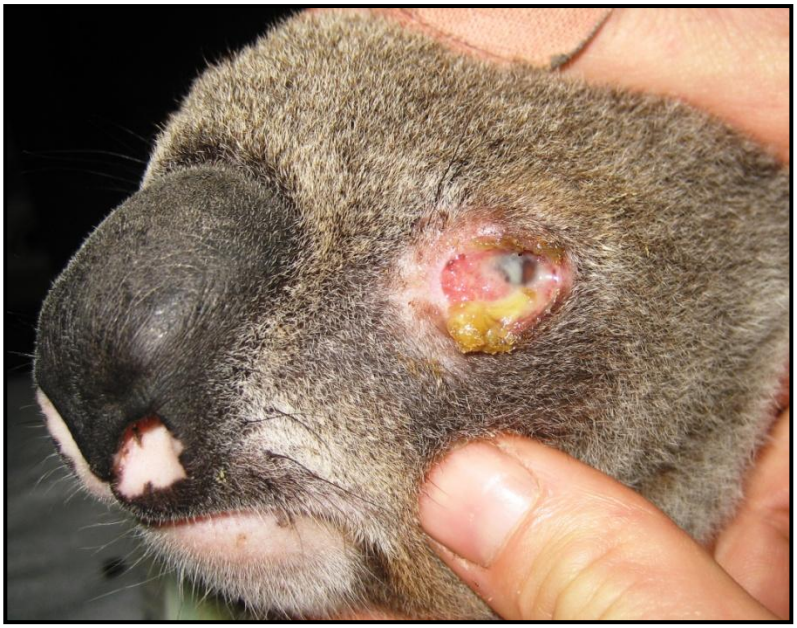

Plate 5.3: ***Anatomical Chlamydia Disease Score*** **(Eyes) = 3** Chronic kerato-conjunctivitis with active inflammation and muco-purulent discharge.

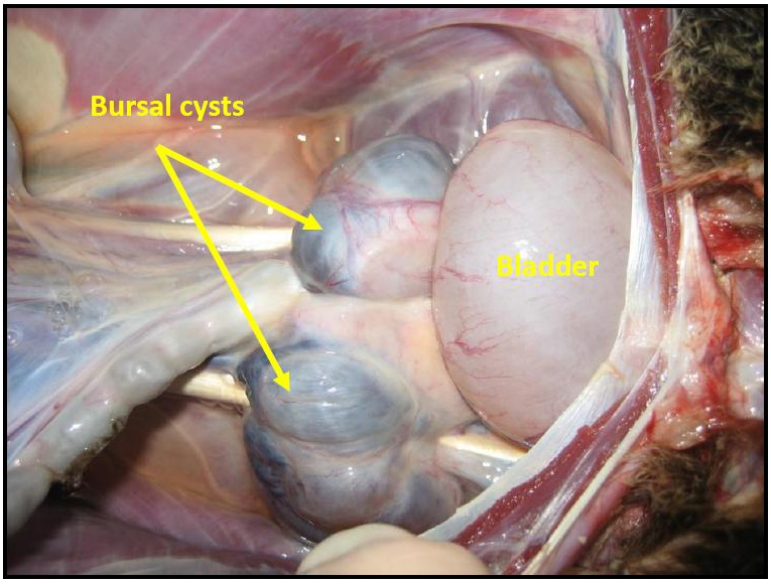

Plate 5.4: ***Anatomical Chlamydia Disease Score*** **(Urogenital tract) = 1** Cystic ovarian bursitis- overall this koala was in good health and body condition (BCS 8)

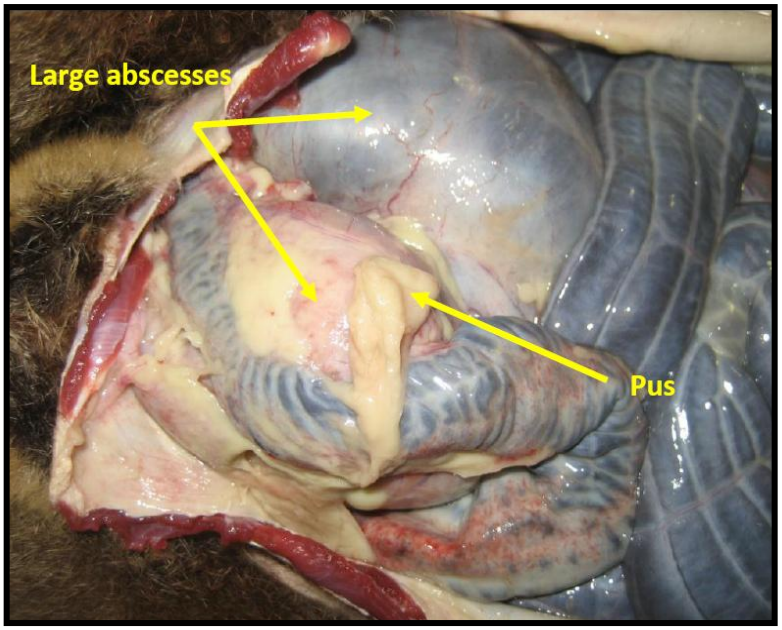

Plate 5.5: ***Anatomical Chlamydia Disease Score*** **(Urogenital tract) = 4** Severe reproductive disease (ruptured abscesses causing a peritonitis) and chronic cystitis

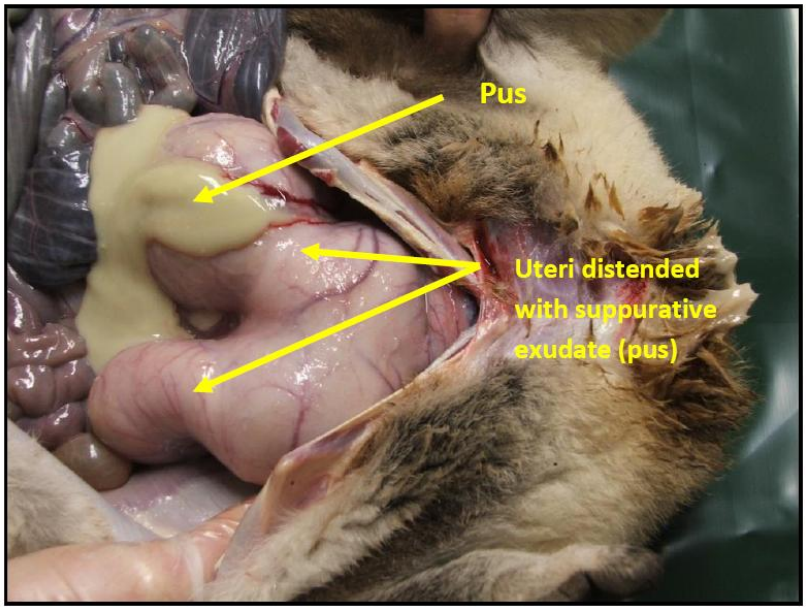

Plate 5.6: ***Anatomical Chlamydia Disease Score*** **(Urogenital tract) = 4** Koala with pyometron and chronic cystitis (Photo: A. Gillett)

5.2.5 Post-mortem examinations

Koalas that required euthanasia on humane grounds were given an intravenous injection of sodium pentobarbitone 325 mg/ml (Lethabarb® Euthanasia Injection, Virbac (Australia)) prior to recovery from anaesthesia. Post-mortem examinations were conducted on the majority of koalas that were euthanased or that died during the period of this study. Gross pathology was recorded and photographed, and representative samples of tissues were collected and fixed in 10% neutral buffered formalin. Tissue samples were submitted to Queensland Medical Laboratories (QML) or the University of Queensland Diagnostics Services Laboratory (School of Veterinary Science) for blocking and preparation of histology sections using standard methods. Dr. Jon Hanger assisted with interpretation of gross and histologic pathology.

For female koalas with reproductive disease, the Australian Wildlife Hospital (AWH) algorithm was followed for euthanasia decisions. It should be noted that these criteria are not necessarily referable to high *overall disease score*. The AWH decision algorithm for female koalas with reproductive disease is in Appendix 3.

5.3 Results

The major clinical findings from each koala with detectable illness are listed in Tables 5.4-5.7.

Koala Name	**Sex**	**Approx. Age** (when illness was diagnosed)	**Body Condition Score** (when illness was diagnosed)	**Overall Disease Score** (when illness was diagnosed)	**Summary of Diagnoses**
Fat Tony	M	8-10 yrs	8	3	Oral mass of indeterminate aetiology
Brianna	F	8-10 yrs	5	4	Reproductive disease, chronic cystitis, mild multifocal sebaceous hyperplasia (esp. ventral skin)
Stefan*	M	4-5 yrs	4	4	1st exam: Cystitis Subsequent exam: cystitis and prostatic abscess
Lydia	F	10 yrs	5	3	Reproductive disease
Red	M	5-6 yrs	5	4	Chronic cystitis, oxalate nephrosis
Todd	M	8-10 yrs	6	2	Mild kerato-conjunctivitis
Indie	M	2 yrs	7	2	Cystitis
Janet	F	6-7 yrs	4	4	Reproductive disease, chronic cystitis
Shirl**	F	10+ yrs	4	5	Cryptococcosis (nasal), tick burden ++, anaemia, focal bronchopneumonia, pharyngeal paralysis
Pnau	M	6 yrs	6	3	Chronic cystitis, renal disease
Maggie**	F	2 yrs	5	3	Reproductive disease
Lisa	F	10 yrs	7	3	Reproductive disease
Claude	F	10 yrs	5	2	Marked multifocal hyperplasia- ventral skin, particularly pouch, otherwise NAD
Megan	F	3-4 yrs	7	3	Reproductive disease, mild cystitis
Miss Radio	F	10 yrs	6	3	Reproductive disease, chronic cystitis
Poppy	F	3-4 yrs	7	2	Reproductive disease
Renee	F	12 mths	7	2	Chlamydial rhinitis
Paula	F	18 mths	4	4	AIDS (?)- Ill-thrift, caeco-colic dysbiosis, stomatitis, candidiasis
Val	F	10 yrs	5	3	Reproductive disease

***Stefan had cystitis and was treated at the Australian Wildlife Hospital at his first veterinary health examination. At a subsequent examination (16mths later), new lesions were detected (cystitis and prostatitis). He was treated again and later released.**

****These koalas were healthy at their first veterinary health examination; however disease was subsequently diagnosed at a follow-up examination.**

Table 5.4: Summary of diagnoses of koalas with detectable illness in the Brendale population

Koala Name	Sex	Approx. Age (when illness was diagnosed)	Body Condition Score (when illness was diagnosed)	Overall Disease Score (when illness was diagnosed)	Summary of Diagnoses
Aria**	F	8 yrs	7	2	Reproductive disease
Dion	M	10 yrs	6	2	Kerato-conjunctivitis
Edna	F	10 yrs	7	3	Reproductive disease, mild cystitis
Frankie	F	2-3 yrs	8	2	Reproductive disease
Felix	F	5-7 yrs	8	2	Reproductive disease
Gus	M	7-9 yrs	7	2	Generalised seborrhoeic dermatitis with otitis externa
Igor**	M	8-10 yrs	6	2	Subclinical cystitis
Jasmine**	F	8 yrs	6	3	Reproductive disease, cystitis
Kaia	F	5-6 yrs	8	3	Reproductive disease
Linda**	F	2 yrs	7	2	Reproductive disease
Mandy**	F	2-3 yrs	7	4	Reproductive disease, cystitis
Kevin	M	4 yrs	8	2	Subclinical cystitis
Natashi	F	4-5 yrs	3	4	Reproductive disease, cystitis
Liam	M	2 yrs	8	2	Subclinical cystitis

****These koalas were healthy at their first veterinary health examination; however disease was subsequently diagnosed at a follow-up examination.**

Table 5.5: Summary of diagnoses of koalas with detectable illness in the Narangba population

Koala Name	**Sex**	**Approx. Age** (when illness was diagnosed)	**Body Condition Score** (when illness was diagnosed)	**Overall Disease Score** (when illness was diagnosed)	**Summary of Diagnoses**
Echo	F	18 mths	6	1	Non-specific illness
Louise	F	7-8 yrs	8	2	Reproductive disease
Althena	F	7-8 yrs	6	3	Reproductive disease, unilateral kerato-conjunctivitis, cystitis
Connor	M	18 mths	2	4	GI candidiasis, ill-thrift, non-regenerative anaemia, AIDS (?)
Sharon**	F	3 yrs	7	5	Peracute *Salmonella* septicaemia
Angela^	F	5-6 yrs	3	5	Reproductive disease, severe regenerative anaemia, trypanosomiasis, stomatitis, hepatitis, histological evidence suggestive of an immuno-suppressive disorder
James	M	3 yrs	7	2	Mild acute cystitis
Emma	F	8 yrs	6	2	Reproductive disease
Dale	M	8-10 yrs	7	3	Septic arthritis (left shoulder), cystitis
Hamid	M	8 yrs	6	3	Cystitis
Glen	M	8-10 yrs	8	2	Fungal infection (scrotum), ear mites
Peter	M	5 yrs	7	2	Mild cystitis, prostatic cyst (?)
Maree	F	8-10 yrs	6	3	Reproductive disease, cystitis

^ Angela was first examined at post-mortem

****This koala was healthy at the first veterinary health examination; however disease was subsequently diagnosed at a follow-up examination.**

Table 5.6: Summary of diagnoses of koalas with detectable illness in the East Coomera population

Koala Name	**Sex**	**Approx. Age** (when illness was diagnosed)	**Body Condition Score** (when illness was diagnosed)	**Overall Disease Score** (when illness was diagnosed)	**Summary of Diagnoses**
Graeme	M	7-8 yrs	3	4	Poor body condition, GI candidiasis, trypanosome parasitaemia, regenerative anaemia, AIDS (?), cystitis
Kellie	F	4 yrs	8	3	Reproductive disease, cystitis, unilateral kerato-conjunctivitis
Ned	M	5 yrs	8	3	Bilateral kerato-conjunctivitis, mild subclinical cystitis. (AIDS (?)-post-mortem examination)
Andrew^	M	5-6 yrs	3	5	AIDS (?), mild non-suppurative prostatitis

^ Andrew was first examined at post-mortem

Table 5.7: Summary of diagnoses of koalas with detectable illness in the Clagiraba population

5.3.1 Chlamydiosis

Chlamydial disease was detected in 37% (35/94) of koalas at their initial veterinary health examination. A further 6 koalas were diagnosed with chlamydiosis at subsequent examinations, that is, 6 previously healthy koalas developed new chlamydial disease during the period of the study. The severity of chlamydiosis varied significantly in the affected koalas, from mild/resolved pathology that was considered unlikely to be causing significant discomfort, to serious/severe disease that was considered to be significantly impacting affected koalas. Approximately one half of the affected koalas (46%; 19/41) were adjudged to have mild/resolved disease.

For the purposes of this study, we defined “resolved disease” as disease, which, in the clinical judgment of the consulting vet, was not causing any significant illness in the koala and no evidence of significant active inflammation was found. For example a diagnosis of:

- Chronic, inactive cystitis was based on bladder wall thickening on ultrasound but an absence of significant inflammatory cells in the urine sediment.
- Chronic, inactive reproductive disease was based on cystic reproductive disease with no evidence of illness and no significant blood changes indicative of active inflammation.
- Chronic, inactive kerato-conjunctivitis was based on minimal proliferation, minimal hyperaemia, scarring of the conjunctiva, cornea and palpebra, and absence of ocular discharge.

Of the sexually mature female koalas, 57% (24/42) had detectable reproductive disease (detected at either the initial or a subsequent health examination). Of these, 11 that had cystic reproductive pathology and in some cases mild to moderate enlargement of the uteri, were in good body condition (BCS 7-8). Five of these 11 koalas (Aria, Lisa, Megan, Edna and Kellie) had mild concurrent chlamydial disease (either mild cystitis, or mild cystitis and mild unilateral kerato-conjunctivitis (Kellie)), and the remaining six koalas had no other concurrent detectable illness. Two females (Val and Lydia) had large bilateral oviductal

and/or bursal cysts with no concurrent disease and a body condition score of 5 Plate 5.7). The reproductive pathology of both of these koalas was not severe, and their fair body condition may have simply been the result of old age (both had advanced tooth wear and were estimated to be 10 years of age, or older).

