Assessing the significance of endemic disease in conservation—koalas, chlamydia, and koala retrovirus as a case study

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Abstract
It can be difficult to establish the conservation significance of endemic infectious diseases—those that are well established in a population—in contrast with infectious diseases that are still invading. This difficulty can have important implications for designing policy to address species declines. The infectious diseases of koalas provide an ideal case study to examine issues involved in identifying the role of endemic disease in conservation biology. Koala populations are in decline, amidst claims for many years that infectious diseases, particularly those with chlamydial etiology, play a key role in this loss. However, weak associations between prevalence of infection, clinical signs of disease, and population decline mean that it remains unclear whether infectious disease is a primary driver of koala population decline. There are multiple causes of koala decline including drought, habitat destruction, and disease. Well-designed experiments, linked to appropriate models, are necessary to determine the true role of infectious disease in the current koala population declines and whether a focus on disease is likely to be a feasible, let alone the most cost-effective, means of preventing further declines.

Keywords
chlamydia, endemic disease, koala, koala retrovirus, Phascolarctos cinereus, population declines

1 INTRODUCTION

Infectious diseases are increasingly recognized as an important issue in conservation biology (McCallum, 2012). In some cases (e.g., Tasmanian devil facial tumor disease [McCallum, 2008] and chytridiomycosis in frogs [Kilpatrick, Briggs, & Daszak, 2010]), the evidence that a disease has caused substantial population declines is unequivocal. Discussion of management and policy implications of infectious diseases in wildlife have largely concentrated on these dramatic examples of emerging epidemic infectious disease (Langwig et al., 2015). Managing endemic infections, which have been present in an ecological community for an extended period, raises different issues. In many situations, parasites and pathogens are a normal component of the ecology of a species of concern, posing no additional threat to survival (Fancourt, Nicol, Hawkins, Jones, & Johnson, 2014). Conservation policy should aim to conserve these parasites and pathogens as an important component of overall biodiversity (Windsor, 1995). In other cases, whether an endemic infectious disease is an important consideration in the conservation of a host species is uncertain. Infected individuals may have lower survival and/or fecundity than uninfected individuals, but this does not necessarily mean disease has significant
effects at the population level; importantly, even when clinical disease is evident in a population, it is not axiomatic that disease is the proximate cause of population decline.

The koala *Phascolarctos cinereus* is an iconic species in which disease has been suggested as a key agent of decline (Rhodes et al., 2011), while other studies have concluded the contrary (Penn et al., 2000); the species thus provides an ideal case study to investigate the impact of endemic diseases. Koalas are impacted by a range of pathogens (including *Haemophilus pneumoniae*, *Cryptococcus sp.*, and *Burkholderia pseudomallei*) and parasites (e.g., ticks, mites, and the tapeworm, *Bertiella obesa*) (Carrick, 1996). However, many of these occur sporadically and do not appear to generate mass mortality. Of greater concern are those conditions (chlamydiosis and possibly koala retrovirus [KoRV]) that are endemic in koala populations, and may have a significant impact on koala population dynamics (Carrick, 1996).

Koalas are infected by bacteria in the Family *Chlamydiaceae*, which may result in urogenital, respiratory, and ocular disease, leading to cystitis, sterility, pneumonia, and blindness and ultimately to reduced reproductive output and increased mortality (Weigler et al., 1988). Koalas are also infected by KoRVs, trypanosomes, and herpesviruses, but impacts on survival and fecundity are poorly understood (Denner & Young, 2013; McInnes, Gillett, Hanger, Reid, & Ryan, 2011; Vaz et al., 2011). While infectious disease has been suggested as a significant, perhaps dominant, factor in drastic declines (and in some instances, local extinction) of many koala populations (Craig et al., 2014; Rhodes et al., 2011), the role of chlamydial disease and retroviral infection of koalas as fundamental drivers of population decline remains unclear (White & Timms, 1994).

