

Infectious Disease in Koalas: Implications for Conservation

Jon Hanger and Jo Loader

Australian Wildlife Hospital

A program of Australia Zoo Wildlife Warriors Worldwide Ltd

Abstract

Infectious disease in koalas is undoubtedly one of the critical threatening processes contributing to their dramatic population declines in Queensland and New South Wales. Two of the most important infections: koala retrovirus (KoRV) and *Chlamydia* are still relatively poorly understood, although they are the subject of active research at a number of universities. We still have much to learn about their associated disease pathogenesis, the interaction between these agents, their ecological impact and distribution. This information is important not only to assist in our efforts to treat affected koalas, but also to add weight to our arguments for greater protection of habitat. Our concerns about the impact of infectious disease in koalas continue to be validated by both koala admissions to the Australian Wildlife Hospital, and also our investigations of koala health in a number of wild koala populations. We will present an overview of the prevalence of disease in some populations that we are studying in south-east Queensland (SEQ) and the implications for koala conservation generally.

Introduction

Although the loss of koala habitat and consequent decline of the species has been a concern of conservationists for many years (Melzer *et al.*, 2000), the actual magnitude of the decline and severity of the situation is just starting to be accepted by some of the regulatory authorities. Similarly, the high prevalence of disease in koalas has been recognised for well over a century, but its importance as a key threatening process has only recently received acknowledgement. Despite this, regulators and legislation at all levels of government continue to fail to address koala declines in any meaningful way. Although the principles and intent of ecologically sustainable development (ESD) are espoused commonly in government policy and statute, their application (in terms of effective and measurable outcomes) is virtually non-existent. As a consequence, the localised extinction of koalas is commonplace, and the tipping points for wider, or regional, extinction seem to be looming.

There is no question that the protection of remaining koala habitat and restoration of effective corridors between habitat remnants are the most critical things that must be done to conserve

koalas. In focusing on the prevalence and impact of disease in this paper, our intent is not in any way to detract from the importance of habitat issues. However, if the critical threat that disease plays in population decline and extinction is not considered in population modeling, conservation planning and prioritisation of research funding, it will be a recipe for disaster. We will focus our discussion on the two most important infectious agents in koalas: koala retrovirus and *Chlamydia*, because arguably they are having the greatest impact on wild koala populations.

Koala Retrovirus (KoRV)

Retroviruses are fragile organisms that are able to integrate their own genetic sequences into the DNA strands of the cell that they have infected. In doing so, they are able to hijack host cell processes to produce many more virus particles; in effect, turning the host cell into a virus factory. The genomes of retroviruses contain regions that strongly promote the transcription of the viral DNA sequence by the host cell. Whilst this process is designed to promote the production of virus particles, it may also “accidentally” switch on genes of the host cell, and this in turn may cause cancer. Conversely, the viral DNA may disrupt a host cell gene, leading to the death of the cell, or altered cell function. Simplistically, the clinical syndromes that are observed in koalas reflect these basic molecular processes.

The following conditions in koalas are thought to be caused by infection with KoRV:

1. Leukaemia (a cancer of the blood forming cells)
2. Myelodysplasia (abnormalities in production of blood cells)
3. Immunodeficiency syndrome (koala “AIDS”)
4. Other cancers, including lymphoma, osteochondroma, mesothelioma.

A range of other conditions may be associated with KoRV infection in koalas, but it is beyond the scope of this paper to list them all. The relationship between KoRV infection and chlamydial disease is discussed below.

Epidemiology of KoRV

Infection with KoRV appears to be close to 100% in Queensland and NSW koalas, and somewhat less than that in Victorian and South Australian populations, based on recent work by the KoRV Research Group at the University of Queensland. Interestingly, the incidence of the conditions listed above is essentially zero in Victorian koalas (Bodley, K. 2009 pers. comm., 12 May), based on approximately 1500 wild koala examinations (throughout Victoria) each year.

All koalas tested by Rachel Tarlinton during her PhD studies were viraemic with KoRV (virus particles present in the blood), but the level of viraemia varied considerably between koalas (Tarlinton 2006). She also demonstrated that there was a significant correlation between a high level of viraemia and development of neoplastic diseases such as leukaemia (Tarlinton *et al.*, 2005).

Koala retrovirus has endogenised in koalas in Queensland and New South Wales. That is: it has infected germ line cells (spermatozoa or oocytes) and is transmitted genetically (by inheritance) from parents to offspring. Although this is a known mechanism of transmission, KoRV may also spread from koala to koala (horizontal spread) by close contact, and from infected mothers to their joeys via the milk, similarly to the way that many other viruses spread (Hanger 1999). Whether KoRV can be transmitted by biting insects has yet to be determined.