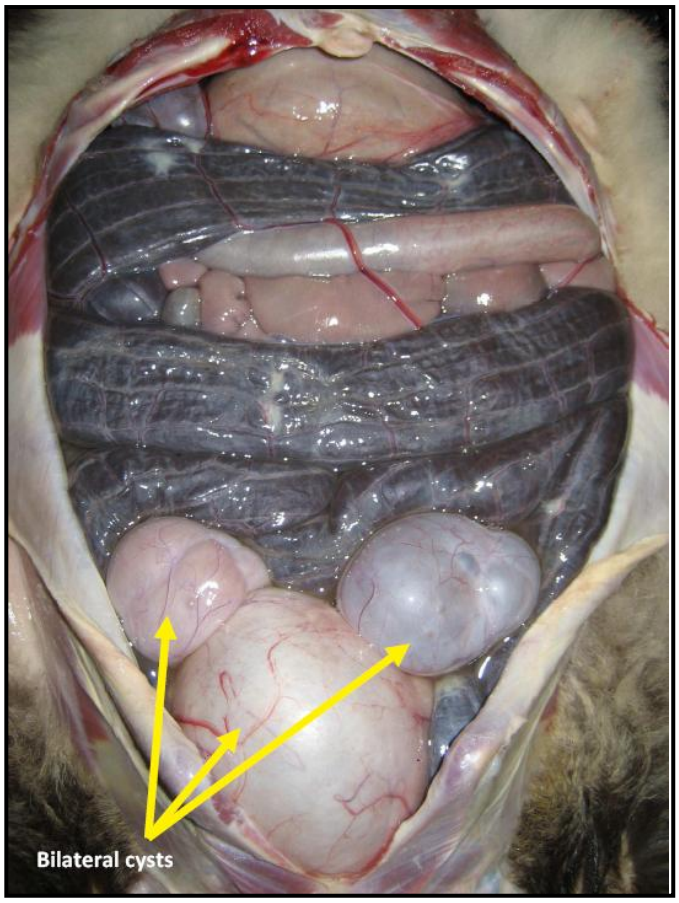

Plate 5.7: Reproductive disease: size of cysts is not necessarily indicative of the severity of pathology (Koala 'Lydia')

Koalas with more serious reproductive pathology (including moderate to severe uterine involvement, severe metritis and/or abscesses), or reproductive pathology with chlamydiosis at another site or concurrent non-chlamydial disease of moderate to marked severity, were generally in poor to fair body condition. Some examples included a:

- 2 year old koala with severe metritis, suppurative cloacal discharge and a unilateral uterine cyst (but no other concurrent disease) - body condition score of 5 (Maggie).
- 5-6 year old koala with a unilateral bursal cyst and severe chronic cystitis - body condition score of 3 (Natashi).
- 6-7 year old koala with a unilateral uterine cyst and severe chronic cystitis- body condition score of 4 (Janet).
- 3 year old koala with bilateral ovarian bursal cysts and severe chronic cystitis- body condition score of 4 (Mandy) (Plate 5.8).
- 5-6 year old koala with reproductive tract disease detected histologically, severe regenerative anaemia, possible trypanosome infection and evidence of immunosuppression- body condition score of 3 (Angela).

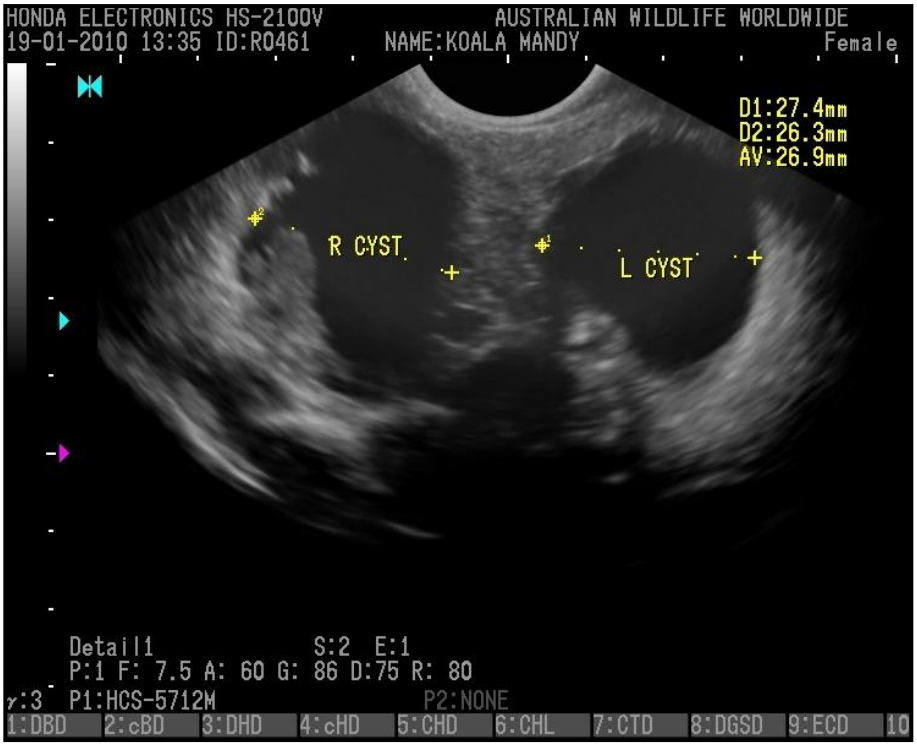

Plate 5.8: Sonogram of bilateral cystic reproductive disease (Koala 'Mandy')

Reproductive disease was detected in only two male koalas (4%; 2/45). One male had a prostatic abscess (in addition to cystitis) detected during ultrasound examination (Stefan) (Plate 5.9). This koala was treated by ultrasound-guided needle aspiration of the abscess, followed by flushing of the abscess with sterile saline and finally instillation of oxytetracycline (Engemycin, Intervet Australia Pty Ltd, Bendigo) into the abscess cavity. The other male (Andrew) had a mild non-suppurative prostatitis detected histologically (after post-mortem examination), which was not evident clinically or grossly at post-mortem examination.

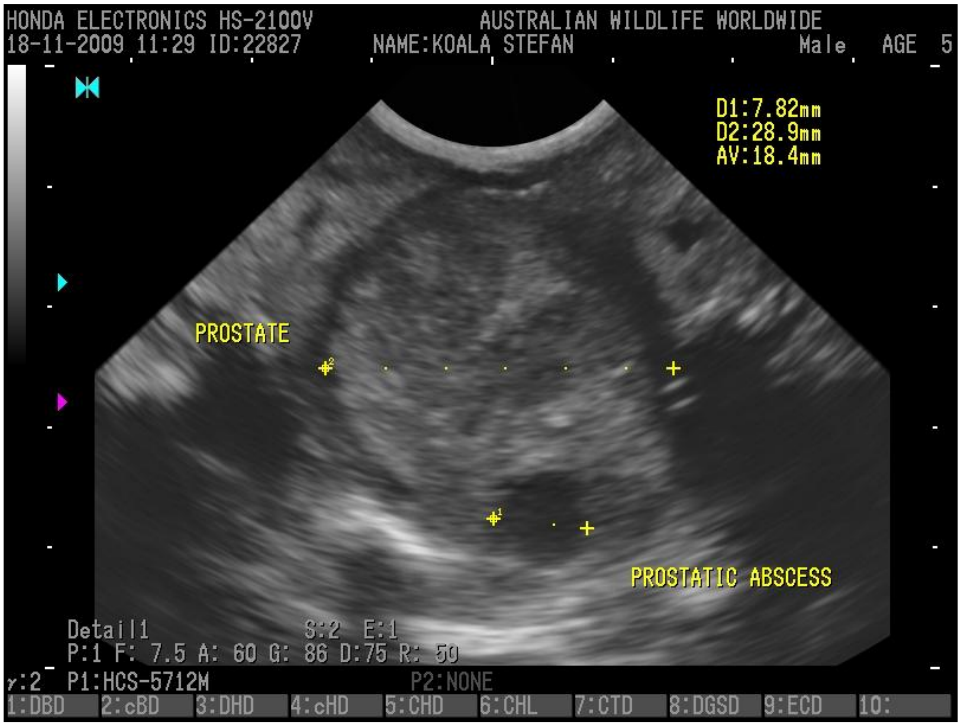

Plate 5.9: Sonogram of a prostatic abscess (Koala 'Stefan')

Urinary tract disease was the most commonly clinically detected manifestation of chlamydiosis in male koalas. Of the 12 male koalas (27%; 12/45) with urinary tract pathology, 9 had mild cystitis while 3 had severe chronic active cystitis with or without renal disease. Of these three koalas, one male (Hamid) had marked thickening of the bladder and inflammatory cells in the urine sediment, and was in care at the conclusion of this study. Another koala had marked gross haematuria and marked crystalluria (oxalate crystals) in the urine sediment. This koala (Red) died after less than one month in care due to oxalate nephrosis. The remaining male (Pnau) had chronic cystitis and renal pathology, which was

refractive to treatment, and he was euthanased after a period in care. Post-mortem examination revealed an early/mild chronic nephritis, renal pelvic and ureteral dilatation, and confirmed the clinical diagnosis of severe haemorrhagic chronic active cystitis.

One female koala (Jasmine) had a joey and no detectable illness at her initial veterinary examination, but had a Clearview® *Chlamydia* MF test score of 3^{+} for her urogenital tract swab. This koala was released without treatment. She was recaptured five months later with dirty tail, and found to have severe chronic active cystitis, severe metritis and cystic reproductive disease. Due to the severity of disease (*overall Chlamydia disease score* of 2 and an *anatomical Chlamydia disease score* of 4), the koala was euthanased.

Kerato-conjunctivitis was evident in 5% (5/94) of the koalas examined. Unilateral lesions were observed in four koalas, all of which had mild or resolved disease with no ocular discharge (Todd, Dion, Kellie, and Althena). One koala (Ned) had bilateral kerato-conjunctivitis with moderate to marked proliferation of both conjunctivae, muco-purulent discharge, hyperaemia, corneal opacity and prolapse of the nictitating membranes, as well as mild cystitis. This koala was treated for chlamydiosis, and released back into the wild. He was found dead at the base of a tree approximately two months later. Post-mortem and histological examination found the koala had chronic renal failure in addition to a number of pathological features suggestive of immunosuppression (mouth ulcers, poor

lymphoid cellularity in the lymphoid tissues, and invasion of tongue, oesophagus and small intestine by *Candida*-like yeasts).

Chlamydial rhinitis was detected in only one koala (Renee), and was characterised by nasal discharge (Plate 5.10). A Clearview® swab of the discharge was positive (3^{+}) for chlamydial antigen. The koala was successfully treated for chlamydiosis and subsequently released.

Plate 5.10: Chlamydial rhinitis characterised by nasal discharge (Koala 'Renee')

5.3.2 Other disease

Although chlamydiosis was the most common disease detected in the koalas examined, a number of koalas had illnesses that were not associated with, or directly attributable to, chlamydiosis.

a) Koala AIDS:

In some cases, the diagnosis of "AIDS(?)" was made by the consulting veterinarian based on the findings of the clinical assessment. AIDS is a putative diagnosis in koalas, and is thought to be associated with koala retrovirus (KoRV) infection (Hanger *et al.,* 2003). This diagnosis was based on the finding of two or more of the following: ill-thrift or unexplained weight loss, unexplained anaemia, caeco-colic dysbiosis/typhlocolitis, stomatitis, mouth, hand and foot ulcers, hyperkeratosis, severe debilitating chlamydiosis, generalised or life-threatening fungal infections, serious candidiasis and other opportunistic infections (for which there appeared to be no alternative predisposing cause) (Hanger *et al.,* 2003; J. Hanger pers. comm., 1st April 2009). Six of the 94 koalas examined for this study had the diagnosis of "AIDS(?)" applied, either as a clinical diagnosis, or as a post-mortem diagnosis.

Two male koalas (Connor and Graeme) had chronic ill-thrift, a brown, dry coat, anaemia (non-regenerative and regenerative, respectively), gastrointestinal candidiasis and an *overall disease score* of 4. Graeme also had cystitis and trypanosome parasitaemia. Both koalas failed to respond to treatment,

deteriorated, and were subsequently euthanased. Post-mortem findings were consistent with chronic immune-mediated disease (both koalas), with immuno-suppressive features also present in the koala Graeme, and consequently a presumptive diagnosis of “AIDS(?)” was applied. Another koala (Ned), whose chlamydial pathology is described in section 5.3.1 above, had histopathological features consistent with immunosuppression and was given the diagnosis of “AIDS(?)” also.

One young female koala (<2 years of age) (Paula) had chronic ill-thrift, caeco-colic dysbiosis, stomatitis and candidiasis, with a provisional diagnosis of “AIDS(?)” applied by the consulting veterinarian. This koala was undergoing treatment in hospital at the time of writing. Another young female (Echo) was in a fair body condition at the time of capture. Although there was no definitive diagnosis made for this koala, she displayed ill-thrift, chronic poor coat condition, low weight gains and maladaptation following initial release into the wild (as part of a translocation program). After another protracted stay in hospital on supplemental nutrition, the koala’s body condition improved and she was re-released. At the time of writing this koala was being monitored by radio-telemetry and was apparently well.

One aged female koala (Shirl) that had been considered healthy at the first two health examinations was found depressed and weak at the base of a tree more than one year after her first capture. This koala had a joey in her pouch, a

significant tick burden, pharyngeal paralysis, severe anaemia, pancytopaenia, ataxia, inability to walk, and nasal cryptococcosis (manifested by nasal discharge). Although this koala displayed some clinical features consistent with the putative diagnosis of “AIDS(?)”, pancytopaenia and bone marrow smear evaluation were suggestive of primary marrow disease (which may commonly result in reduced cellular defences) (J. Hanger pers. comm., 12th May 2010). In view of her advanced age, and other clinical findings the ultimate diagnosis remained open. She was euthanased on humane grounds.

Post-mortem examinations were conducted on three koalas that had been captured and radio-tracked but had died prior to ante-mortem examination. All were found dead at the base of a tree and consequently were given an *overall disease score* of 5. Post-mortem examination of one koala (Sharon) found strands of fibrin in the peritoneal cavity, petechial haemorrhages on the retroperitoneal surface, renal capsule and parietal pleura, and patchy haemorrhage of the mucosal surface of the duodenum which was consistent with, but not diagnostic of septicaemia. Blood and tissue cultures resulted in heavy growths of *Salmonella* sp., and the cause of death was diagnosed as per-acute *Salmonella* septicaemia. Some histological findings were suggestive of immunosuppression, but were not sufficient to warrant the presumptive diagnosis of “AIDS(?)”.

Two other koalas (Angela and Andrew) had more definitive evidence of immunosuppression characterised by general depletion of lymphocytes in all lymphoid tissues. Both koalas also had evidence of reproductive disease histologically which was not obvious grossly. Additionally, Angela had mouth ulceration, severe regenerative anaemia, and histological necropsy findings consistent with severe trypanosomiasis and hepatitis.

b) Other conditions:

Skin conditions were evident in three adult koalas (Claude, Brianna and Gus). Claude had marked multifocal sebaceous hyperplasia over the inguinal skin, particularly the pouch region (Plate 5.11). At subsequent health examinations, these lesions were more numerous and significantly more extensive with respect to the area of skin involved. They seemed to have had no adverse affects on the health or reproductive output of this koala as she produced a joey each year. The skin condition affecting Brianna was also characterised as multifocal sebaceous hyperplasia and was of a mild nature and scattered over the ventral skin. In addition, this koala had chronic cystitis and reproductive disease, and was subsequently euthanased.

One aged male koala (Gus) had generalised seborrhoeic dermatitis consistent with allergic skin disease, with secondary infection by mixed fungi and bacteria Plate 5.12). At the first veterinary health examination, the skin condition was mild with some alopecic regions around the chest and left forearm. The

condition worsened over time and within 18 months he had marked alopecia over his entire body, particularly the ventral surface, face, digits and rump. Gus was euthanased on humane grounds due to poor body condition, advanced age, progressive skin disease and the imminent destruction of his native habitat (translocation was not considered to be a viable or compassionate option for this koala).

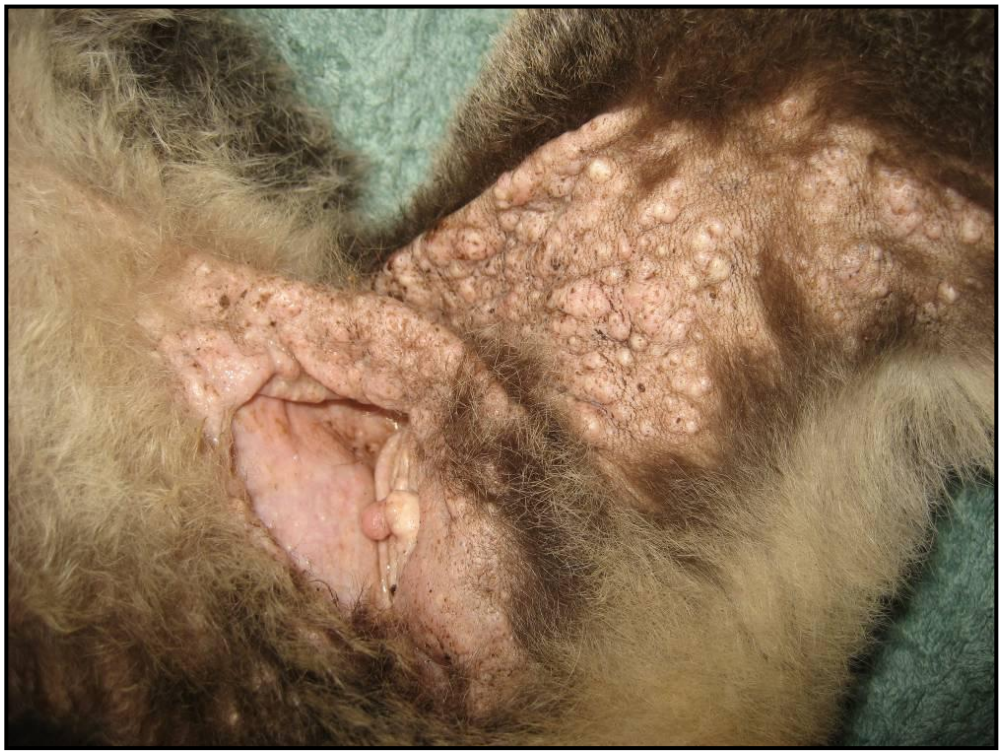

Plate 5.11: Sebaceous hyperplasia - inguinal and pouch region (Koala 'Claude')

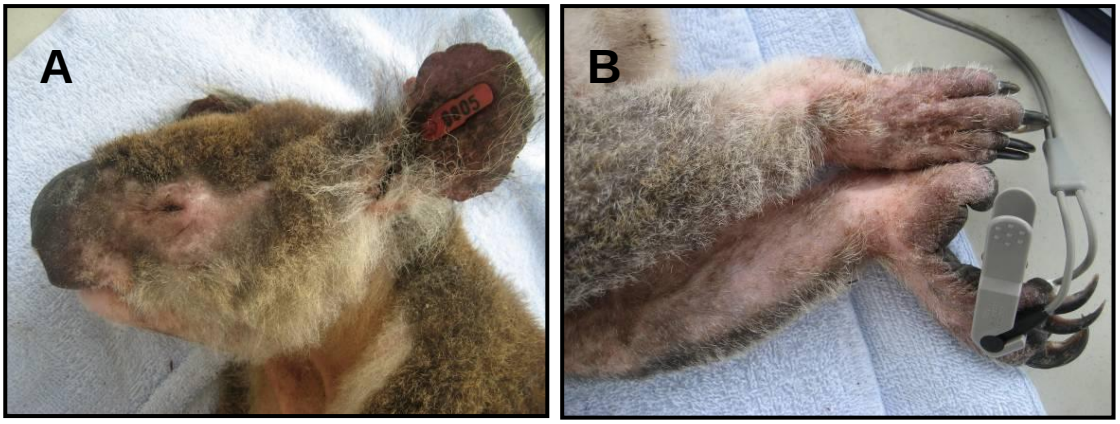

Plate 5.12: Generalised seborrhoeic dermatitis (Koala 'Gus') **A,** Alopecia of periorbital skin, ears and neck. **B,** Alopecia of hind legs and digits

5.4 Discussion

Disease severity in the koalas examined ranged from mild clinical disease likely to respond to treatment or resolve spontaneously (*overall disease score* 1 or 2), to severe disease generally requiring immediate euthanasia (*overall disease score* 4 or 5). Although some chlamydiosis cases could be classified as mild and/or resolved disease (i.e. where it was considered to have no major impact on the health of the koala), infertility[2] resulting from chlamydial infection of the reproductive tract is nevertheless important from a conservation perspective. With the exception of one koala with chlamydial rhinitis (Renee), all females with mild/resolved (and severe) chlamydiosis had detectable reproductive disease.