### 1.1 Historical trends in koala abundance and distribution

Koalas were widely distributed over much of eastern Australia at the time of European colonization. Hunting for the fur trade, in concert with habitat destruction, altered fire regimes and disease, led to significant declines, with northern populations severely diminished and koalas largely absent across the southern part of their range by the early 20th century (Menkhorst, 2008). As early as the late 1930s, disease had also been suggested as a factor in their collapse in Queensland (Pratt, 1937), though it is difficult to disentangle the impact of disease from hunting and land clearing.

A series of reintroductions and translocations, commencing in the 1920s but not accelerating until 1944, reestablished populations in much of southern Australia (Menkhorst, 2008), while the cessation of hunting allowed northern populations to recover. At present, koalas remain widely but patchily distributed over the eastern third of Australia in forested and wooded areas (Figure 1). Populations in New South Wales, the Australian Capital Territory, and Queensland have declined markedly since 1995, leading the Australian Minister for the Environment to list these populations as vulnerable to extinction in 2012 (Threatened Species Scientific Committee 2012). Inland koala populations have been particularly impacted by drought (Seabrook et al., 2011), while urban expansion and related dog- and car-associated mortality have had a profound impact on coastal populations (Carrick, 1990; McAlpine et al., 2006). The extent to which infectious disease has been a primary driver of decline, or the extent to which the impact of disease on koala mortality and fecundity at an individual level has exacerbated declines at a population level due to these anthropogenic or climate change factors remains controversial.

### 1.2 Chlamydial and retroviral infections in koalas

*Chlamydia* is a genus of intracellular bacteria that affects a variety of invertebrates, birds, and mammals. There are two species reported to infect koalas (currently described as *Chlamydia pneumoniae* and *Chlamydia pecorum*), with *C. pecorum* generally accepted to be more pathogenic (Polkinghorne, Hanger, & Timms, 2013). Chylandia is generally accepted to be a sexually transmitted infection in koalas; however, it is likely that infection also occurs via direct transfer of the pathogen from the eyes and reproductive tract of one animal to another, and vertically, with mothers infecting offspring via pap-feeding (Blanshard & Bodley, 2008); the relative importance of these two routes of transmission remains unclear. An association between koala keratoconjunctivitis and chlamydiae was first reported in 1974 (Cockram & Jackson, 1974), although clinical signs that may be associated with chlamydial infection in koalas (conjunctivitis and cystitis; Figure 2) had been observed as early as the late 19th century (Lavin et al., 1990). However, it was not until Brown and Grice (1984) established Koch’s postulates a decade later that the association was confirmed. While koalas have likely coexisted with *C. pneumoniae* for thousands of years (Mitchell et al., 2010), the relationship with *C. pecorum* is more nuanced. Recent genomic evidence suggests that while the majority of *C. pecorum* strains present in koalas are likely to have an ancient origin, certain strains were shared with livestock (Bachmann et al., 2015), implying that these strains may have a more recent origin, moving between livestock and koalas post-European colonization.

Nine KoRV subtypes in three clades have thus far been described from wild or captive koala populations (Chappell et al., 2017). One of these, KoRV-A, appears to have been integrated into the genome of some koala populations (endogenized), and is found as multiple copies at 100% prevalence in some populations (Queensland and New South Wales) (Legione et al., 2017; Oliveira, Satija, ...
FIGURE 1 Distribution and abundance of koalas

Note: Inset bar charts show Australian Government estimated abundance (in thousands) of koalas in each state in 1990 and 2010. Green shading shows jurisdictions in which koalas are listed as vulnerable to extinction, whereas brown shading shows jurisdictions in which they are not listed. Modified under a Creative Commons 3.0 license (Department of the Environment and Energy, 2012)

Kouwenhoven, & Eiden, 2007). Deoxyribonucleic acid (DNA) evidence from museum skins suggests it was ubiquitous in these populations by the late 19th century (but how much earlier KoRV was present in these populations is unknown) (Avila-Arcos et al., 2013). Prevalence in koalas sampled from southern Australia is substantially lower (Denner & Young, 2013). Ishida, Zhao, Greenwood, and Roca (2015) estimated a maximum age for KoRV-A invasion of the koala germ line as 22,200–49,900 years ago, reflecting a lengthy association. Other subtypes are found with fewer copies and lower prevalence and can be geographically restricted in their distribution; these other subtypes appear to be primarily horizontally transmitted (Chappell et al., 2017).