Where it came from, when it arrived, and how its impact will play out in the koala population, we still do not know.

KoRV and Chlamydiosis

It seems a reasonable hypothesis that *severe* chlamydial disease is more common in koalas because of the consequences of KoRV infection, specifically its effects on immune responses, and that normal immune function (in koalas without AIDS) results in more minor chlamydial pathology (similar to that seen in chlamydiosis in other species). This contention is supported by the generally minor nature of chlamydial pathology in southern koalas (Bodley, K. 2009 pers. comm. 12 May), where KoRV is less prevalent. Although Tarlinton's work suggested an association between chlamydial disease and high KoRV viraemia, the statistical significance of that association has not yet been demonstrated. Current research, which is using a larger and more sophisticated data set, may clarify the situation.

Chlamydia

Chlamydial infection in koalas is common and affects most mainland and many island koala populations. Prevalence of *infection* and prevalence of *disease* varies between populations, but severe disease is more common in northern koalas (Qld and NSW) than in southern koalas (Vic and SA), irrespective of prevalence of infection (Timms 2000). Severe cystitis, keratoconjunctivitis and active reproductive tract disease is common in northern koalas, and very uncommon in southern koalas (Bodley, K. & Lynch, M. 2009 pers. comm. 12 May). Although cystic change and fibrosis of the reproductive tract leading to infertility is common in southern koalas, the severe debilitating pathology seen commonly in northern koalas is not.

Chlamydial disease in southern koalas is more consistent (in terms of severity of pathology) with chlamydial disease in humans (*Chlamydia trachomatis*) and other species (Timms, P. 2009,

pers. comm. 12 May). That is: it is of a relatively minor nature and rarely causes debilitating disease. In contrast: severe chlamydial disease seen commonly in northern koalas is quite unusual, compared with other species.^a

Chlamydial infection in koalas is commonly associated with ocular infections (keratoconjunctivitis), urinary tract infection (cystitis and nephritis) and reproductive tract disease (prostatitis, metritis, pyometron, cyst formation and fibrosis). Infertility is a common sequel to reproductive tract infection in both northern and southern koalas. Less common manifestations include respiratory infections and granulomas, and the epizootic koala “flu” is thought to result from acute infection, possibly with a strain of *Chlamydia pneumoniae* (Nicholson, V. 2009, pers. comm. 1 May).

There are many other papers and articles that describe in detail chlamydial epidemiology, pathology, and treatment in koalas. It is beyond the scope of this paper to provide such detail. The most important points regarding chlamydial disease in koalas are:

1. It is common and widespread in most koala populations.
2. Severe debilitating disease is more common in koalas in Qld and NSW than in southern states.
3. KoRV co-infection probably increases the risk of serious disease.

Prevalence of infection versus prevalence and incidence of disease

Koalas can be infected with *Chlamydia* and also KoRV without detectable disease, hence prevalence of *disease* is less than prevalence of *infection*. Many studies have reported *Chlamydia* infection prevalence in wild koala populations using a variety of detection methods (Devereaux *et al.*, 2003; Timms 2000; White & Timms 1994), and some have also described the prevalence of overt disease (Jackson *et al.*, 1999). However, with the exception of a study by Jones (2008), no wild koala population health surveys, to date, have routinely used techniques such as ultrasound and cystocentesis to detect pathology which might otherwise be inapparent. These techniques are essential in the routine assessment of koalas for urogenital tract disease. Hence, many of the aforementioned studies are likely to have underestimated disease prevalence due to insensitivity of detection methods. Furthermore, some koalas, which, at the time of examination show no signs of disease, but are *Chlamydia* positive, may progress to clinically diseased in time.

^a This generalisation does not apply to disease resulting from cross-species transmission of some types of *Chlamydia*, such as psittacosis in humans, which is caused by infection with avian strains of *Chlamydia psittaci*, and can cause severe and occasionally fatal infections.

Prevalence of Disease in Two SEQ Koala Populations

One of our current research projects is investigating the prevalence and incidence of disease in two koala populations in the Moreton Bay Regional LGA, SEQ, one in the suburb of Brendale, the other in Narangba (hereafter referred to as Populations A and B respectively). In an attempt to capture all of the koalas in each population, comprehensive searches of both sites were undertaken. After capture, each koala was transported to the Australian Wildlife Hospital and subjected to a thorough clinical assessment using a standardised veterinary protocol (Appendix 1). This included a complete physical examination under general anaesthesia, and a range of ancillary diagnostic tests designed to detect most known conditions in koalas. Clinical data were recorded for 25 koalas in Population A (13 male, 12 female) and 17 koalas in Population B (7 male, 10 female).