[2] Note: For the purpose of this study, the term 'infertility' was used to describe female koalas that were considered to be incapable of breeding due to pathology of the reproductive tract.

Overwhelming empirical evidence suggests that if cystic reproductive disease or more severe reproductive pathology is detected, then the koala will be infertile (J. Hanger pers. comm., 3rd January 2010). Similarly, Higgins (2008) suggested that ovarian bursal cysts are a common indicator of infertility. However theoretically, in cases in which only unilateral pathology is detected (depending on the extent of uterine fibrosis), a koala may still be able to breed. This may have been the case with one female koala (Linda) that had a unilateral bursal cyst and a one month old joey in her pouch. Alternatively, the reproductive lesions may have developed quickly after parturition. Higgins (2008) also suggested that a small and "unused" pouch, in addition to the presence of reproductive cysts, is currently the most reliable indicator of female infertility.

Chlamydiosis is generally considered to be more severe in koalas than any other species (Hanger and Loader 2009). Urogenital disease caused by *Chlamydia trachomatis* in human females may cause fibrosis of the reproductive tract and lead to infertility, but it does not tend to cause debilitating disease as is often seen in the koala (Mertz *et al.,* 1997; Reddy *et al.,* 2004; Gray-Swain and Peipert 2006; Baecher-Lind *et al.,* 2009; Hanger and Loader 2009). It has been suggested that KoRV may play a role in the severity of chlamydiosis in koalas (Hanger 1999). Of the female koalas examined, those with detectable bursal cysts and no concurrent chlamydial disease were generally found to be reasonably healthy and well nourished. However, while these koalas may have had an appropriate immunological response to their chlamydial infection they

may still be left with the damage to the reproductive tract (J. Hanger pers. comm., 3rd November 2009). In contrast, those koalas with moderate to marked uterine pathology and/or reproductive abscesses tended to be in poor body condition and often had additional health issues.

Considering that the majority of female koalas with detectable illness were likely to be infertile, and that those regarded as fertile may have been ill enough to have a reduced ability to successfully raise a joey, this is likely to be having a profound effect on the overall population health and viability. Not only is disease responsible for illness and mortalities, it is also responsible for reducing birth rates in wild populations. This is of great consequence to SEQ koalas as they are already under significant pressures from urbanisation (particularly dog attack and motor vehicle incidents) and extensive habitat loss.

This study demonstrated the value of using ultrasonography to diagnose reproductive disease. Higgins (2008) indicated that ovarian bursal cysts of around 15mm can be reliably palpated in anaesthetised koalas, although reproductive pathology can be easily overlooked using this technique alone. Ultrasonography increases the sensitivity for detection of both reproductive and bladder disease and also assists in diagnosing or further defining the nature of masses detected by palpation of the abdomen. However, it is still not sensitive enough to detect all reproductive pathology; if the pathology is not associated with significant structural changes then it may remain undetected by both

ultrasonography and palpation. When confirmation of reproductive disease cannot be achieved by these means, histological examination of the tract can be useful; however this is usually only possible at post-mortem. It is also important to note that the size of the reproductive cysts is generally not indicative of the severity of pathology. In other words, large cysts are not necessarily indicative of severe pathology, and *vice versa*. However cysts may become large enough to block the pelvic canal or impinge on other organs.

Reproductive disease was sonographically evident in only one male koala (Stefan) which had a prostatic abscess. However, this is not necessarily indicative of the *actual* prevalence of reproductive pathology in male koalas. Reproductive disease may be more common in males than our results suggest, but it is more difficult to detect (other than histologically at post-mortem examination) than in female koalas. Sonographically, prostatitis is unlikely to be evident unless there are abscesses or significant structural changes in the gland. Such changes occur far less commonly in the prostate than they do in the female reproductive tract (J. Hanger pers. comm., 3rd March 2010). Alternatively, pathology may only be evident (grossly or histologically) at post-mortem (eg koala Andrew). Hence it is likely that a proportion of the koalas found to be "healthy" after a clinical assessment may in fact have had prostatitis.

The objective of the Clearview® *Chlamydia* MF test is to detect chlamydial antigen. Therefore, even if a chlamydial infection has resolved, the test may still

detect residual antigen in biological material and provide a positive result. Although the Clearview® test is not diagnostic for disease *per se* (koalas may be in early stages of infection, without significant disease present), the test was useful as it allowed decisions to be made about whether to treat an animal which had no detectable disease. Previously a koala would only be treated on the basis of detecting disease, irrespective of the Clearview® result. However, based on the development of severe chlamydiosis in one female koala (Jasmine) that had a Clearview® test of 3^+, but no detectable illness at the first examination, it is our policy that all koalas with a Clearview® result of $>1^+$ are treated as a precautionary measure. A koala may have no detectable illness, but have a moderate to strong Clearview® result; hence it is likely that they may be in the acute stages of infection and disease development. Furthermore, it is likely that koalas with strong Clearview® positivity are shedding high numbers of organisms, and therefore are likely to be contagious to other koalas. From an infection control perspective, it makes good sense to treat these animals irrespective of whether disease is detected or not.

When trying to correlate disease severity and prevalence with environmental factors and KoRV status/titre (which is the focus of other current research projects), the application to each case of an *overall disease score* is useful. It enables disease severity to be ranked objectively, rather than grouping cases based just on a general description of pathology. For example, ‘Felix’ from the Narangba koala population had bursal cysts with no uterine involvement and a

body condition score of 8, while ‘Maggie’ from the Brendale koala population had severe metritis, a uterine cyst and a body condition score of 5. Hence, when disease scores were applied ‘Felix’ and ‘Maggie’ were designated scores of 2 and 4 respectively. Although both of these koalas had reproductive disease, it was obvious that ‘Maggie’ had disease of a more serious nature. This is an important distinction to make if subtle associations with other factors, such as KoRV infection/viral titre, are to be detected.

Koalas were commonly found to have evidence of immune dysfunction, and/or illness consistent with an AIDS-like condition. These koalas were placed into the category of “KoRV-associated disease” as currently no other clear explanation exists for their syndromes. None of the koalas was found to have neoplastic disease (eg leukaemia/lymphoma), which according to Tarlinton *et al.* (2005), has been associated with high levels of KoRV infection. Relatively acute conditions such as these are more likely to be detected in longitudinal studies. Understanding KoRV is clearly a priority for research, not only because KoRV-associated disease is poorly defined and the pathogenesis is only speculative, but because it has significant ramifications for our understanding of disease more generally in the koala population.

5.5 Conclusions

In summary, this study found that:

- Chlamydial disease in koalas is not only a significant cause of morbidity and mortality in the species, but it is also responsible for significantly reducing birth rate (as a result of reproductive pathology) which has serious implications for population viability.

- Chlamydial reproductive disease appears to be more common in female koalas than males, but this may be an artifact of the relatively crude method of detection (ultrasound). It is likely that reproductive tract disease in both males and females is underestimated by use of ultrasonography. This contention is validated by the finding of pathology histologically in koalas (post-mortem) which had not previously had reproductive pathology detected clinically.

- Clearview® *Chlamydia* MF test positivity *should* be used as an indication for treatment of koalas in which disease is not apparent, and test negativity *should not* preclude treatment in koalas with detectable pathology.

- Almost half of the koalas with detectable chlamydiosis had mild pathology; however without veterinary intervention, in some koalas, these conditions may have progressed to serious disease.

- The use of ultrasonography for the detection of bladder and reproductive disease is useful, and markedly increases the sensitivity of detection clinically, however it will not enable detection of all pathology.

- The application of an *overall disease score* (plus an *overall Chlamydia disease score* and an *anatomical Chlamydia disease score* for koalas with chlamydiosis) to each koala is useful as it allows a quantitative analysis of disease severity (thereby distinguishing koalas from one another that have similar conditions but that differ in severity), and gives greater resolution for the purposes of correlation with other factors.

- KoRV-associated disease was commonly seen in the koalas examined; however longitudinal studies may be more likely to detect some conditions such as neoplastic disease, which may have a relatively short clinical course before causing death.

The following chapter quantitatively summarises the health of the koalas (described in this chapter) and includes health data from Kangaroo Island koalas in South Australia for comparison.

CHAPTER 6: The Prevalence and Incidence of Disease in Wild Koala Populations

6.1 Introduction

Koala populations in Queensland and New South Wales are experiencing dramatic declines (Lunney *et al.,* 2002; Gordon *et al.,* 2006; Lane 2008; DERM 2009a). This is primarily attributed to ongoing habitat loss and fragmentation, and the increasing pressures of urbanisation (Pahl *et al.,* 1990; Reed and Lunney 1990; Dique *et al.,* 2003b). Infectious disease, particularly chlamydiosis, has also been implicated in koala population declines (Brown *et al.,* 1987; Gordon *et al.,* 1990; Gordon and Hrdina 2005); however, the impact of disease in wild populations has not been properly evaluated. Mortality studies of free-living and captive koalas have been reported (Backhouse and Bolliger 1961; Weigler *et al.,* 1987; Canfield 1989; Canfield 1990; Canfield 1991; Stalder *et al.,* 2008), and these and admission data from wildlife hospitals and shelters suggest a high prevalence of disease in wild koala populations (Australian Wildlife Hospital unpublished data; Stalder *et al.,* 2008; DERM 2009b). Although these studies are important in understanding the nature of diseases affecting koalas, they only subjectively reflect the health of wild populations in that they do not give accurate information on disease prevalence, nor measure the rate of occurrence of new disease cases over time (disease incidence).

The koala retrovirus (KoRV) and *Chlamydia* are two of the most widely recognised infections affecting koala populations today (Lee and Carrick 1989; Timms 2005; Hanger and Loader 2009). Similar to the range of diseases caused by retroviruses in other species (Hardy *et al.,* 1976; Rezanka *et al.,* 1992), KoRV is thought to be causally associated with a spectrum of conditions in koalas including neoplastic disorders and an immunodeficiency syndrome (Hanger 1999; Hanger *et al.,* 2003). In 2005, Tarlinton and co-workers found a significant correlation between high KoRV plasma viral load and the development of lymphoma and leukaemia in koalas. This study also demonstrated that all koalas sampled in Queensland are viraemic with KoRV; a finding which may have significant consequences for koala populations in this part of their range.

A few studies have documented a relatively high prevalence of chlamydial infection in wild populations (White and Timms 1994; Jackson *et al.,* 1999; Devereaux *et al.,* 2003), with infection rates in some populations as high as 85% (Jackson *et al.,* 1999). However, the prevalence of overt disease was estimated to be far less common at 10-20% of infected animals (Timms 2005). Overt signs of chlamydiosis include, but are not limited to, incontinence and staining of the rump (a consequence of cystitis: also known as ‘wet bottom’ or ‘dirty tail’) and proliferative conjunctivitis (Cockram and Jackson 1974; Brown and Grice 1984; Brown *et al.,* 1987). Like KoRV-associated disease, chlamydiosis is often present in koalas without evidence of physical signs (Markey *et al.,* 2007; Hanger and Loader 2009). Considering that many studies have relied on the

presence of overt signs to estimate chlamydial disease status (Gordon *et al.,* 1990; White and Kunst 1990; White and Timms 1994; Jackson *et al.,* 1999; Dique *et al.,* 2003c; Lane 2008), there is a potential risk of underestimating disease occurrence. Often, it is only by the use of diagnostic techniques, such as urinalysis and ultrasound examination, that urogenital tract and reproductive pathology is detected (Jones 2008; Markey *et al.,* 2007; Hanger and Loader 2009).

This study aimed to determine more accurately the prevalence and incidence of disease in four koala populations in SEQ. This was achieved by conducting thorough veterinary health examinations using a standardised protocol, in conjunction with radio-telemetry for in-situ monitoring of koalas. Veterinary examinations included techniques not previously utilised in koala population health assessment. This is the first time that health investigations of wild koala populations using ultrasonography and bone marrow assessment have been reported. For comparison, this study also briefly describes the health of koalas from Kangaroo Island in South Australia that were assessed using an abridged veterinary examination.

6.2 Materials and Methods

6.2.1 Assessment of disease prevalence

Koalas from four geographically separate populations in SEQ (Brendale, Narangba, East Coomera and Clagiraba) were captured for veterinary health examinations (as described in section 4.4). In addition, each koala was observed for overt physical signs of disease:

1. Prior to disturbance (for capture); and
2. During and/or after capture.

The reason for this was to make an estimate of the proportion of koalas *overtly* ill, for comparison with:

1. The proportion actually found to be ill *after* thorough veterinary examination; and
2. Other studies reporting disease prevalence based only on overt signs, rather than thorough veterinary assessment.

Many previous studies (Gordon *et al.,* 1990; White and Kunst 1990; White and Timms 1994; Jackson *et al.,* 1999; Dique *et al.,* 2003c) have relied only on the observation of overt and classical signs of chlamydiosis to assess the disease prevalence of wild koala populations. Very few studies (Jones 2008; Hanger and Loader 2009) have reported on the pathology in wild koalas using thorough

health assessment, but clearly this is necessary to accurately estimate disease prevalence.

6.2.2 Health examinations and sampling of Kangaroo Island koalas

Opportunistically, veterinary health examinations and sampling of 50 Kangaroo Island koalas were conducted at the Kangaroo Island Veterinary Clinic in Kingscote, South Australia, over a two day period in December 2009. As part of an ongoing management program on Kangaroo Island to control koala population numbers, male and female koalas were being surgically sterilised. All adult koalas and independent young (around 2kg and above) were anaesthetised by veterinary staff at the Kangaroo Island Veterinary Clinic using a combination of isoflurane and medical oxygen. Female koalas were surgically sterilised laparoscopically, enabling visual assessment of the reproductive tract. Once the surgery was completed, blood and bone marrow smears were collected using the same methods outlined in sections 4.4.2 and 4.4.3.

Due to the short time available to perform each health examination, an abridged clinical assessment was performed so as not to compromise the efficiency of the desexing program. Therefore only limited clinical data were obtained and reported on. This included sex, weight, body condition score, reproductive status, estimation of age based on tooth wear, and physical lesions. Blood and bone marrow smears were evaluated at a later date.

6.2.3 Collection of disease prevalence and incidence data

The health of four SEQ koala populations was assessed using a cross-sectional study at each site to determine disease **prevalence** (section 4.2). Prevalence is a measure of the proportion of diseased individuals in a population at a given point in time. For logistical reasons, all koalas could not be located and captured on the same day, and so the prevalence of disease in each koala population had to be determined over an extended period (timeframes for prevalence data collection are defined in section 6.3). Hence, inferred disease prevalence was derived from the *initial* health examination for each koala, irrespective of when it was caught.

Note: This inferred prevalence will only accurately estimate true prevalence if the prevalence is not changing significantly over time. This is our assumption for the purposes of this exercise but it may or may not be true.

It was also necessary to estimate the rate of new disease or lesions per annum (represented by disease **incidence** data) by conducting a longitudinal study. Included in this incidence data was the incidence of death, otherwise known as the mortality rate. Due to time constraints in this study, incidence data was only obtained from the Moreton Bay (Brendale and Narangba) koala populations. This data was acquired by performing veterinary health examinations on koalas every six months (although in some cases this was not possible- see section 4.5).

6.2.4 Calculation of disease prevalence

The prevalence of disease (*P),* expressed as a percentage, in each koala population was calculated using the following equation:

$$P = d / t \times 100/1$$

where:

d denotes the number of diseased individuals in the population at a given time (ie at their first health assessment), and *t* is the total number of individuals in the population.

6.2.5 Calculation of disease incidence

The incidence of new disease is expressed as a percentage or proportion of the population showing new lesions per year. In order to correct for the variation in radio-tracking periods between koalas, it was necessary to calculate the average number of days (*d)* the koalas were monitored up until their final health examination.