A high prevalence of chlamydiosis was found in both populations: 44% (5 male, 6 female) and 41% (2 male, 5 female) of koalas were found to have chlamydial disease in Population A and B, respectively (Table 1). Despite the high level of chlamydial disease in both populations, of the koalas with detectable illness, 45% (1 male, 4 female) of Population A, and 57% (1 male, 3 female) of Population B exhibited no *overt* signs of disease (Table 2). These results indicate that without thorough investigative veterinary techniques, subclinical disease would in some cases have remained undetected.

	Population A	Population B
Total No. of Koalas	25 (13 male: 12 female)	17 (7 male: 10 female ^b)
Healthy Koalas (no detectable disease)	52% (7 male: 6 female)	59% (5 male: 5 female)
Diseased Koalas Requiring Veterinary Intervention	48% (6 male: 6 female)	41% (2 male: 5 female)
Chlamydial Disease	44% (5 male: 6 female)	41% (2 male: 5 female)
No. of Females with a Joey	33% (4 females)	40% (4 females)
Euthanased/Died due to Severity of Disease	32% (3 male: 5 female)	11% (0 male: 2 female)

Table 1: Koala Health Summary and Outcomes of Population A and Population B

^b This number includes one new case of disease detected at the second (6 month) health check

Of the female koalas, 50% (6/12) from Population A, and 50% (5/10) from Population B had reproductive tract disease. Of these, only 33% (2/6) of Population A and 40% (2/5) of Population B demonstrated overt physical signs of chlamydial disease, and those signs were referable to cystitis (the koalas had “dirty-tail”)(Table 2). The remainder of cases required palpation and/or ultrasonography to make the diagnosis. In other words, overt signs of reproductive tract disease are rare, and sometimes cystitis is also not apparent as dirty-tail.

Chlamydial Disease	Population A		Population B	
	Males	Females	Males	Females
Overt Chlamydial Disease	4/5 (80%)	2/6 (33%)	1/2 (50%)	2/5 (40%)
Subclinical Disease	1/5 (20%)	4/6 (66%)	1/2 (50%)	3/5 (60%)
Conjunctivitis only	1/5 (20%)	0/6 (0%)	1/2 (50%)	0/5 (0%)
Cystitis only	4/5 (80%)	0/6 (0%)	1/2 (50%)	0/5 (0%)
Multifocal Chlamydial Disease	0/5 (0%)	4/6 (66%)	0/2 (0%)	2/5 (40%)
Reproductive Tract Disease (likely to be infertile)	0/5 (0%)	6/6 (100%)	0/2 (0%)	5/5 (100%)
Total Koalas with Chlamydiosis	5	6	2	5^c
	TOTAL (POP A)= 44% 11 out of 25 individuals in population A with chlamydiosis		TOTAL (POP B)=41.2% 7 out of 17 individuals in population B with chlamydiosis	

Table 2: Summary of Koalas with Chlamydial Disease in Population A and Population B

Finally, the definitive indicator of fertility (production of a joey) was shown by only 33% (4) and 40% (4 - one of which subsequently became infertile) of females of breeding age in populations A and B respectively. In population A, one female was not yet of breeding age, and one had a lesion on ultrasound which could not, at the time, be distinguished from a pregnancy. That female has not since produced a joey, so, in retrospect, the lesion is likely to have been a pathological change. In population B, two females were not of breeding age.

^c This number includes one new case of disease detected at the second (6 month) health check.

Our disease results contrast starkly with those of Lane (2008), who performed population surveys and disease prevalence estimates in the Pine Rivers, Caboolture and Redcliffe Shires in 2007 (in which our study populations are located). The primary tool for detection of disease presence in that study was a pair of binoculars, and, predictably, their estimate of disease prevalence was low (approximately 10% of “urban” and “bushland” koalas combined). The fact that they reported low observed disease prevalence, without qualifying that their sensitivity for detection of disease was very limited, means that *true* disease prevalence may be (and has been) misinterpreted as also being low.

For this reason prevalence studies that rely on the observation of overt signs of chlamydial disease will invariably underestimate disease prevalence in the sample group. Koalas that have subclinical reproductive tract pathology are clearly of significance to disease prevalence studies, and to the assessment of population health. In most cases, these females will be infertile, population fecundity will be affected proportionately with prevalence, and therefore impacts on population viability can be significant, as demonstrated by our data.

Incidence of disease

With disease in wild populations it is often useful to investigate the *incidence* of disease, that is: the number of new cases per population over a given time period. For example: in our koala disease study described above, the incidence of new cases of infertility in female koalas was 10% per year (one new case among the 10 female koalas during the year). For chlamydiosis, a chronic disease, data about both *incidence* (of new cases) and *prevalence* (of present disease) are useful: the first give us an estimate of what might happen to a population over time, the latter, a snapshot or cross-sectional view of the population at a given moment in time. In contrast, the *incidence* of some acute KoRV-associated diseases (such as leukaemia) is more important than the *prevalence*, because they kill koalas relatively quickly, therefore the chance of detecting affected animals in a cross-sectional study is small. With KoRV-associated AIDS, prevalence data is just as important, because it (probably) takes longer to kill koalas, and information on how many koalas in a population have AIDS at any given point in time, is useful.

If we are to be well-informed about the impacts of disease on koala populations and the likely consequences for survival (or extinction), then we need to gather data on both incidence (by longitudinal studies – over time) and prevalence (by thorough cross-sectional studies). Such studies, if conducted on wild populations, generally require radio-telemetry (hence are labour-intensive) and experienced veterinary support (for health examinations and mortality investigation), and are therefore expensive. Needless to say, it is high time that both State and Federal governments got serious about funding such research on koalas, given our embarrassing lack of knowledge on the topic.

Implications of disease for population survival

Infectious disease may result in a range of impacts on both the individuals affected and the population as a whole. Impacts on individuals may be insignificant, minor, or serious and life-threatening. Similarly, impacts on populations may be insignificant, minor, or may lead ultimately to extinction. Those impacts are dependent upon factors such as prevalence of infection, incidence of new infection, pathogenicity of the organism, modes of transmission, population dynamics, and genetic diversity, to name a few.

To put it simply:

Impacts on the individual:

- Insignificant
- Minor debility
- Infertility
- Major debility
- Death

Impacts on the population

- Insignificant
- Reduced fecundity
- Population decline
- Increased vulnerability to extinction
- Inevitable extinction

It is our view that both KoRV and *Chlamydia* are highly significant in both their potential impacts on individuals, and on populations. We believe that, in respect of Qld and NSW koala populations, both should be considered critical threats to long-term viability. It is likely that it is only a matter of time before the same can be said of the Victorian and South Australian koala populations.

Our data, as well as that published by other researchers, suggest that prevalence of disease has little to do with habitat quality. It is common dogma in koala conservation circles that “habitat stress” leads to disease; that high levels of disease are largely due to loss of habitat, urbanisation and consequent stress and “crowding” of surviving koalas. We suggest that this is at best an oversimplification, and is certainly not substantiated by hard data. Although some believe that this paradigm promotes the imperative for habitat conservation (which it may well do), it nevertheless implies that if we conserve habitat, the impact of disease will be abated or abolished. Furthermore, it naturally leads to the assumption that disease will not be a threat to

population viability in large habitat fragments. It is our view that this is a dangerous assumption, and probably not true.

Implications of disease for conservation planning and management

Factoring Disease into PVAs

Given the high level of disease in koalas and the prevalence of infertility in female koalas it is crucial that these factors are included in population viability analyses (PVAs). The variability in prevalence of infection and disease between koala populations means that accurate factoring of these into PVA equations requires more thorough assessment of prevalence across metapopulations. As we have mentioned before, many published infection and disease surveys have probably underestimated disease prevalence due to limited veterinary investigation. It is important to note, that, even with ultrasound imaging of the female reproductive tract, early or subtle lesions may not be detected. In other words, a proportion of koalas are probably infertile even though lesions are not apparent using advanced techniques. Disease models used for PVAs must therefore account for this.

Modeling for the impacts of KoRV are somewhat more difficult: the organism is at 100% prevalence (for argument's sake); we have no definitive predictors of disease; arguably, the most important condition (AIDS) is often a presumptive and sometimes tenuous diagnosis; the molecular epidemiology, transmission and pathogenesis of disease are poorly understood; and the impacts, at a population level, have not, to date, been measured. Needless to say, the whole KoRV situation is very worrying from a species conservation point of view, not least because it is difficult to model.

Controlling KoRV and Chlamydia impacts

At the individual level, clinically controlling the impacts of KoRV, in terms of disease production or progression has not been attempted to our knowledge. Feline Leukaemia Virus (FeLV) infection in cats, which causes a similar constellation of clinical consequences in cats as KoRV infection of koalas, is a good example for comparison. Control of FeLV infection in cats relies on prevention by vaccination, and removal of persistently infected cats from situations which allow exposure of uninfected cats. Treatment of persistently viraemic cats with the anti-retroviral drug AZT/zidovudine (Retrovir®) or interferon- α has been shown to have some clinical benefit experimentally. Otherwise, treatment of such cats relies upon treatment of opportunistic infections and other measures to prevent these infections from occurring. Whether these benefits can be translated to koalas that are viraemic with, or affected by KoRV remains to be tested, and does not realistically provide solutions to conservation issues at this stage. Whether vaccination of koalas can reduce the incidence of disease (in infected animals) or

prevent infection (of uninfected animals) are certainly topics worthy of research prioritisation, because they may have some benefits in terms of conservation at the population level.