This was calculated using the following formula:

$$d = \Sigma m/h$$

where:

m is the total days of monitoring for each "healthy" koala, and *h* is the total number of koalas in the population *minus those euthanased at their first health*

examination. In other words, koalas' euthanased at their first health examination due to severe illness were not included in the calculations to determine disease incidence.

The incidence *(I)* of new disease cases/lesions in the population each year was then calculated mathematically by the following equation:

$$I = (x/n) / (d/365) \times 100/1$$

where:

x = number of new cases of disease since the first health examination

n = number of healthy koalas in population at the first health examination

d = average number of days the koalas were monitored from their first to their final health examination

6.2.6 Chi-square analysis

Statistical analyses were performed using a chi-square test to compare the prevalence of disease between koala populations.

6.3 Results

The results for each koala population are discussed separately and then overall conclusions drawn at the end of this chapter in section 6.5.

6.3.1 Disease in the Brendale koala population

6.3.1.1 Prevalence of disease (Brendale)

Between July 2008 and February 2010, 34 resident Brendale koalas (17 male, 17 female) were captured and subjected to a veterinary health examination while under general anaesthesia (summarised in Table 6.1). Of these koalas, 50% (17/34 individuals) were considered to be healthy at their first examination; however the remainder of the population was found to have chlamydiosis or another unrelated pathological condition. Of the koalas with detectable illness (17/34 individuals), all but three had chlamydiosis.

	Males	Females	Total
Total no. of koalas	17	17	34
Healthy koalas (no detectable disease)	11/17 (65%)	6/17 (35%)	17/34 (50%)
Diseased koalas requiring veterinary intervention	6/17 (35%)	10/17 (59%)	16/34 (47%)
Chlamydial disease	5/17 (29%)	9/17 (53%)	14/34 (41%)
Disease (other)	1/17 (6%)	3/17 (23%) *one female koala had chlamydiosis in addition to other pathology	3/34 (9%)
No. of sexually mature females with a joey at 1st health examination	N/A	5/14 (36%)	14/17 (82%) females were sexually mature
Euthanased/died due to severity of disease	3/17 (18%)	8/17 (47%)	11/34 (32%)

Table 6.1: Health summary of the Brendale koala population at the first veterinary examinations

a) Overt disease vs. non-overt disease (Brendale)

Not all koalas with illness diagnosed by veterinary examination showed overt signs of disease. Prior to disturbance for capture, overt signs of disease were apparent in only 9% (3/34) of koalas, or only 18% (3/17) of koalas which were subsequently found to be diseased. In an additional five koalas, illness was apparent after disturbance and/or during capture, but had not been apparent prior to disturbance. Hence, only 8 of the 17 diseased koalas (47%) had overt signs of disease, and 9 (53%) showed no overt signs of illness, despite lesions

being subsequently detected during veterinary health assessments. As a proportion of the total population, 24% (8/34) of koalas displayed overt physical signs of disease, whereas 50% (17/34) were actually found to be diseased. Figure 6.1 represents the proportion of healthy koalas compared to those with detectable illness in the Brendale population.

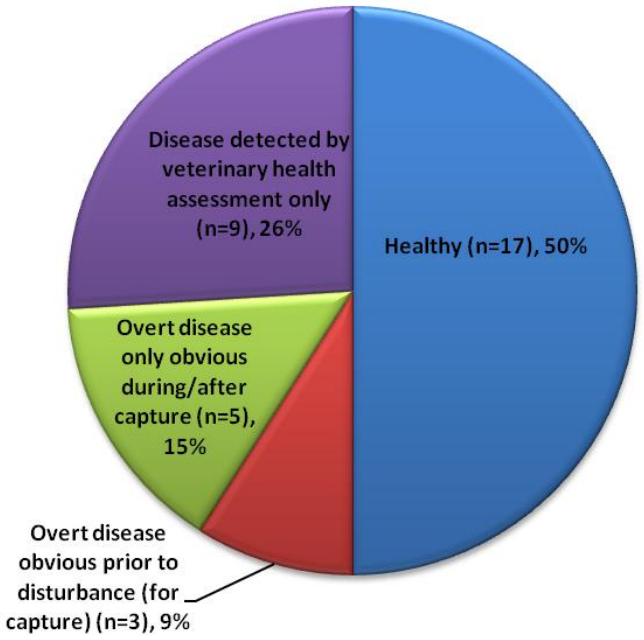

Figure 6.1: The proportion of healthy vs. diseased koalas in the Brendale population (n=34) at the first health examinations

b) Chlamydiosis (Brendale)

With respect to chlamydiosis, the proportion of koalas actually diagnosed with chlamydial disease was 41% (14/34). Of those koalas with detectable chlamydiosis, 20% (1/5) of males and 67% (6/9) of females showed no overt signs of disease. Hence 50% (7/14) of koalas with chlamydiosis did not show any overt clinical signs of disease, with disease only being detected by thorough veterinary health assessment. These results are shown in Table 6.2.

Chlamydial Disease	Males	Females
Overt chlamydial disease	4/5 (80%)	3/9 (33%)
Chlamydial disease (with no overt physical signs of chlamydiosis)	1/5 (20%)	6/9 (67%)
Conjunctivitis only	1/5 (20%)	0/9 (0%)
Cystitis only	4/5 (80%)	0/9 (0%)
Rhinitis only	0/5 (0%)	1/9 (11%)
Multifocal chlamydial disease	0/5 (0%)	4/9 (44.5%)
Reproductive disease	0/5 (0%)	8/9 (89%)
Total koalas with chlamydiosis	**5**	**9**
	TOTAL = 41% **14 out of 34 koalas in the population were diagnosed with chlamydiosis at the first health examination**	

Table 6.2: Summary of koalas with chlamydial disease in the Brendale population at the first health examinations

c) Prevalence of chlamydial disease in male vs. female koalas (Brendale)

The prevalence of detectable disease was higher in female koalas with 53% (9/17) affected by chlamydiosis compared to 29% (5/17) of males. Of the female koalas with chlamydial disease, 44.5% (4/9) had multifocal chlamydial disease (a combination of cystitis and reproductive disease, but none had kerato-conjunctivitis), 44.5% (4/9) had reproductive disease only and 11% (1/9) had chlamydial rhinitis. Kerato-conjunctivitis was not observed in any female within the study population. In contrast, cystitis was the most commonly detected pathological condition in male koalas, affecting 80% (4/5) of individuals with chlamydiosis. Only one male had kerato-conjunctivitis. Unlike female koalas in the study population, multifocal chlamydiosis was not detected in any of the male koalas. The proportions of different chlamydial disease lesions diagnosed in male versus female koalas with detectable chlamydiosis are shown in Figure 6.2.

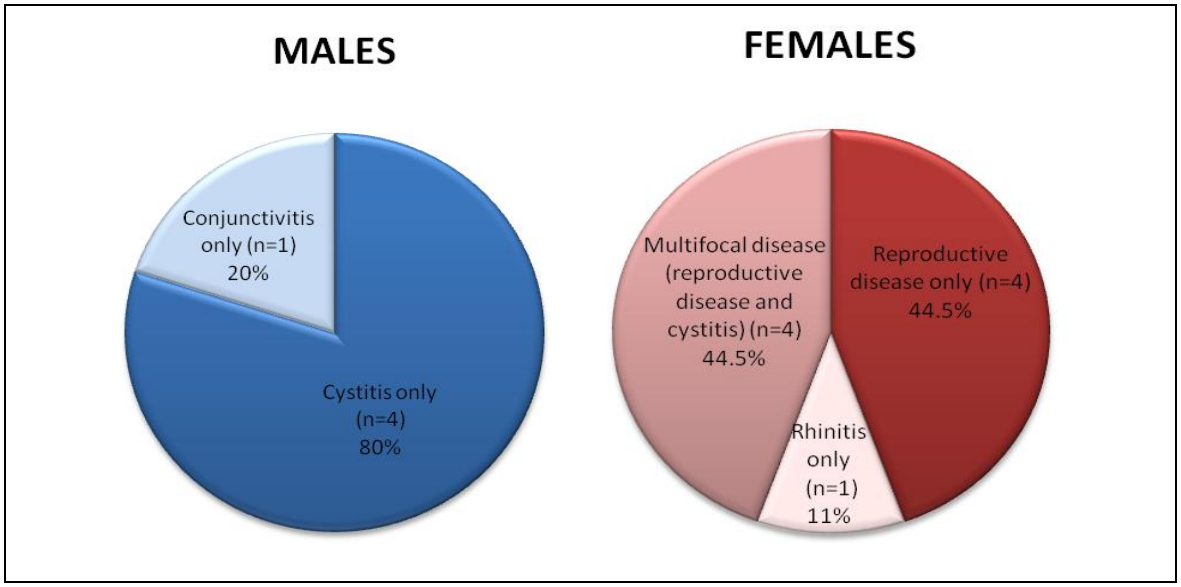

Figure 6.2: Comparison of chlamydial disease diagnosed in males vs. female koalas in the Brendale population at the first health examinations

d) Non-chlamydial disease (Brendale)

Pathologic lesions apparently unrelated to chlamydial infection were detected in four koalas: one mature male had a oral mass of indeterminate aetiology, but subsequently succumbed to acute renal disease during treatment (Fat Tony), two female koalas had skin conditions (Claude and Brianna), and a juvenile female koala had non-specific illness possibly attributable to the putative AIDS-like syndrome of koalas (Paula) (Hanger *et al.,* 2003), and was in care at the Australian Wildlife Hospital at the conclusion of this study.

e) Health and fecundity of female koalas (Brendale)

Of the 17 female koalas sampled in the population, three were considered to be sexually immature at the time of first capture (Renee, Lex and Paula). The proportion of sexually mature female koalas with a joey at the initial health examination was 36% (5/14). In the females with no joey present, reproductive disease (a major cause of infertility in koalas) was diagnosed in 89% (8/9). This represents 57% (8/14) of the sexually mature females in the population. The reproductive status of one koala (Xena) could not be definitively established by ultrasound examination (this koala was either pregnant or had reproductive disease) at the first clinical assessment. Due to a radio-collar failure, no confirmation of this koala's disease status could be achieved at a follow-up health examination. Of the females with reproductive disease, only 25% (2/8) had overt signs of chlamydiosis: in both cases illness was only evident because both individuals had concurrent cystitis, resulting in dirty tail, in addition to reproductive disease.

f) Outcome for koalas with detectable illness (Brendale)

The proportion of koalas' euthanased at their first health examination was 29% (2 male, 8 female). Of the remaining koalas with detectable illness, one koala died in care (Red), four koalas were treated and subsequently released, and one koala (Paula) was still in care at the conclusion of this study.

g) KoRV viraemia titre (Brendale)

Of the 34 koalas in the population, a KoRV viraemia titre was conducted on 10. All koalas tested were viraemic with KoRV with titres ranging from 2.42 x 10^5 to 1.42 x 10^9 genome equivalents/ml. (Note: retrovirus genomes are diploid genomes, therefore the qPCR titre (estimated copies per ml) needs to be divided by two to get an estimate of the number of virus particles per ml).

6.3.1.2 Incidence of new disease in the Brendale koala population

In order to determine the annual incidence of new disease cases emerging in the Brendale koala population, follow-up veterinary health examinations were conducted between December 2008 and April 2010.

The incidence of new disease lesions diagnosed in the population since the initial veterinary examinations was 19%. (In other words, each koala in the population had a 19% chance *each year* of becoming ill, or developing a new lesion). This included one aged female (Shirl) diagnosed with cryptococcosis, one young female which developed reproductive disease (bilateral metritis and a unilateral uterine cyst) after production of her first joey (Maggie), and a male koala that had previously been treated for chlamydiosis but had new lesions develop subsequently (cystitis and prostatitis) (Stefan). Of the healthy female koalas at the first health examination, one of the nine developed reproductive disease during the period of this study. This equates to a corrected annual incidence of infertility of 12%.

The incidence of death (mortality rate) in the population that was not disease related was 13%. One male koala was hit by a car and later euthanased at the Moggill Koala Hospital (Tiny Tim), while another male drowned in a sewage pit (Zed). When euthanasia (due to the severity of disease) was included (eg Shirl), the mortality rate was 19%.

6.3.2 Disease in the Narangba koala population

6.3.2.1 Prevalence of disease (Narangba)

The veterinary examinations and sampling of 22 resident Narangba koalas (10 male, 12 female) were conducted between July 2008 and February 2010 (summarised in Table 6.3). Of these koalas, 59% (13/22) were considered healthy, while 41% (9/22) had detectable chlamydiosis or another unrelated pathological condition.

	Males	Females	Total
Total no. of koalas	10	12	22
Healthy koalas (no detectable disease)	6/10 (60%)	7/12 (58%)	13/22 (59%)
Diseased koalas requiring veterinary intervention	3/10 (30%)	5/12 (42%)	8/22 (36%)
Chlamydial disease	3/10 (30%)	5/12 (42%)	8/22 (36%)
Disease (other)	1/10 (10%)	0/12 (0%)	1/22 (5%)
No. of sexually mature females with a joey at 1st health examination	N/A	5/11 (46%)	11/12 (92%) females were sexually mature
Euthanased due to severity of disease	0/10 (0%)	3/12 (25%)	3/22 (14%)

Table 6.3: Health summary of the Narangba koala population at the first veterinary examinations

a) Overt disease vs. non-overt disease (Narangba)

The proportion of koalas that had observable signs of disease preceding capture was 9% (2/22), or 22% (2/9) of koalas subsequently found to be diseased. Following capture (but prior to veterinary check), two additional koalas were found to have disease that was not obvious prior to disturbance. Once veterinary examinations had been conducted, a further five koalas that exhibited no overt signs of disease, were found to have detectable illness. This indicates that only 44% (4/9) of diseased koalas showed overt signs of illness, compared with 56% (5/9) of koalas that had no overt pathology. Figure 6.3 represents the proportion

of healthy koalas compared to those with detectable illness in the Narangba population.

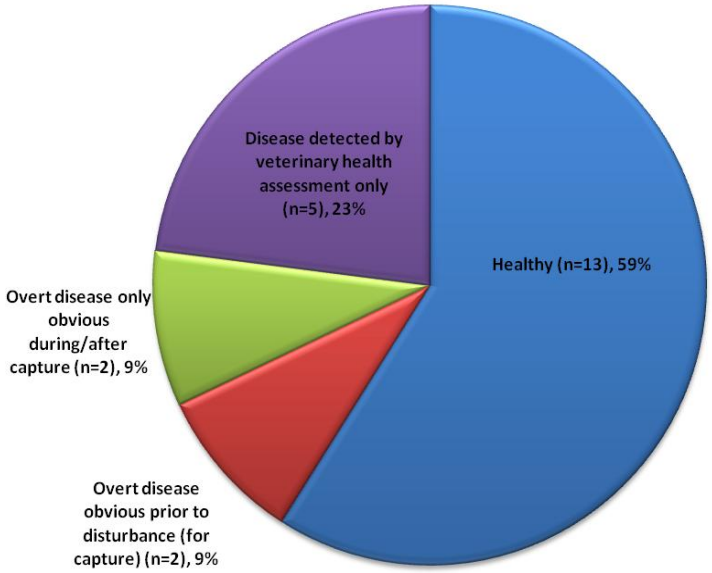

Figure 6.3: The proportion of healthy vs. diseased koalas in the Narangba population (n=22) at the first health examinations

b) Chlamydiosis (Narangba)

Of the koalas with detectable chlamydiosis (by veterinary examination), 67% (2/3) of males and 60% (3/5) of females showed no overt signs of disease. As a proportion of the total population, 14% (3/22) of koalas displayed overt physical signs of chlamydial disease, whereas 36% (8/22) were actually found to have chlamydiosis (Table 6.4).

Chlamydial Disease	Males	Females
Overt chlamydial disease	1/3 (33%)	2/5 (40%)
Chlamydial disease (with no overt physical signs of chlamydiosis)	2/3 (67%)	3/5 (60%)
Conjunctivitis only	1/3 (33%)	0/5 (0%)
Cystitis only	2/3 (67%)	0/5 (0%)
Rhinitis only	0/3 (0%)	0/5 (0%)
Multifocal chlamydial disease	0/3 (0%)	2/5 (40%)
Reproductive disease	0/3 (0%)	5/5 (100%)
Total koalas with chlamydiosis	**3**	**5**
	TOTAL = 36% **8 out of 22 koalas in the population were diagnosed with chlamydiosis at the first health examination**	

Table 6.4: Summary of koalas with chlamydial disease in the Narangba population at the first health examinations

c) Prevalence of chlamydial disease in males vs. females (Narangba)

Chlamydial disease was detected in 42% (5/12) of female koalas compared to 30% (3/10) of males. Of these koalas, reproductive disease was the most common condition diagnosed in females (5/5; 100%). Two of these females also had concurrent disease (both reproductive disease and cystitis). Conversely, multifocal disease was not evident in any of the male koalas. Of the three male koalas with chlamydiosis, one had kerato-conjunctivitis and two were found to have subclinical cystitis. The proportion of chlamydial disease conditions

diagnosed in male versus female koalas with detectable chlamydiosis is highlighted in Figure 6.4.