At present, one interesting hypothesis that remains to be tested is: that female koalas with high KoRV titres tend to give rise to offspring that also have high KoRV titres; and that the converse is also true. If this is proven to be true, and the assumption that high KoRV titres are associated with increased risk of disease is also true, then reduced incidence of KoRV-associated disease could be achieved by selectively breeding female koalas with low KoRV titres. The option of breeding KoRV-free koalas (at least in Qld and NSW) is an opportunity that appears to have passed us by some time ago.

Control of chlamydial infection in individual koalas is somewhat more effective: the infection can be essentially eliminated by appropriate antibiotic therapy (as it can in other species), but the actual pathology itself is more difficult, and sometimes impossible to treat. Consequently, many koalas affected by chlamydiosis are euthanased (or should be), due to the chronic and permanent nature of their pathology. An important confounding issue in the treatment of chlamydiosis in koalas is our inability to meaningfully assess their KoRV disease status (particularly with regard to immune function), which almost certainly has effects on their chlamydial disease status.

Treatment of closed populations of koalas with antibiotics, with the intent of eliminating infection from the population, is certainly hypothetically possible. The time when such management interventions are required for conservation (and not just considered frivolous suggestions) may soon be upon us. Development of a vaccine to prevent chlamydiosis in koalas is the subject of a current research project. If successful it may provide an additional tool for control of chlamydial disease at the individual and population level.

Although some of the island populations of koalas may be free of infection with KoRV or *Chlamydia* at present, the reliance on these populations alone, for preservation of the species, comes with the hazard of substantial lack of genetic diversity. This has already manifested in the high prevalence of congenital abnormalities, and could certainly result in a reduced ability to respond to an incursion of either pathogen in the future. For comparison, low genetic diversity in Tasmanian devils is suggested to be important in their inability to defend against the facial tumour cells, which in many respects is an “infection” (Siddle *et al.*, 2007).

Priorities for research and funding

In summary, some of the tools that we may have available to control infection and/or disease in both individuals and populations include:

1. Vaccination

2. Treatment/elimination of infection
3. Selective breeding for disease resistance

All require a significant investment in research time and funding, and a substantially improved understanding of the epidemiology of, and interactions between, the infections which they seek to control. To date, the level of funding, and therefore our state of knowledge, has been quite poor, given the iconic status of the animal and the magnitude of the threat facing its continued existence in the wild.

In comparison to the response to the epizootic of Tasmanian devil facial tumour disease (DFTD) (\$22 million of government funding committed to date) (Lunney *et al.* 2008), our response to the threat of koala disease (particularly KoRV-associated disease) has been minimal. Some of the reasons probably include:

1. That disease in koalas has been recognised for years (over a century, in fact), and it is almost accepted as “part of being a koala”.
2. That DFTD causes dramatic and overt pathology, and has spread rapidly; in contrast, KoRV-associated disease is insidious and often overlooked even with veterinary assessment.
3. Severe habitat impacts on remnant koala populations are masking the impacts of KoRV and Chlamydia.
4. Regulatory authorities in Queensland and New South Wales are largely ignorant of the threat, mainly due to the poor level of veterinary support and disease surveillance provided to key koala rehabilitation centres.
5. The koala is still geographically widespread, and in some areas “over-abundant”.

Whatever the reasons, our current poor understanding of the real impacts of disease means that we cannot assume that the koala’s hold on existence is any less tenuous than that of the Tasmanian devil, whose extinction from the wild is considered likely (Lunney *et al.*, 2008).

It is important to reiterate that the urgent need for further disease research (and the funding to support it) does not in any way lessen the imperative to apply effort and funding to habitat protection and restoration.

In Summary

The key messages regarding infectious disease in koalas are:

1. That it must be considered a key threatening process (that may ultimately contribute to extinction), until proven otherwise;
2. That the impact of KoRV on koala population health and survival is unknown, but potentially catastrophic;
3. That this potential *drives* the imperative for effective habitat conservation and restoration, rather than detracting from it;
4. That State and Federal government understanding and acknowledgement of the potential impacts of disease on the conservation of koalas is poor.
5. That there is an urgent need for the application of appropriate effort and government funding to better understand and (hopefully) mitigate the impacts of epizootic infectious disease in koalas.