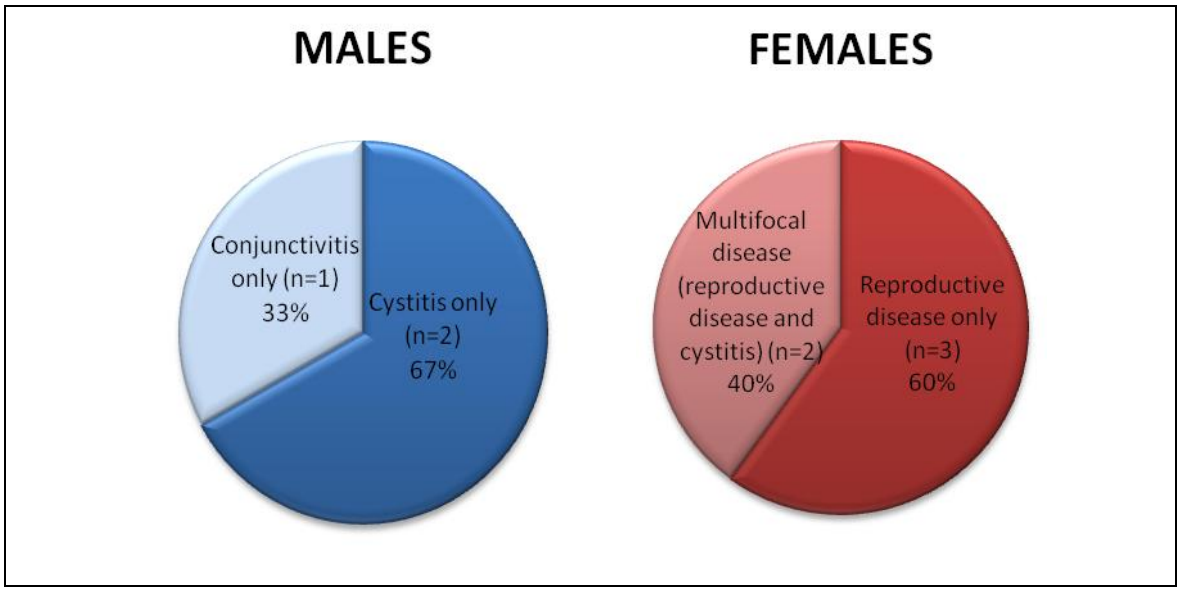

Figure 6.4: Comparison of chlamydial disease found in males vs. female koalas in the Narangba population at the first health examinations

d) Non-chlamydial disease (Narangba)

One male koala was diagnosed with generalised seborrhoeic dermatitis at the initial veterinary examination (Gus). This was associated with areas of fur thinning and alopecia, but was not considered to be of major health significance.

e) Health and fecundity of females (Narangba)

The proportion of sexually mature female koalas, of total female koalas (excluding dependent young) at the first health examination was 92% (11/12). (One female (Linda) was considered to be sexually immature). Of these koalas, 45.5% (5/11) had either a pouch or back young, and one was pregnant (Mandy) (as determined by ultrasound examination). The remaining five (45.5%) koalas had reproductive disease and were therefore considered to be infertile.

f) Outcome for koalas with detectable illness (Narangba)

Sick koalas that were considered unsuitable for treatment and were subsequently euthanased on humane grounds, comprised 14% (3/22) of the total population. All of these koalas were females with severe reproductive disease, two of which also had chronic cystitis. Of the remaining koalas with detectable chlamydiosis, three male koalas were treated at the Australian Wildlife Hospital and were subsequently released. Two female koalas with chronic, resolved reproductive disease (that were otherwise healthy) underwent ovariohysterectomy and were subsequently placed into permanent captive care (under the DERM Queensland Species Management Program). The male koala with mild seborrhoeic dermatitis was released without treatment immediately after the initial veterinary examination but was euthanased 18 months later at the final veterinary examination (Gus) (see 5.3.2b).

g) KoRV viraemia titre

Of the 22 koalas in the population, a KoRV viraemia titre was conducted on 15. All koalas tested were viraemic with KoRV with titres ranging from 4.3 x 10^3 to 7.7 x 10^9 genome equivalents/ml. (Note: retrovirus genomes are diploid genomes, therefore the qPCR titre (estimated copies per ml) needs to be divided by two to get an estimate of the number of virus particles per ml).

6.3.2.2 Incidence of new disease in the Narangba koala population

In order to determine the annual incidence of new disease cases emerging in the Narangba koala population, follow-up veterinary health examinations were conducted between March 2009 and February 2010.

The incidence of new disease diagnosed in the population since the initial veterinary examinations was 34%. This included four females that developed reproductive disease (with one of these females also having concurrent cystitis) (Linda, Mandy, Jasmine and Aria), and one male that developed subclinical cystitis (Igor). Of the healthy female koalas at the first health examination, four of the seven developed reproductive disease during the period of this study. This equates to a corrected annual incidence of infertility of 55%. When combined with the Brendale incidence data, the incidence of infertility was 32%.

The incidence of death (mortality rate) in the population when euthanasia (due to the severity of disease) was included was 20%. Other than the koalas that

were euthanased (Aria, Jasmine and Mandy), no koalas died during the period of this study.

6.3.3 Disease in the East Coomera koala population

6.3.3.1 Prevalence of disease (East Coomera)

Veterinary health examinations were conducted on 34 East Coomera koalas (15 male, 19 female) between September 2009 and May 2010 (summarised in Table 6.5). Of these koalas, 65% (22/34) were considered healthy, while 35% (12/34) had a pathological condition of clinical significance.

	Males	Females	Total
Total no. of koalas	15	19	34
Healthy koalas (no detectable disease)	9/15 (60%)	13/19 (68%)	22/34 (65%)
Diseased koalas requiring veterinary intervention	4/15 (27%)	3/18 (17%) *Note one koala was first examined at post-mortem, so was not included in this calculation	7/33 (21%)
Chlamydial disease ***Note: some koalas may have had chlamydiosis in addition to other pathology**	4/15 (27%)	5/19 (26%)	9/34 (26%)
Disease (other)	3/15 (20%)	2/19 (11%)	5/34 (15%)
No. of sexually mature females with a joey at 1st health examination	N/A	9/16 (56%)	16/19 (84%) females were sexually mature
Euthanased due to severity of disease	2/15 (13%)	0/19 (0%) *Note one koala was first examined at post-mortem, so was not included in this calculation	2/34 (6%)

Table 6.5: Health summary of the East Coomera koala population at the first veterinary examinations

a) Overt disease vs. non-overt disease (East Coomera)

The proportion of koalas that had observable signs of disease preceding capture was 6% (2/34) (Hamid and Maree), or 17% (2/12) of koalas subsequently found to be diseased. Following capture (but prior to veterinary check), one additional koala (Althena) was found to have disease that was not obvious prior to disturbance. Once veterinary examinations had been conducted, a further nine koalas that exhibited no overt signs of disease, were found to have detectable illness. This indicates that only 25% (3/12) of diseased koalas showed overt

signs of illness, compared with 75% (9/12) of koalas that had no overt pathology. As a proportion of the total population, 9% (3/34) of koalas displayed overt physical signs of disease, whereas 35% (12/34) of koalas were actually found to be diseased.

Figure 6.5 represents the proportion of healthy koalas compared to those with detectable illness in the East Coomera population.

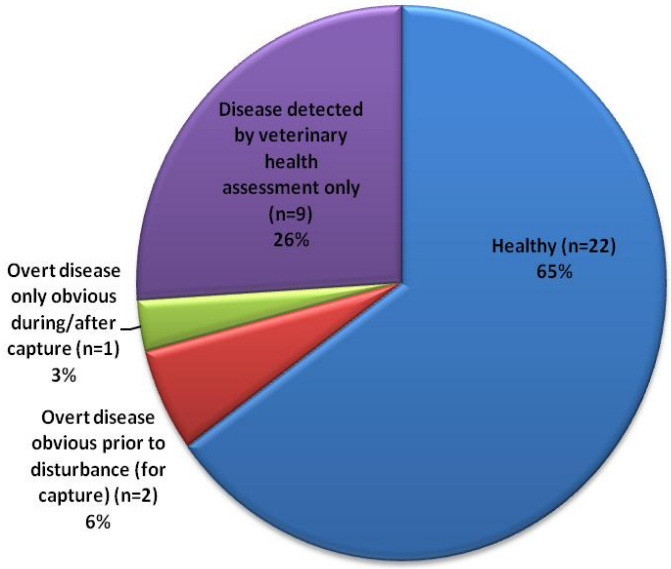

Figure 6.5: The proportion of healthy vs. diseased koalas in the East Coomera population (n=34) at the first health examinations

b) Chlamydiosis (East Coomera)

The proportion of koalas diagnosed with chlamydial disease was 26% (9/34). Of the males with detectable chlamydiosis, 75% (3/4) showed no overt physical

signs of disease. Of the females with detectable chlamydiosis, 60% (3/5) koalas showed no overt signs of disease, with disease only being detected by thorough veterinary health assessment (Table 6.6).

Chlamydial Disease	Males	Females
Overt chlamydial disease	1/4 (25%)	2/5 (40%)
Chlamydial disease (with no overt physical signs of chlamydiosis)	3/4 (75%)	3/5 (60%)
Conjunctivitis only	0/4 (0%)	0/5 (0%)
Cystitis only	4/4 (100%)	0/5 (0%)
Rhinitis	0/4 (0%)	0/5 (0%)
Multifocal chlamydial disease	0/4 (0%)	2/5 (40%)
Reproductive disease	0/4 (0%)	5/5 (100%)
Total koalas with chlamydiosis	**4**	**5**
	TOTAL = 26% **9 out of 34 koalas in the population were diagnosed with chlamydiosis at the first health examination**	

Table 6.6: Summary of koalas with chlamydial disease in the East Coomera population at the first health examinations

c) Prevalence of chlamydial disease in males vs. females (East Coomera)

Of the female koalas with detectable chlamydiosis, 100% (5/5) had reproductive disease. Two of these females had multifocal chlamydiosis: a combination of cystitis, kerato-conjunctivitis and reproductive disease (Althena), or cystitis and

reproductive disease (Maree). Of the four male koalas with detectable chlamydiosis, all had cystitis (Dale, Hamid, Peter and James) (Figure 6.6).

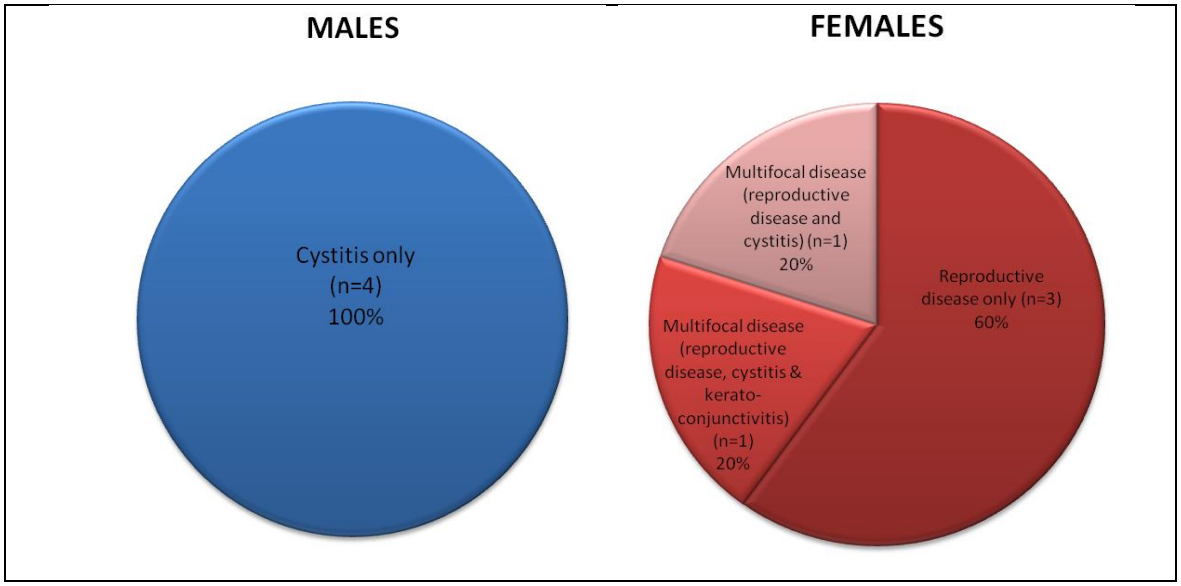

Figure 6.6: Comparison of chlamydial disease found in males vs. female koalas in the East Coomera population at the first health examinations

d) Non-chlamydial disease (East Coomera)

Pathology apparently unrelated to chlamydial infection was detected in five koalas (3 male, 2 female). One male (Dale) had septic arthritis in the left shoulder (in addition to cystitis), one had a fungal lesion on his scrotum (Glen), while the other male had chronic ill-thrift, non-regenerative anaemia, gastrointestinal candidiasis and some evidence of renal failure (Connor). This koala was euthanased after demonstrating a poor response to treatment.

Of the females, one juvenile koala had non-specific illness and was in fair body condition at the time of the initial examination (Echo), while the other was first examined at post-mortem after being found dead at the base of a tree (Angela). This koala was in poor body condition, had severe regenerative anaemia, reproductive disease and evidence of immunosuppression.

e) Health and fecundity of females (East Coomera)

Of the 19 female koalas examined in the population, three were sexually immature at the time of first capture (Avi, Caitlin and Jo). The proportion of sexually mature female koalas with a joey at the initial health examination was 56% (9/16). In the females with no joey present, reproductive disease was detected in 71% (5/7). This represents 31% (5/16) of the sexually mature females in the population. The two remaining females without a joey present (Echo and Kiwi Sarah) had no detectable reproductive lesions at the time of their first health examination.

In the females with reproductive disease, 40% (2/5) had overt signs of chlamydiosis. Both of these koalas had multifocal chlamydial disease, a combination of kerato-conjunctivitis, cystitis, and reproductive disease (Althena), or cystitis and reproductive disease (Maree).

f) Outcome for koalas with detectable illness (East Coomera)

Of the koalas with detectable illness, three were treated at the Australian Wildlife Hospital and subsequently released (James, Echo and Emma), two were euthanased after responding poorly to medical treatment (Connor and Dale), one koala was deceased at the first veterinary health examination (Angela), three koalas with chronic, resolved chlamydial lesions were left untreated and monitored in the field (Althena, Louise and Peter), one koala was treated and monitored in the field (Glen), and two koalas were still in care at the conclusion of this study (Hamid and Maree).

6.3.4 Disease in the Clagiraba koala population

6.3.4.1 Prevalence of disease (Clagiraba)

Four resident Clagiraba koalas (3 male, 1 female) were subjected to a veterinary health examination between October 2009 and March 2010 (summarised in Table 6.7). Morbidity in the population was high, with all koalas found to have detectable illness.

	Males	Females	Total
Total no. of koalas	3	1	4
Healthy koalas (no detectable disease)	0/3 (0%)	0/1 (0%)	0/4 (0%)
Diseased koalas requiring veterinary intervention	2/2 (100%) *Note: one koala was first examined at post-mortem, so was not included in this calculation	1/1 (%)	3/3 (100%)
Chlamydial disease	3/3 (100%)	1/1 (100%)	4/4 (100%)
Disease (other)	2/3 (75%)	0/1 (0%)	2/4 (50%)
No. of sexually mature females with a joey at 1st health examination	N/A	0/1 (0%)	0/1 (0%)
Euthanased due to severity of disease	1/2 (50%) *Note: one koala was first examined at post-mortem, so was not included in this calculation	1/1 (100%)	2/3 (75%)

Table 6.7: Health summary of the Clagiraba koala population at the first veterinary examinations

a) Overt disease vs. non-overt disease (Clagiraba)

Prior to disturbance for capture, overt disease was observed in one koala in the population (Ned). In an additional two koalas (Kellie and Graeme), illness was apparent during or after capture, but prior to clinical examination. One koala (Andrew), which was initially examined after being found dead at the base of a tree, was also found to have overt signs of disease. As a proportion of the total population, 100% (4/4) of koalas had overt signs of disease. Figure 6.7

represents the proportion of healthy koalas compared to those with detectable illness in the Clagiraba population.

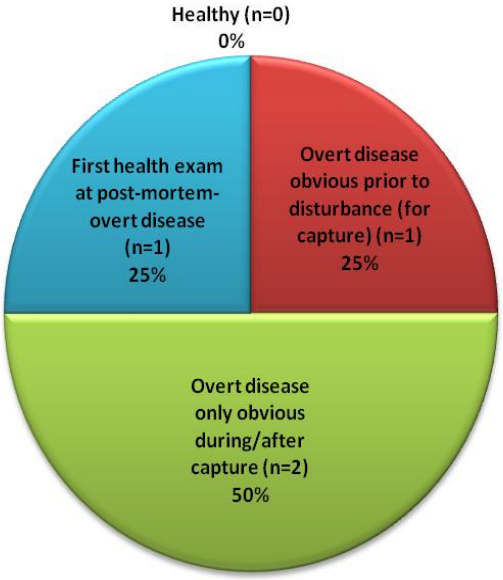

Figure 6.7: The proportion of healthy vs. diseased koalas in the Clagiraba population (n=4) at the first health examinations

b) Chlamydiosis (Clagiraba)

Chlamydial disease was diagnosed in all koalas examined in the population. Of these koalas, two of the three males showed no overt signs of chlamydial disease. One koala (Graeme) had subclinical cystitis; but had evidence of overt illness apparently unrelated to chlamydial infection (poor body condition and poor coat condition), and the other koala (Andrew) had mild non-suppurative

prostatitis. In comparison, the only female koala examined in the population (Kellie) had obvious signs of chlamydiosis (kerato-conjunctivitis). Overall, 50% (2/4) of koalas showed no overt physical signs of chlamydial disease (Table 6.8).

Chlamydial Disease	Males	Females
Overt chlamydial disease	1/3 (33%)	1/1 (100%)
Chlamydial disease (with no overt physical signs of chlamydiosis)	2/3 (67%)	0/1 (0%)
Conjunctivitis only	0/3 (0%)	0/1 (0%)
Cystitis only	1/3 (33%)	0/1 (0%)
Rhinitis	0/3 (0%)	0/1 (0%)
Multifocal chlamydial disease	1/3 (33%)	1/1 (100%)
Reproductive disease	1/3 (33%) *Note: This does not necessarily mean this koala was sterile	1/1 (100%)
Total koalas with chlamydiosis	**3**	**1**
	TOTAL = 100% **4 out of 4 koalas in the population were diagnosed with chlamydiosis at the first health examination**	

Table 6.8: Summary of koalas with chlamydial disease in the Clagiraba population at the first health examinations

c) Prevalence of chlamydial disease in males vs. females (Clagiraba)

Chlamydiosis was diagnosed in 100% of both male and female koalas. Of the male koalas with chlamydiosis, two were found to have cystitis; however one individual also had kerato-conjunctivitis. One male had mild prostatitis which

was detected by histological examination post-mortem. The only female examined in the population had multifocal chlamydiosis: a combination of reproductive disease, cystitis and kerato-conjunctivitis. The proportion of chlamydial disease conditions diagnosed in male versus female koalas with detectable chlamydiosis is highlighted in Figure 6.8.

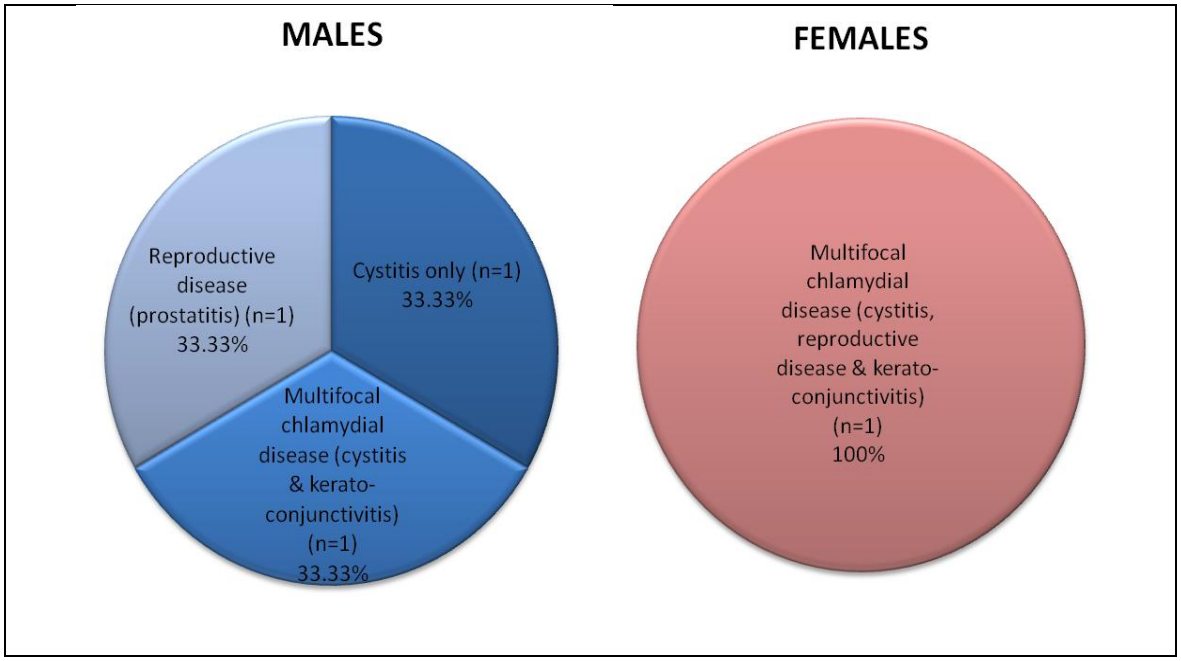

Figure 6.8: Comparison of chlamydial disease found in males vs. female koalas in the Clagiraba population at the first health examinations

d) Non-chlamydial disease (Clagiraba)

Pathology apparently unrelated to chlamydial infection was detected in two koalas: one aged male (Graeme) was in poor body condition and had gastrointestinal candidiasis, trypanosome parasitaemia, subclinical cystitis and regenerative anaemia. In addition, this koala also had non-specific illness possibly attributable to the AIDS-syndrome of koalas (Hanger 1999; Hanger *et al.,* 2003). The other male (Andrew) was diagnosed post-mortem with non-specific chronic immune-mediated and immunodeficiency disease, possibly also attributable to KoRV infection and the koala AIDS-syndrome.

e) Health and fecundity of females (Clagiraba)

Only one female koala (Kellie) was captured for a veterinary health examination. This koala was sexually mature but had reproductive disease and a small, undeveloped pouch, and was considered to be infertile.

f) Outcome for koalas with detectable illness (Clagiraba)

The first examination of one koala (Andrew) was conducted post-mortem after he was found dead at the base of a tree. Of the three remaining koalas all were treated at the Australian Wildlife Hospital, but all responded poorly to treatment. Two koalas (Graeme and Kellie) were euthanased on humane grounds, while one koala (Ned) died some months after his release back into the wild after a

period of treatment in hospital. This koala had developed lesions consistent with chronic immunosuppression/ immunodeficiency (koala “AIDS”).

6.3.5 Disease in the Kangaroo Island koala population

Veterinary health examinations of 50 Kangaroo Island koalas (28 male, 22 female) were conducted in December 2009. Based on the abridged clinical examination and sampling procedures that were conducted, no pathological condition was detected in any of the koalas. All koalas were in excellent body condition (BCS 8-10), except for one koala that was in fair condition (BCS 6), but had no other specific conditions detected.

Anatomical abnormalities, possibly associated with the low genetic diversity of koalas on Kangaroo Island (Houlden *et al.,* 1996; Seymour *et al.,* 2001; Cristescu *et al.,* 2009), were detected in three koalas. This included one male and one female with maxillary prognathism (undershot mandible), and one male with testicular aplasia.

Of the females sampled, four were sexually immature, three were pregnant, 11 had joeys and the reproductive status of four koalas was not recorded. Excluding the four females with no data on their reproductive status, 100% (14/14) of the sexually mature female koalas examined were considered fecund prior to desexing.

Blood samples were given to the Koala Retrovirus Research Group at UQ and the following data were kindly provided: KoRV provirus was detected in 18 of the 50 koalas' sampled (36%) using standard (non-quantitative) and nested PCR (K. Jones pers. comm., 10th May 2010). PCR band intensity on gels were relatively weak, which is consistent with exogenous infection (P. Young pers. comm., 11th May 2010).

6.3.6 Chi-square analysis of disease prevalence between five koala populations

A chi-square test indicated that there was no significant difference in disease prevalence between the Brendale, Narangba and East Coomera koala populations ($p>0.05$). However, pair-wise comparisons revealed the disease prevalence to be significantly different between the Clagiraba and Narangba and East Coomera populations, but not between the Clagiraba and Brendale populations. When the disease prevalence of each SEQ population was compared with the Kangaroo Island koala population, the differences were significant. Table 6.9 shows the results of the chi-square analysis.

Koala Population	Brendale	Narangba	East Coomera	Clagiraba
Brendale (Qld)				
Narangba (Qld)	χ^2= 0.444, p-value= 0.505			
East Coomera (Qld)	χ^2= 1.503, p-value= 0.220	χ^2= 0.180, p-value=0.672		
Clagiraba (Qld)	χ^2= 3.619, p-value= 0.057	χ^2= 4.727, p-value= 0.029	χ^2= 6.147, p-value= 0.013	
Kangaroo Island (S.A)	χ^2= 31.343, p-value= 2.16×10^{-8}	χ^2= 23.376, p-value= 1.33×10^{-6}	χ^2= 20.588, p-value= 5.69×10^{-6}	χ^2= 54.000, p-value= 2.00×10^{-13}

Table 6.9: Chi-square analysis of the prevalence of disease in five koala populations

6.4 Discussion

The proportion of koalas with illness detected by a diagnostic work-up was markedly higher, in each of the study populations, than estimations of disease prevalence when relying on overt physical signs of disease. These results demonstrate the importance of conducting thorough veterinary health examinations to determine the prevalence of disease in wild koala populations. Without the utilisation of diagnostic techniques including cystocentesis for urinalysis, blood and bone marrow assessment and ultrasound examination, a substantial proportion of disease in koalas may be overlooked. Nevertheless, although the sensitivity for detection of disease is greater when a comprehensive veterinary examination is performed, it will not necessarily detect 100% of pathology.

According to Dique *et al.* (2003c), koala populations in the old Pine Rivers Shire (now part of the Moreton Bay LGA), including those residing in the suburb of Brendale, were considered to be “extremely healthy” and “robust” compared to other populations in SEQ, particularly the Koala Coast population. Their estimate of chlamydial disease prevalence in bushland habitat (patches >100ha), based on observations through binoculars of the classical signs of chlamydiosis (dirty tail and conjunctivitis) was 7.4% (6/81). This figure was similar to the results of the present study, in which overt physical signs of disease were apparent in only 9% (3/34) of the Brendale koalas *prior to* disturbance for capture. By comparison, the actual proportion of Brendale koalas with detectable chlamydiosis (detected using thorough veterinary investigative techniques) was 41% (14/34). This is approximately five times the prevalence suggested by the observation of overt signs of disease solely. Similarly, the proportion of Narangba and East Coomera koalas with detectable chlamydiosis (8/22; 36% and 9/34; 26%, respectively) was around four times higher than the number observed with overt signs prior to disturbance. White and Kunst (1990) indicated that the use of binoculars to observe clinical signs of chlamydiosis prior to disturbance was likely to underestimate disease prevalence. This suggestion is strongly validated by the results of our study.

In 1999, a study by Jackson *et al.* investigated the prevalence of overt chlamydial disease in the Mutdapilly koala population, south-west of Brisbane, and found that *after* capture had occurred, 17% of koalas had obvious signs of

chlamydiosis. White and Kunst (1990) reported similar results for a koala population in Sheldon, SEQ, with 18% of koalas exhibiting overt signs of chlamydiosis after capture. This is comparable to two of the koala populations used in our study (those of Narangba and Brendale), in which the prevalence of overt chlamydial disease in each population *after* capture (but prior to veterinary examination) was 18% and 21%, respectively. Nevertheless, results from this study also indicated that the prevalence of chlamydiosis (and other diseases) in wild koala populations is likely to be vastly underestimated when relying exclusively on the presence of overt physical signs of disease, rather than conducting comprehensive veterinary examinations.

Hence, a useful rule of thumb is that if disease prevalence is measured by observation of overt signs without capture of the koala, then the actual figure is likely to be at least five times that figure. If koalas are captured, closely observed, but not subjected to a thorough veterinary examination, then it is safe to double that figure as a crude estimate of actual prevalence. It is important to note, however, that the true prevalence of disease must be somewhat higher again, to account for cases that are beyond the detection sensitivity of our methods, but that may still have significant health impacts.

In each koala population studied, reproductive disease was diagnosed in a higher proportion of females than in males. Although ultrasound imaging is useful for the detection and confirmation of reproductive disease in female

koalas, particularly when cysts or abscesses are too small to palpate, or when uterine abnormalities are present, limitations exist such that subtle pathological changes may not be evident. Therefore the actual number of infertile females in each study population is likely to be somewhat higher than our results would indicate. Ultimately, however, the most effective measure of fertility is the production of a joey which should occur annually for fertile females in our experience.

An advantage of conducting prevalence studies is that they are useful for the detection of chronic diseases; however mortalities and acute/sub-acute illnesses are less likely to be detected. This data is more likely to be reflected in longitudinal studies. From the long-term monitoring of the Brendale and Narangba koalas, it was found that 19% and 34% of koalas, respectively, developed illness each year. For example, one female koala (Aria) was healthy with a pouch joey at the first veterinary examination, however at a subsequent examination, approximately one year later, she had developed reproductive pathology. Disease in this koala would not have been detected without long-term monitoring.

Of all the populations studied, the proportion of sexually mature female koalas with reproductive disease was highest in the Brendale and Narangba populations with 57% (8/14) and 45.5% (5/11) affected, respectively. The annual incidence of newly developed infertility in previously healthy female

koalas in the Narangba population was 55% and at Brendale, 12%. The combined incidence was 32%. In other words, approximately one third of the healthy koalas developed reproductive disease/infertility annually. In comparison with women, this is orders of magnitude greater. A review by Beagley and Timms (2000) reported that in the United States of America (USA), around one million women annually experience pelvic inflammatory disease caused by infection with *C. trachomatis*, of which 50, 000 (5%) become infertile each year[3] (which equates to around 0.1% of fertile, reproductive age women (U.S. Department of Commerce 2002)). Although *Chlamydia* is the most common STD in humans, and the most common cause of tubal infertility in women, severe disease is rare, and infertility occurs infrequently compared with koalas (Hanger and Loader 2009; P. Timms pers. comm., 12th May 2009).

Mortalities reflected in the incidence data of the Brendale koala population indicated that 13% of koalas died from causes unrelated to disease (eg motor vehicle injury and other misadventure), or 19% when death by euthanasia (due to illness) was also included. None of the koalas in the Narangba population died during the study period other than those that were euthanased due to disease (20%). Mortality data is extremely useful for population viability analyses and aids in identifying the most important threatening processes. This data is

[3] Chlamydial disease is most well-studied in humans with respect to prevalence and incidence and is the most common cause of acquired infertility in women (P. Timms pers. comm., 24th May 2010)

fundamental for the development of management plans for populations under threat. The causes of death in many studies may not be ascertained for the following reasons:

- Radio-telemetry or other forms of real-time monitoring may not be used, hence mortality events remain undetected;
- Koalas that are only infrequently monitored by radio-telemetry may be in an advanced state of decomposition by the time they are found, making a cause of death less likely to be determined;
- Access to experienced veterinary expertise may not be available or taken advantage of by field researchers due to budgetary or other constraints.

KoRV viraemia PCR titres were conducted on 25 koalas from each of the Brendale and Narangba populations. All were viraemic with KoRV (G. Simmons unpublished data). These results are consistent with the study by Tarlinton *et al.* (2006) in which it was reported that all koalas sampled in Queensland were viraemic with KoRV. The same study found that KoRV was absent in all 26 of the koalas sampled on Kangaroo Island. In contrast, results from the Kangaroo Island koalas sampled in our study found a 36% prevalence of koalas infected with KoRV using PCR for detection of provirus, however at the time of writing, qPCR to quantitatively detect viraemia had not yet been conducted (K. Jones unpublished data). Due to the low heterozygosity of koalas on Kangaroo Island (Seymour *et al.* 2001), a genetic parasite such as KoRV has the potential to be

devastating for these animals. A real-life example of such a scenario is the recent emergence and rapid spread of Devil Facial Tumour Disease (DFTD) in Tasmanian devils (*Sarcophilus harrisii*), which is thought to be largely due to low genetic diversity in the species, leading to a failure to recognise the transmissible tumour as foreign (Siddle *et al.,* 2007). This epizootic has demonstrated how rapidly new diseases can devastate a population, particularly one with low genetic diversity (Lunney *et al.,* 2008), and has caused ecologists to rethink common dogmas relating to disease ecology and species extinction (Smith *et al.,* 2006).

When comparing the health of the four koala study populations in SEQ to the Kangaroo Island koala population in South Australia, it is evident SEQ koalas are a sick population. Although the Kangaroo Island koalas have genetic issues to contend with, our results indicate that they are a much healthier population when compared with their northern counterparts. Whilst the health examinations of these koalas did not follow exactly the same protocol as that applied to the SEQ koalas, they nevertheless aimed to detect most known conditions in koalas. Reproductive disease was not grossly evident in any of the Kangaroo Island female koalas examined for this study and has only been rarely observed in the past (Higgins *et al.,* 2005; G. Johnsson, pers. comm., 9th December 2009). As Kangaroo Island koala populations are considered to be *Chlamydia*-free (Brown *et al.,* 1984; Timms 2005) the observed lesions were probably unrelated to chlamydial infection (although only a small proportion of Kangaroo

Island koalas have been tested for *Chlamydia* to date). Interestingly, all of the sexually mature female koalas examined (excluding the four koalas with no data recorded on their reproductive status) from Kangaroo Island had joeys and/or were pregnant. This is in stark contrast to the fecundity of SEQ females where only 36%, 54.5% and 56% of sexually mature females had joeys or were pregnant at the first health examination in the Brendale, Narangba and East Coomera populations, respectively. Arguably, the Kangaroo Island koala population is indicative of what the health of koala populations "should" be, with the SEQ study populations representing koala populations severely affected by disease epizootics. There are limitations of the comparison: the Kangaroo Island population is somewhat "artificial" as koalas did not occur there naturally at the time of European settlement. Also, chlamydial disease has not been reported in the koala population on the island.

Although a detailed analysis of habitat disturbance at each of the four SEQ study sites and Kangaroo Island was not conducted, subjectively there appeared to be relatively few differences between sites. All sites had some degree of disturbance, evidence of prior clearing, a degree of weed incursion, and varying proximity to urban development. Nevertheless they all consisted of suitable habitat with no evidence of overbrowsing or overcrowding, with the exception of Kangaroo Island. Kangaroo Island has had some areas of habitat affected by overbrowsing in the past, although in localised areas this has reduced significantly as a result of the koala management practices that have been

applied for more than a decade (Duka and Masters 2005). Interestingly, the most “pristine” habitat site, at Clagiraba, had the highest prevalence of diseased animals (100%), albeit the sample size was small (only four koalas). Given that the level of habitat disturbance at each of the sites (including Kangaroo Island) did not appear to differ markedly, KoRV represents the most likely explanation for this dichotomy. However, further research on both KoRV and other potential factors affecting disease prevalence and severity is clearly an important priority.