Finally, we encourage all koala conservationists and carers to use and share your knowledge, statistics and experience to lobby government, unrelentingly, for change.

Epilogue

At the time of writing, the Australian Koala Foundation has an application for federal listing of the Koala Coast koala population as vulnerable under the Federal *Environment Protection and Biodiversity Conservation Act*. Letters in support of this application, expressing concern also for your local koala populations, can only help the cause, so we urge you, respectfully, to put pen to paper and let them know what is happening at the coalface.

References

Devereaux, L.N., Polkinghorne, A., Meijer, A. & Timms, P. 2003, 'Molecular Evidence for Novel Chlamydia Infections in the Koala (*Phascolarctos cinereus*)', *Systematic and Applied Microbiology*, vol. 26, pp. 245-253.

Hanger, J. 1999, 'An Investigation of the Role of Retroviruses in Leukaemia and Related Diseases in Koalas', PhD thesis, University of Queensland, St. Lucia.

Jackson, M., White, N., Giffard, P. & Timms, P. 1999, 'Epizootiology of *Chlamydia* infections in two free-range koala populations', *Veterinary Microbiology*, vol. 65, pp. 255-264.

Jones, K. 2008, 'A study of the Reintroduction, Dispersal, Health and Survival of Juvenile and Hand-Raised Koalas (*Phascolarctos cinereus*) in Rural South-East Queensland', Honours thesis, University of Queensland, Gatton.

Lane, C. 2008, *Caboolture, Pine Rivers & Redcliffe Councils Report for Koala Habitat Survey and Mapping: Final Report May 2008*, GHD Pty Ltd, Brisbane.

Lunney, D., Jones, M. & McCallum, H. 2008, 'Lessons from the Looming Extinction of the Tasmanian Devil', *Pacific Conservation Biology*, vol. 14, no. 3, pp. 151-153.

Melzer, A., Carrick, F., Menkhorst, P., Lunney, D. & St. John, B. 2000, 'Overview, Critical Assessment, and Conservation Implications of Koala Distribution and Abundance', *Conservation Biology*, vol. 14, no. 3, pp. 619-628.

Siddle, H.V., Kreiss, A., Eldridge, M.D.B., Noonan, E., Clarke, C.J., Pyecroft, S., Woods, G.M & Belov, K. 2007, 'Transmission of a fatal clonal tumor by biting occurs due to depleted MHC diversity in a threatened carnivorous marsupial', *PNAS*, vol. 104, no. 41, pp. 16221-16226.

Tarlinton, R., Meers, J., Hanger, J. & Young, P. 2005, 'Real-time reverse transcriptase PCR for the endogenous koala retrovirus reveals an association between plasma viral load and neoplastic disease in koalas', *Journal of General Virology*, vol. 86, pp. 1-5.

Tarlinton, R. 2006, 'Characterisation of the epidemiology and molecular biology of the koala retrovirus (KoRV)', PhD thesis, University of Queensland, St. Lucia.

Timms, P. 2000, 'Koala Chlamydia from East to West', *Spirit and the Land Proceedings 2000*, Noosa Lakes Convention Centre, Noosa 23-25 October 2000, Australian Koala Foundation, Brisbane, pp. 41-51.

White, N.A. & Timms, P. 1994, 'Chlamydia psittaci in a Koala (*Phascolarctos cinereus*) Population in South-east Queensland,' *Wildlife Research*, vol. 21, pp. 41-47.

APPENDIX 1

Date of Examination: <hr style="border: none; border-top: 1px solid black;"/>	<p>Australian Wildlife Hospital</p> <h2 style="color: red; margin: 0;">Koala Examination Sheet</h2>	
Animal Details <input type="checkbox"/> See Accession Form (or complete details below)		
Animal's Name	Accession No	
Gender <input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Intersex	QPWS Form No	
Re-Admission <input type="checkbox"/> No <input type="checkbox"/> Yes (previous Accession No.....)		
Rescuer Details		
Rescuer Name	Affiliation/Group	
Rescuer Address (optional)	Telephone (home)	
Email Address	Telephone (mobile)	
Caller Details		
Caller Name	Telephone	
Reason for calling		
Rescue Details		
Date of Rescue	Time of Rescue	AM/PM
Exact location of rescue		
Grid Reference	LGA	
Reason for Rescue		
Position of koala	<input type="checkbox"/> In tree <input type="checkbox"/> On ground <input type="checkbox"/> In captivity	
	<input type="checkbox"/> Other	
Identifying Features:		
Ear Tag	<input type="checkbox"/> No <input type="checkbox"/> Yes - Tag No..... <input type="checkbox"/> Left <input type="checkbox"/> Right	
Microchip	<input type="checkbox"/> No <input type="checkbox"/> Yes - No.....	
Other identifying features:		
Summary of Diagnoses:		
1.		
2.		
3.		
4.		
KoRV Suspicion		
<input type="checkbox"/> ? Aids	<input type="checkbox"/> Aplastic anaemia	<input type="checkbox"/> Eyes
<input type="checkbox"/> Lymphoma	<input type="checkbox"/> Other Neoplasm	<input type="checkbox"/> Urinary / Renal
<input type="checkbox"/> Plantar hyperkeratosis	<input type="checkbox"/>	<input type="checkbox"/> Respiratory
<input type="checkbox"/> Leukaemia	<input type="checkbox"/>	<input type="checkbox"/> Reproductive
<input type="checkbox"/> Myelodysplasia	<input type="checkbox"/>	<input type="checkbox"/>
Chlamydia		
Other		
<input type="checkbox"/> Trauma		
<input type="checkbox"/> Orphan		
<input type="checkbox"/> Healthy		
<input type="checkbox"/>		
<input type="checkbox"/>		
<input type="checkbox"/>		
Initial Outcome:		
<input type="checkbox"/> Dead on arrival	<input type="checkbox"/> Euthanased	<input type="checkbox"/> Admit to Hospital
<input type="checkbox"/> Died during examination	<input type="checkbox"/> Immediate release	<input type="checkbox"/> Sent to Carer on / / 20
Final Outcome:		
<input type="checkbox"/> Euthanased on / / 20	<input type="checkbox"/> Sent to carer on / / 20	<input type="checkbox"/> Released on / / 20
<input type="checkbox"/> Died on / / 20	<input type="checkbox"/> Permanent care @	<input type="checkbox"/> Transferred to on / / 20
Release Details:		
Release Date:	Released by:	
Distance from rescue site:	Release authorised by:	
Exact address of release:		

Distant Examination:

Demeanour	<input type="checkbox"/> B.A.R.	<input type="checkbox"/> Depressed	<input type="checkbox"/> Excited	<input type="checkbox"/> Moribund
Behaviour	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal			
Posture	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal			
Gait	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal			
Symmetry	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal			
Breathing	<input type="checkbox"/> Normal	<input type="checkbox"/> Shallow	<input type="checkbox"/> Rapid	<input type="checkbox"/> Laboured
Coat	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal			
Discharges	<input type="checkbox"/> Nil <input type="checkbox"/> Present			
Wounds/Bleeding	<input type="checkbox"/> Nil <input type="checkbox"/> Present			
Other lesions:	<input type="checkbox"/> Nil <input type="checkbox"/> Present			
Abdomen:	<input type="checkbox"/> Normal	<input type="checkbox"/> Bloating	<input type="checkbox"/> Sunken	

General Physical Examination:

Anaesthesia	Induction Anaesthetic Agent Dose			
	Route: <input type="checkbox"/> i.m. <input type="checkbox"/> i.v. <input type="checkbox"/> facemask <input type="checkbox"/> Tube			
	Maintenance Anaesthetic Agent Dose			
	Route: <input type="checkbox"/> i.m. <input type="checkbox"/> i.v. <input type="checkbox"/> facemask <input type="checkbox"/> Tube			
Vital Signs	Mucous Membrane: <input type="checkbox"/> Pink <input type="checkbox"/> Pale <input type="checkbox"/> Cyanotic <input type="checkbox"/> Red			
	HR	RR	Rectal Temp ° C	
	CRT	SpO ₂		
	Pulse: Rate	Rhythm: <input type="checkbox"/> Regular <input type="checkbox"/> Occasional arrhythmia <input type="checkbox"/> Frequent/constant arrhythmia		
	Amplitude: <input type="checkbox"/> Normal <input type="checkbox"/> Increased <input type="checkbox"/> Decreased		Tone: <input type="checkbox"/> Normal <input type="checkbox"/> Increased <input type="checkbox"/> Decreased	
Hydration	<input type="checkbox"/> Normal	<input type="checkbox"/> <5% dehydrated	<input type="checkbox"/> 5-10% dehydrated	<input type="checkbox"/> > 10% dehydrated
Weight / Age / Body Score	Body Score / 10		Weightkg	Ageyrs/mths
Tooth Wear:	Class		Estimated Ageyears	
Chest auscultation:	Heart.....			
	Lungs Other respiratory findings: <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal			
Neurological	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal			

General Physical Examination (continued):