6.5 Conclusions

This study aimed to determine accurately the prevalence and incidence of disease in four SEQ koala populations by conducting thorough health examinations using a standardised veterinary protocol. Overall, the results of the study demonstrated that:

- Disease is threatening the survival of at least some koala populations in SEQ;
- The prevalence of disease in the four SEQ koala study populations is higher than has been estimated in koala populations investigated in previous studies (White and Timms 1994; Dique *et al.* 2003c; Jackson *et al.,* 1999; Lane 2008);
- The incidence of new cases of infertility caused by chlamydial infection in female koalas is high when compared with human females;

- Relying on overt physical signs of disease, detected using usual survey techniques, to estimate disease prevalence in SEQ koala populations will underestimate true disease prevalence by a factor of 5 (approximately);

- Conducting veterinary health examinations of koalas under general anaesthesia by an experienced wildlife veterinarian is essential for obtaining accurate information on koala health;

- Diagnostic techniques, such as ultrasound, are extremely useful for the detection of reproductive disease but will not detect all pathology. Hence the prevalence of infertility in koalas is likely to be somewhat higher than can be detected by ultrasonography and substantially higher than can be detected by palpation alone;

- Chlamydiosis was the most common and important disease affecting koalas in the Brendale, Narangba, East Coomera and Clagiraba populations, however disease that is putatively associated with infection by KoRV was more subtle but regularly encountered.

- The reproductive potential of these koalas, and consequently, the long-term viability of these koala populations, is diminished due to the high levels of infertility caused by chlamydial infection in sexually mature females.

The following chapter discusses the significance of disease in wild koala populations, and provides recommendations and priorities for future research.

CHAPTER 7: General Discussion

The apparently high susceptibility of koalas to diseases, particularly infectious diseases such as chlamydiosis, has long been attributed to “stress” caused by a range of factors including habitat disturbance, nutritional deficiency, overcrowding and “harassment” by predators (Weigler *et al.,* 1988; Melzer *et al.* 2000; Department of Environment and Climate Change NSW 2008). This theory has been expounded in scientific literature and government reports for many years and has now become generally accepted (ANZECC 1998; EPA 2007; Predavec 2008; Natural Resource Management Ministerial Council 2009). However, it has never before been substantiated with accurate health data from wild koala populations that are being subjected to these factors. Certainly, the results from this study do not support the theory: specifically, the one koala population that was subject to overcrowding and possibly nutritional stress (the Kangaroo Island population) had a zero prevalence of detectable disease; while conversely, the koalas living in relatively good habitat in SEQ at low densities (with no evidence of overbrowsing) were affected by high levels of disease. Such results clearly suggest that koalas living in good habitat cannot be assumed to be healthy and hence sustainable.

It is apparent that chlamydiosis and other diseases of koalas, such as leukaemia, do not always produce overt clinical signs, and hence may be missed in health surveys which lack capture of the animals and detailed

veterinary investigations. Such surveys may then report falsely low estimates of disease prevalence in these koala populations. Even with this limitation, some studies report prevalences of chlamydial disease (i.e severe chlamydial disease) in the order of 9-17 % (Weigler *et al.,* 1988; Jackson *et al.,* 1999), which for any other species, including humans, would be considered extreme. Our data suggest that the real prevalence of disease, as detected by thorough veterinary examination, may be in the order of twice the prevalence detected as overt disease (*after* capture but *prior* to veterinary examination). In other words, the true prevalence in the populations studied by Weigler *et al.* (1988) and Jackson *et al.* (1999), in which capture of the koalas occurred, was more likely to be at least 18-34%, if our data are indicative. The true prevalence of disease, if the disease status is determined by looking through binoculars (without disturbance for capture), is likely to be in the order of five times the prevalence detected as overt disease. A study by Dique *et al.* (2003c) detected chlamydial disease in 7.4-12.7% of koalas in bushland and urban surveys conducted in Pine Rivers (in the Moreton Bay LGA), using those methods. Our study found a 41% prevalence of chlamydial disease within the Brendale population, which is also in the Pine Rivers District.

Even thorough veterinary examination may fail to diagnose a proportion of cases, particularly if the disease is in its early stages, subtle or causes minimal structural or functional change to the animal. Hence disease prevalence studies that incorporate thorough veterinary assessment, will also marginally

underestimate actual prevalence. Nevertheless, the application of a robust, repeatable and thorough veterinary assessment protocol is essential if the disease threat in the wild koala populations is to be accurately determined.

Such high prevalence of disease may help to explain recent declines in some SEQ populations. For instance, a recent study by DERM (2009a) reported the decline of koalas in areas of secure habitat in the Koala Coast (SEQ) where minimal habitat loss had occurred. Very limited mortality data were available from these sites to accurately determine the primary cause of their decline, but it was suggested that declines in these bushland sites may be the result of reduced immigration from urban populations (Thompson 2006; DERM 2009a). However, extrapolation of our data to these sites would suggest that disease may also have played a significant role in their decline.

Our hypothesis for the high occurrence of disease in koalas in SEQ, which may also explain the difference in severity and prevalence of disease between northern and southern populations, is that infection with KoRV causes impairment or dysregulation of immune function (Hanger and Loader 2009). The high prevalence and severity of disease found in Queensland and N.S.W koalas, in which KoRV is at 100% prevalence and has endogenised multiple times, contrasts starkly with the situation in the more healthy Victorian and SA koala populations (Bodley and Lynch, pers. comm. cited in Hanger and Loader 2009), in which KoRV appears to be at much lower prevalence, and has not apparently

endogenised in all koalas infected (Tarlinton 2005; G. Simmons pers. comm., 8th May 2010).

The long-term impacts of such a high prevalence of disease in koala populations can perhaps be better understood by noting the similarities with the effects of disease in Tasmanian devil populations. A study by Lachish *et al.* (2007) investigated the prevalence of Devil Facial Tumour Disease (DFTD) in Tasmanian devils, on the Freycinet Peninsula in eastern Tasmania. Of the 448 devils captured, 36 (8%) had characteristic DFTD tumours present. However, as with koalas, disease is often asymptomatic (such as early-stage lesions) and hence may not be detected. Lachish *et al.* (2007) also found one devil population at the Mt William National Park to have a DFTD prevalence of 33% (although there was no indication as to how many devils were sampled in this study). Lunney *et al.* (2008) suggested that once DFTD was in a population, the disease would affect at least 50% of adults within a four-year period. Although disease in koalas tends not to be as overt or invasive as DFTD in devils, it is arguably just as serious a threat to their population viability.

The level and severity of disease in the koala populations examined in our study is almost unprecedented compared to other species, with >50% of koalas in some populations affected. This has ramifications for individual animal welfare, due to the severity of the diseases affecting these koalas, as well as population viability, because of the high level of infertility from chlamydiosis. In addition to

anthropogenic impacts, it is no wonder that SEQ koalas are in rapid decline. When Lunney *et al.* (2008, p. 151) proclaimed that '…extinction in the wild is now regarded as likely for the Tasmanian devil…', it could also be argued that this will be the case for koalas in SEQ, and perhaps further afield.

It has previously been suggested that *Chlamydia* is part of the 'normal biology' of a koala (Carrick 1996). However, regardless of this view, the high prevalence and unusual severity of chlamydiosis, and its consequent infertility in wild SEQ koala populations means that if koala populations are not actively managed to reduce the impacts of this disease, ongoing local extinctions are likely. Whether the impact of chlamydial disease, or disease more generally, has increased over the years is very difficult to determine as there are no historical records that accurately estimate their prevalence and incidence in wild koala populations. Early records of disease outbreaks in the late 19th and early 20th centuries thought to be responsible for the death of "millions" of koalas have been reported (Le Souef and Burrell 1926, cited in Gordon and Hrdina 2005), however objective data for comparison are not available.

7.1 Conclusions and recommendations

This study has confirmed that disease is a critical threatening process impacting four koala populations in SEQ. Chlamydial disease is a significant cause of morbidity and mortality in the koala and also causes a high prevalence of infertility in females. If the data on disease prevalence and incidence derived

from this study is indicative of the situation for koalas more broadly, the reduction in fecundity and death of koalas caused by chlamydiosis (and other diseases), is significantly contributing to their decline. Further investigations into the health of koala populations throughout Queensland are necessary to validate this hypothesis. It is also imperative that we achieve a better understanding of the epidemiology and pathogenesis of chlamydiosis and KoRV-associated disease in koalas and the interaction of these infections. They are clearly significant epizootics, and a failure to better understand them may hamper future conservation efforts. Some recommendations for disease research include:

- Conducting thorough veterinary health examinations under general anaesthesia of all koalas captured for scientific purposes. This maximises the scientific benefit of each capture event, and reduces the welfare impacts on koalas by avoiding unnecessary captures. Assessment of disease impacts across the geographic range of koalas is warranted.

- Further investigation and comparison of disease pathogenesis and severity in northern vs southern koalas and their potential contributing factors (KoRV, habitat factors etc) may improve our understanding of the severe pathology often encountered in northern koalas. Conducting veterinary examinations of koalas in Victorian populations (that are known to have *Chlamydia)* for comparison with SEQ koala populations may be

more informative than comparing with the Kangaroo Island koala population which is ostensibly free of chlamydial disease;

- Development of an immune function test for koalas would assist in better defining the AIDS-like syndrome in koalas, which appears very common, and is devastating for the affected individuals. Further research aimed at defining the aetiology and pathogenesis of the syndrome is clearly warranted, as the KoRV aetiology is only putative.

- Development of an effective chlamydial vaccine for use as a tool to reduce disease impacts may be critical for the sustainable management of some koala populations;

- Development of a population viability model for SEQ koala populations that incorporates our current understanding of both *Chlamydia* and KoRV-associated disease; and,

- In the long-term: establishment of ex situ “insurance” populations (similar to those established to conserve Tasmanian devils) that are comprised of koalas resistant to disease (by virtue of vaccination, specific pathogen exclusion or genetic selection for disease resistance).

Despite this thesis being focused on the significance and magnitude of the disease threat to SEQ koala populations, it in no way intends to divert attention from, or diminish the importance of habitat protection. In fact, the existence of a

significant threatening process that is very difficult to control (disease) makes it even more imperative that habitat is protected effectively (which is relatively easy to control). It is paradoxical, however, that despite the recent government recognition of the seriousness of local koala declines (in SEQ) and in spite of the strong recommendations of koala experts of the need for a moratorium on koala habitat clearing (Qld Premier's Koala Crisis Taskforce, Jon Hanger, pers. comm., 26th February 2009), large areas of koala habitat remain unprotected.

Perhaps the most crucial recommendation arising from this study is that all levels of government must recognise the magnitude of the problems facing koalas in SEQ and the consequences of failure to respond adequately. The application of more funding for disease research is critical, and it must be sufficient to address the important deficiencies in our knowledge. Only then can effective conservation management plans be developed and implemented.

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Appendices

1. Summary of the main clinical findings from the initial veterinary health examination of each koala (prevalence data)

2. Koala examination data sheet

3. Australian Wildlife Hospital decision algorithm for female koalas with reproductive disease

APPENDIX 1: Summary of the main clinical findings from the initial veterinary health examination of each koala (prevalence data)

BRENDALE KOALA POPULATION:

Koala Name	Sex	Approx. Age at 1st Health Examination	Body Condition Score	Main Clinical Findings of 1st Health Examination
Fat Tony	M	8-10 yrs	8	Oral mass of indeterminate aetiology
Nigel	M	7-9 yrs	8	No abnormalities detected (NAD)
Bond	M	10 yrs	7	NAD
Mona	F	4 yrs	7	NAD- 5 month old joey
Tiny Tim	M	8-10 yrs	7	Left renal cyst, white plaques in oro-pharynx, otherwise NAD
Brianna	F	8-10 yrs	5	Reproductive disease, chronic cystitis, mild multifocal sebaceous hyperplasia (esp. ventral skin)
Michael	M	8-10 yrs	7	NAD
Stefan	M	4 yrs	4	Chronic cystitis
Lydia	F	10 yrs	5	Reproductive disease
Red	M	5-6 yrs	5	Cystitis, oxalate nephrosis
Todd	M	8-10 yrs	6	Mild kerato-conjunctivitis
Indie	M	2 yrs	7	Cystitis
Janet	F	6-7 yrs	4	Reproductive disease, chronic cystitis
Shirl	F	10 yrs	8	NAD- 2 month old joey
Pnau	M	6 yrs	6	Chronic cystitis, renal disease
Sumo	M	4 yrs	9	NAD
Jacquie	F	8-10 yrs	5	NAD- 11 month old joey
Kurt	M	7-8 yrs	8	NAD
Lisa	F	10 yrs	7	Reproductive disease
Claude	F	10 yrs	5	Sebaceous hyperplasia multifocal-pouch and inguinal region, otherwise NAD- 7 month old joey
Maggie	F	11 mths	8	NAD
Megan	F	3-4 yrs	7	Reproductive disease, mild cystitis
Miss Radio	F	10 yrs	6	Reproductive disease, chronic cystitis
Al	M	11 mths	7	NAD
Poppy	F	3-4 yrs	7	Reproductive disease
Lex	F	15 mths	8	NAD
Lance	M	2 yrs	8	NAD
Zed	M	2 yrs	8	NAD
Wilbur	M	12 mths	8	NAD
Xena	F	4 yrs	7	Pregnancy (?) or early reproductive disease (?)
Renee	F	12 mths	7	Chlamydial rhinitis
Paula	F	18 mths	4	AIDS (?)
Dom	M	2 yrs	7	NAD
Val	F	10 yrs	5	Reproductive disease

NARANGBA KOALA POPULATION:

Koala Name	Sex	Approx. Age at 1st Health Examination	Body Condition Score	Main Clinical Findings of 1st Health Examination
Aria	F	5-6 yrs	7	NAD- 1 month old joey
Bec	F	6-8 yrs	6	NAD- 9 month old joey
Christine	F	5-6 yrs	7	NAD- 10 month old joey
Greg	M	10 mths	8	NAD
Dion	M	10 yrs	6	Unilateral kerato-conjunctivitis
Edna	F	10 yrs	7	Reproductive disease, cystitis
Frankie	F	2-3 yrs	8	Reproductive disease
Felix	F	5-7 yrs	8	Reproductive disease
Gus	M	7-9 yrs	7	Generalised seborrhoeic dermatitis with otitis externa
Horatio	M	2 yrs	7	NAD
Igor	M	7-8 yrs	6	NAD
Jasmine	F	7-8 yrs	6	NAD- 10 month old joey
Marty	M	18 mths	8	NAD
Kaia	F	5-6 yrs	8	Reproductive disease
Linda	F	14-16 mths	8	NAD
Mandy	F	18 mths	7	NAD- pregnant
Kevin	M	4 yrs	8	Subclinical cystitis
Natashi	F	4-5 yrs	3	Reproductive disease, cystitis
Liam	M	2 yrs	8	Subclinical cystitis
Stratty	M	2 yrs	8	NAD
Mac	M	12 mths	8	NAD
Kate	F	18 mths	8	NAD- 1month old joey

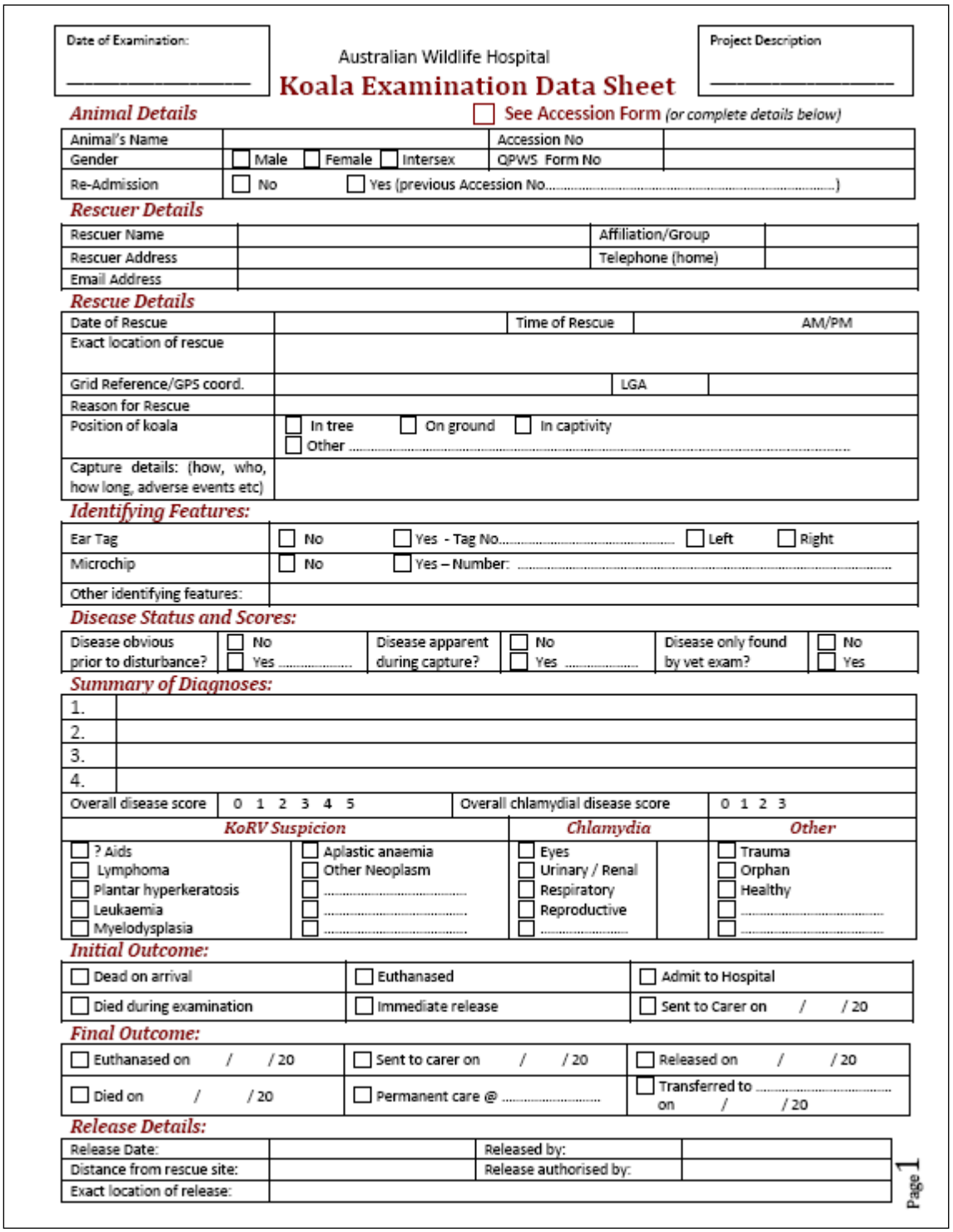
EAST COOMERA KOALA POPULATION:

Koala ID	Sex	Approx. Age at 1st Health Examination	Body Condition Score	Main Clinical Findings of 1st Health Examination
John Junior	M	2-3 yrs	8	NAD
Echo	F	18 mths	6	Non-specific illness
David	M	4-5 yrs	9	NAD
Kiwi Sarah	F	2-3 yrs	7	NAD
Louise	F	7-8 yrs	8	Reproductive disease
Warren	M	11-12 mths	8	NAD
Alicia	F	7-8 yrs	7	NAD- <1 week old joey
Steve	M	7-8 yrs	8	NAD
James	M	3 yrs	7	Mild acute cystitis
Althena	F	7-8 yrs	6	Reproductive disease, unilateral kerato-conjunctivitis, cystitis
Caitlin	F	12 mths	7	NAD
Connor	M	18 mths	2	GI candidiasis, ill-thrift, non-regenerative anaemia, AIDS (?)
Gail	F	7-8 yrs	6	NAD- 1.5-2 month old joey
Jo	F	16-18 mths	8	NAD
Carmel	F	3 yrs	7	NAD- 8-9 month old joey
Jason	M	8-9 mths	8	NAD
Kim	F	5-6 yrs	4	NAD- <2 weeks old joey
Donna	F	9 yrs	7	NAD- 1.5-2 month old joey
Sharon	F	3 yrs	7	NAD- 2-3 week old joey
Angela	F	5-6 yrs	3	DOA- reproductive disease, immunodeficiency disorder (?), severe regenerative anaemia
Emma	F	8 yrs	6	Reproductive disease
Robin	F	2 yrs	7	NAD- 2 month old joey
Avi	F	12-14 mths	8	NAD
Marg	F	4-5 yrs	6	Old healed injury to left forepaw (Digits III, IV & V missing), otherwise NAD
Mr. Mayor	M	2 yrs	8	NAD
Lili	F	3-4 yrs	8	NAD- 1.5-2 month old joey
Dale	M	8-10 yrs	7	Septic arthritis left shoulder, sub-acute cystitis
Hamid	M	8 yrs	6	Chronic cystitis
Chris	M	2-3 yrs	6	NAD
Tim	M	15-18mths	8	NAD
Kevin	M	2 yrs	6	NAD
Glen	M	8-10 yrs	8	Fungal infection (scrotum), ear mites
Peter	M	5 yrs	7	Mild cystitis, prostatic cyst (?)
Maree	F	8-10 yrs	6	Reproductive disease, cystitis

CLAGIRABA KOALA POPULATION:

Koala Name	Sex	Approx. Age at 1st Health Examination	Body Condition Score	Main Clinical Findings of 1st Health Examination
Kellie	F	4 yrs	8	Reproductive disease, cystitis, kerato-conjunctivitis
Ned	M	5 yrs	8	Kerato-conjunctivitis, cystitis
Graeme	M	7-8 yrs	3	Poor body condition, cystitis, gastrointestinal candidiasis, trypanosomiasis, regenerative anaemia, AIDS (?)
Andrew	M	3 yrs	3	DOA- AIDS (?), mild non-suppurative prostatitis

APPENDIX 2: Koala examination data sheet



Date of Examination: _____

Australian Wildlife Hospital

Koala Examination Data Sheet

Project Description _____

Animal Details

☐ See Accession Form *(or complete details below)*

Animal's Name		Accession No	
Gender	☐ Male ☐ Female ☐ Intersex	QPWS Form No	
Re-Admission	☐ No ☐ Yes (previous Accession No.....)		

Rescuer Details

Rescuer Name		Affiliation/Group	
Rescuer Address		Telephone (home)	
Email Address			

Rescue Details

Date of Rescue		Time of Rescue	AM/PM
Exact location of rescue			
Grid Reference/GPS coord.		LGA	
Reason for Rescue			
Position of koala	☐ In tree ☐ On ground ☐ In captivity ☐ Other		
Capture details: (how, who, how long, adverse events etc)			

Identifying Features:

Ear Tag	☐ No	☐ Yes - Tag No.....	☐ Left	☐ Right
Microchip	☐ No	☐ Yes – Number:		
Other identifying features:				

Disease Status and Scores:

Disease obvious prior to disturbance?	☐ No ☐ Yes	Disease apparent during capture?	☐ No ☐ Yes	Disease only found by vet exam?	☐ No ☐ Yes

Summary of Diagnoses:

1.	
2.	
3.	
4.	

Overall disease score	0 1 2 3 4 5	Overall chlamydial disease score	0 1 2 3

KoRV Suspicion		Chlamydia	Other
☐ ? Aids	☐ Aplastic anaemia	☐ Eyes	☐ Trauma
☐ Lymphoma	☐ Other Neoplasm	☐ Urinary / Renal	☐ Orphan
☐ Plantar hyperkeratosis	☐	☐ Respiratory	☐ Healthy
☐ Leukaemia	☐	☐ Reproductive	☐ .....
☐ Myelodysplasia	☐	☐	☐ .....

Initial Outcome:

☐ Dead on arrival	☐ Euthanased	☐ Admit to Hospital
☐ Died during examination	☐ Immediate release	☐ Sent to Carer on / / 20

Final Outcome:

☐ Euthanased on / / 20	☐ Sent to carer on / / 20	☐ Released on / / 20
☐ Died on / / 20	☐ Permanent care @	☐ Transferred to ..... on / / 20

Release Details:

Release Date:		Released by:	
Distance from rescue site:		Release authorised by:	
Exact location of release:			

Page 1

Distant Examination:

Demeanour	☐ B.A.R. ☐ Depressed ☐ Excited ☐ Moribund
Behaviour	☐ Normal ☐ Abnormal ....
Posture	☐ Normal ☐ Abnormal ....
Gait	☐ Normal ☐ Abnormal ....
Symmetry	☐ Normal ☐ Abnormal ....
Breathing	☐ Normal ☐ Shallow ☐ Rapid ☐ Laboured
Coat	☐ Normal ☐ Abnormal ....
Discharges	☐ Nil ☐ Present ....
Wounds/Bleeding	☐ Nil ☐ Present ....
Other lesions:	☐ Nil ☐ Present ....
Abdomen:	☐ Normal ☐ Bloated ☐ Sunken

General Physical Examination:

Anaesthesia	**Induction Anaesthetic Agent**..... Dose ..... Route: ☐ i.m. ☐ i.v. ☐ facemask ☐ Tube **Maintenance Anaesthetic Agent**..... Dose ..... Route: ☐ i.m. ☐ i.v. ☐ facemask ☐ Tube
Vital Signs	**Mucous Membrane**: ☐ Pink ☐ Pale ☐ Cyanotic ☐ Red HR ..... RR ..... Rectal Temp .....° C CRT ..... SpO_2 ..... **Pulse**: Rate ..... Rhythm: ☐ Regular ☐ Occasional arrhythmia ☐ Frequent/constant arrhythmia Amplitude: ☐ Normal ☐ Increased ☐ Decreased Tone: ☐ Normal ☐ Increased ☐ Decreased
Hydration	☐ Normal ☐ <5% dehydrated ☐ 5-10% dehydrated ☐ > 10% dehydrated
Weight / Age / Body Score	Body Score ..... / 10 Weight .....kg Age .....yrs/mths
Tooth Wear:	Class ..... Estimated Age .....years
Chest auscultation:	Heart..... Lungs ..... Other respiratory findings: ☐ Normal ☐ Abnormal ....
Neurological	☐ Normal ☐ Abnormal ....

General Physical Examination (continued):

Musculoskeletal	☐ Normal ☐ Abnormal		
Head / Mouth	Head Symmetry ☐ Normal ☐ Abnormal		
	Ears ☐ Normal ☐ Abnormal		
	Nares ☐ Normal ☐ Abnormal		
	Lips ☐ Normal ☐ Abnormal		
	Tongue ☐ Normal ☐ Abnormal		
	Teeth ☐ Normal ☐ Abnormal		
	Cheek Pouches ☐ Normal ☐ Abnormal		
	Gingiva ☐ Normal ☐ Abnormal		
	Fauces ☐ Normal ☐ Abnormal		
	Palate/tonsils ☐ Normal ☐ Abnormal		
	Pharynx ☐ Normal ☐ Abnormal		
	Larynx ☐ Normal ☐ Abnormal		
Eyes:	**LEFT**	**RIGHT**	
Periorbital skin:	☐ Normal ☐ Alopecic ☐ Pigmented	☐ Normal ☐ Alopecic ☐ Pigmented	
Eyelids:	☐ Normal ☐ Other	☐ Normal ☐ Other	
Palpebral fissure	☐ Normal ☐ ↓ Scarred ☐ ↑	☐ Normal ☐ ↓ Scarred ☐ ↑	
Conjunctiva:	☐ Normal ☐ Proliferated 1 2 3	☐ Normal ☐ Proliferated 1 2 3	
	Other.....	Other.....	
Nictitating:	☐ Normal ☐ Prolapsed 1 2	☐ Normal ☐ Prolapsed 1 2	
Sclera:	☐ Normal ☐ Other	☐ Normal ☐ Other	
Cornea:	☐ Normal ☐ Abnormal	☐ Normal ☐ Abnormal	
Opacity:	☐ Clear ☐ Mild ☐ Marked	☐ Clear ☐ Mild ☐ Marked	
Iris & Pupil:	☐ Normal ☐ Abnormal	☐ Normal ☐ Abnormal	
Discharge:	☐ Nil ☐ Serous ☐ Purulent Amt: 1 2 3	☐ Nil ☐ Serous ☐ Purulent Amt: 1 2 3	
Description:	.....	.....	
	.....	.....	
Chlamydia eye	.....	.....	
disease score:	0 1 2 3	0 1 2 3	
Coat:	Colour: ☐ Light Grey ☐ Brown ☐ Dark Grey		
	Structure: ☐ Normal ☐ Sparse ☐ Clumped/ irregular		
	Texture: ☐ Normal ☐ Greasy ☐ Dry		
	Other:		
Skin/ External parasites:	☐ Normal ☐ Abnormal		
Lymph Nodes	Rostral mandibular ☐ Normal L R ☐ Enlarged L R ☐ Small L R		
	Facial ☐ Normal L R ☐ Enlarged L R ☐ Small L R		
	Mandibular ☐ Normal L R ☐ Enlarged L R ☐ Small L R		
	Superficial cervical ☐ Normal L R ☐ Enlarged L R ☐ Small L R		
	Axillary ☐ Normal L R ☐ Enlarged L R ☐ Small L R		
	Inguinal ☐ Normal L R ☐ Enlarged L R ☐ Small L R		
	Notes		
	.....		

General Physical Examination (continued):

Abdominal Palpation	Abdominal Fill ☐ Normal ☐ Abnormal ..... Stomach Fill ☐ 1 – (empty) ☐ 2 (½ full) ☐ 3 (full) Stomach Consistency ☐ Normal (firm) ☐ Soft ☐ Bloated ☐ ..... Distal colon ☐ Pellets ☐ Empty/ pellets not palpated Proximal colon/caecum ☐ Normal ☐ Abnormal ..... Notes /Other abdominal lesions: ....
Chest Palpation:	Clavicle: ☐ Normal ☐ Abnormal ..... Ribs: ☐ Normal ☐ Abnormal .....
Limbs and Joints	Left Forearm ☐ Normal ☐ Abnormal ..... Left Paw/digits: ☐ Normal ☐ Abnormal ..... Left Hindleg ☐ Normal ☐ Abnormal ..... Left Foot/digits: ☐ Normal ☐ Abnormal ..... Right Forearm ☐ Normal ☐ Abnormal ..... Right Paw/Digits ☐ Normal ☐ Abnormal ..... Right Hindleg ☐ Normal ☐ Abnormal ..... Right Foot/digits: ☐ Normal ☐ Abnormal .....
Scrotum / Pouch	☐ Normal ☐ Abnormal ....
Scent Gland/ Mammary Glands	☐ Active ☐ Inactive ☐ Abnormal ....
Cloaca/Clitoris/ Penis	☐ Normal ☐ Abnormal ....
Dirty Tail Score	*(circle score)* Rump stained: 0 *(no staining)* 1 *(mild)* 2 *(marked)* Rump wetness: 0 *(dry)* 1 *(damp)* 2 *(dripping wet)* Cloaca: 0 *(normal)* 1 *(red/inflamed – slightly protruding)* 2 *(marked protrusion – no ulceration)* 3 *(2 + ulceration/pseudomembrane)* Rump: 0 *(normal)* 1 *(inflamed skin – no decubital ulcers)* 2 *(decubital ulcers or erosions from urine scalding)* Dysuria: 0 *(nil/not observed)* 1 *(apparent discomfort when urinating and/or vocalisation)* Total Score: ..... / 10

Other Koala Details, History or Previous Treatments:

Procedures Performed:

CLINICAL PATHOLOGY

☐ ***Blood*** ☐ In-House ☐ External (Idexx/other.....) PCV_____% TS_____g/litre
Stain: ☐ Giemsa ☐ Diff-Quick ☐ Other Slide Kept? ☐ Y ☐ N
Smear:

☐ ***Bone Marrow*** Stain: ☐ Giemsa ☐ Diff-Quick ☐ Other Slide Kept? ☐ Y ☐ N
Collection Site: ☐ Iliac Crest L R ☐ Other.....
Smear:

☐ ***Abdominal Aspirate*** Stain: ☐ Giemsa ☐ Diff-Quick ☐ Other Slide Kept? ☐ Y ☐ N
Smear:

☐ ***Faecal Analysis***

Gross Examination:	Shape	☐ Normal	☐ Abnormal .....
	Size	☐ Normal	☐ Abnormal .....
	Consistency	☐ Normal	☐ Abnormal .....
	Fragment Size	☐ Normal	☐ Abnormal .....
Tests:		☐ Float ☐ Wet Prep	☐ Stain

☐ ***Urinalysis*** Collection Method: ☐ Cysto ☐ Catheter ☐ Free catch
☐ Urinalysis ☐ Urine Sediment Smear ☐ USG .....
Smear:
Urinalysis: pH: Pr: Hb/Mb: Gluc: Other:

☐ ***Chlamydia (Clearview) Test*** ☐ Test site ..... ☐ + ☐ -
☐ Test site ..... ☐ + ☐ -
☐ Test site ..... ☐ + ☐ -
☐ Test site ..... ☐ + ☐ -
☐ Test site ..... ☐ + ☐ -

Rate positives on a scale of 1 – 4 using the following chart:
4: very strong, ≥ +ve control
3: strong +ve but < +ve control
2: weak +ve, easily seen
1: very weak, barely perceptible

☐ ***Radiographs***

☐ ***Other Diagnostic Aids***

ULTRASOUND

Bladder

☐ Normal ☐ Abnormal .....

Urine/Lumen: ☐ Clear ☐ Flocculent echo ☐ Lumenal cast/other

Wall Thickness at point of widest lumen (transverse section)

	LEFT	LUMEN	RIGHT
Horizontal			
Vertical			
Left Diagonal			
Right Diagonal			

Other remarks:

.....

.....

Female Reproductive Tract RIGHT

☐ Normal ☐ Pregnant ☐ Oestrus/mucosal hyperplasia

☐ Abnormal .....

.....

Female Reproductive Tract LEFT

☐ Normal ☐ Pregnant ☐ Oestrus/mucosal hyperplasia

☐ Abnormal .....

.....

Prostate

☐ Examined ☐ Not examined ☐ Poor image

☐ Normal ☐ Abnormal .....

.....

Frontal plane diameter .....mm (at widest diameter)

		Left	*Right*
Kidneys	Overall Structure:	☐ Normal ☐ Abnormal	☐ Normal ☐ Abnormal ....
	Measurements:	Length .....mm Vert. Diameter .....mm Horiz. Diameter .....mm	Length .....mm Vert. Diameter .....mm Horiz. Diameter .....mm
	Parenchyma echo:	☐ Normal ☐ Hyperechoic ☐ Hypoechoic	☐ Normal ☐ Hyperechoic ☐ Hypoechoic
	Renal pelvis:	☐ Normal ☐ Dilated	☐ Normal ☐ Dilated
	Ureter:	☐ Normal ☐ Dilated	☐ Normal ☐ Dilated
	Lesions:	☐ Nil ☐ Present	☐ Nil ☐ Present ....
UGT Chlamydia disease score	0 1 2 3 4		

Notes/Treatment/Comments

Research Samples Collected

Sample	Purpose	Site	For whom	Stored/Sent	Date	Collector

Electronic data files:

Type	Number	Details	Location
Photos			
Sonograms			

APPENDIX 3: Australian Wildlife Hospital decision algorithm for female koalas with reproductive disease