Musculoskeletal	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal	
Head / Mouth	Head Symmetry	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Ears	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Lips	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Nares	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Tongue	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Teeth	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Gingiva	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Fauces	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Palate/tonsils	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Pharynx	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Larynx	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Cheek Pouches	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
Eyes:	LEFT	RIGHT
Periorbital skin:	<input type="checkbox"/> Normal <input type="checkbox"/> Alopecic <input type="checkbox"/> Pigmented	<input type="checkbox"/> Normal <input type="checkbox"/> Alopecic <input type="checkbox"/> Pigmented
Eyelids:	<input type="checkbox"/> Normal <input type="checkbox"/> Other	<input type="checkbox"/> Normal <input type="checkbox"/> Other
Palpebral fissure	<input type="checkbox"/> Normal <input type="checkbox"/> ↓ Scarred <input type="checkbox"/> ↑	<input type="checkbox"/> Normal <input type="checkbox"/> ↓ Scarred <input type="checkbox"/> ↑
Conjunctiva:	<input type="checkbox"/> Normal <input type="checkbox"/> Proliferated 1 2 3	<input type="checkbox"/> Normal <input type="checkbox"/> Proliferated 1 2 3
Other.....	Other.....
Nictitating:	<input type="checkbox"/> Normal <input type="checkbox"/> Prolapsed 1 2	<input type="checkbox"/> Normal <input type="checkbox"/> Prolapsed 1 2
Sclera:	<input type="checkbox"/> Normal <input type="checkbox"/> Other	<input type="checkbox"/> Normal <input type="checkbox"/> Other
Cornea:	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
Opacity:	<input type="checkbox"/> Clear <input type="checkbox"/> Mild <input type="checkbox"/> Marked	<input type="checkbox"/> Clear <input type="checkbox"/> Mild <input type="checkbox"/> Marked
Iris & Pupil:	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
Discharge:	<input type="checkbox"/> Nil <input type="checkbox"/> Serous <input type="checkbox"/> Purulent Amt: 1 2 3	<input type="checkbox"/> Nil <input type="checkbox"/> Serous <input type="checkbox"/> Purulent Amt: 1 2 3
Description:
Schirmer Tear Test (60 secs):	Lengthmm	Lengthmm
Fluorescein Test:
Coat:	Colour: <input type="checkbox"/> Light Grey <input type="checkbox"/> Brown <input type="checkbox"/> Dark Grey	
	Structure: <input type="checkbox"/> Normal <input type="checkbox"/> Sparse <input type="checkbox"/> Clumped/ irregular	
	Texture: <input type="checkbox"/> Normal <input type="checkbox"/> Greasy <input type="checkbox"/> Dry	
Other:	
Skin:	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal	
Lymph Nodes	Rostral mandibular	<input type="checkbox"/> Normal L R <input type="checkbox"/> Enlarged L R <input type="checkbox"/> Small L R
	Facial	<input type="checkbox"/> Normal L R <input type="checkbox"/> Enlarged L R <input type="checkbox"/> Small L R
	Mandibular	<input type="checkbox"/> Normal L R <input type="checkbox"/> Enlarged L R <input type="checkbox"/> Small L R
	Superficial cervical	<input type="checkbox"/> Normal L R <input type="checkbox"/> Enlarged L R <input type="checkbox"/> Small L R
	Axillary	<input type="checkbox"/> Normal L R <input type="checkbox"/> Enlarged L R <input type="checkbox"/> Small L R
	Inguinal	<input type="checkbox"/> Normal L R <input type="checkbox"/> Enlarged L R <input type="checkbox"/> Small L R
	Notes

General Physical Examination (continued):

Abdominal Palpation	Abdominal Fill <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Stomach Fill <input type="checkbox"/> 1 – (empty) <input type="checkbox"/> 2 (½ full) <input type="checkbox"/> 3 (full)
	Stomach Consistency <input type="checkbox"/> Normal (firm) <input type="checkbox"/> Soft <input type="checkbox"/> Bloated <input type="checkbox"/>
	Distal colon <input type="checkbox"/> Pellets <input type="checkbox"/> Empty/ pellets not palpated
	Proximal colon/caecum <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
Notes /Other abdominal lesions:	
Chest Palpation:	Clavicle: <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Ribs: <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
Limbs and Joints	Left Forearm <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Left Paw/digits: <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Left Hindleg <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Left Foot/digits: <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Right Forearm <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Right Paw/Digits <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Right Hindleg <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
	Right Foot/digits: <input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
Scrotum / Pouch	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
Scent Gland/ Mammary Glands	<input type="checkbox"/> Active <input type="checkbox"/> Inactive <input type="checkbox"/> Abnormal
Cloaca/Clitoris/ Penis	<input type="checkbox"/> Normal <input type="checkbox"/> Abnormal
Dirty Tail Score	<i>(circle score)</i>
	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 vocalization)
	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