The impact of retroviruses on individual koalas is an area of active research. It is not clear whether KoRV infections are responsible for immunosuppression in koalas (Denner & Young, 2013). KoRV infection has been implicated in lymphoma and leukemia in captive koalas, and a link has been suggested in wild koalas, but sample sizes in these reports are limited (Tarlinton, Meers, Hanger, & Young, 2005; Xu et al., 2013).
A study of Victorian koalas did not show a significant link between KoRV infection and chlamydial disease, but only detected the KoRV-A subtype in the population (Legione et al., 2017). A recent study has linked the exogenous KoRV-B subtype with alterations in koala immune function (Maher & Higgins, 2016); this result is supported by a reported association between KoRV-B infection and clinical chlamydial disease in a population of koalas in Queensland (Waugh et al., 2017); however, this association was based on only 36 animals from a population with a limited geographic distribution; work is likely required to further explore this relationship.

1.3 Do these diseases drive population dynamics?

In the ecological literature, it is common to see the term “disease” used as being synonymous with “infection.” However, pathogens may infect individuals without causing overt clinical disease. This is certainly the case with chlamydial infection of koalas (Polkinghorne et al., 2013), where the complexities of infection, and the presence of a number of alternative causes of koala mortality, make it difficult to tease out the role of the disease in driving population declines.

Although there is an overall tendency for koalas with more severe clinical signs of disease to have higher intensities of infection, the relationship between infectious load and clinical signs is poor. Some individuals with no clinical signs of disease have substantially higher intensities of \textit{C. pecorum} infection, as measured by quantitative Polymerase Chain Reaction (PCR), than others with severe clinical signs (Wan et al., 2011). Furthermore, some females with overt urogenital disease show evidence of recent successful reproduction and some individuals with clinical signs of chlamydirosis have become asymptomatic (recovered) spontaneously in the wild (Higgins, Hemsley, & Canfield, 2005; Polkinghorne et al., 2013). At a population level, some populations have relatively high levels of infection but few clinical signs of disease (Ellis, Girjes, Carrick, & Melzer, 1993; Weigler et al., 1988; White & Timms, 1994). Many koala populations with low prevalence of clinical disease have also shown significant declines in abundance (Seabrook et al., 2011).

Stress increases susceptibility to infection by pathogens and reduces tolerance of infection. For example, Zylberberg, Lee, Klasing, and Wikelski (2013) found that avian poxvirus had a greater effect on Galapagos finches in agricultural land than undisturbed habitat and also that immune function of the finches appeared weaker in disturbed habitat. Gervasi, Burgan, Hofmeister, Unnasch, and Martin (2017) demonstrated that elevated corticosterone, a stress hormone, is associated with reduced immune function and increased mortality in zebra finches exposed to West Nile virus. Nutritional stress has been implicated in increased recrudescent viral infections in bats (Plowright et al., 2015), and increased competition and resulting elevated testosterone levels in red grous increase susceptibility to a parasitic nematode (Redpath, Mougeot, Leckie, Elston, & Hudson, 2006).

In koalas, it is plausible that other factors leading to population decline, such as habitat loss and human encroachment on habitat, may precipitate reduced tolerance of infection (Melzer, Carrick, Menkhorst, Lunney, & St. John, 2000); increased clinical disease may therefore be correlated with population decline but not its cause. Recent analysis concluded that urbanization was linked with an increase in clinical disease in koala populations, but noted effects may be delayed until overt disease impacts the population (McAlpine et al., 2017). A noninvasive method of measuring physiological stress in koalas using fecal cortisol metabolites has been developed (Narayan, Webster, Nicolson, Mucci, & Hero, 2013) although its value has been questioned (Johnston et al., 2013). There remains a need for cross-validation of stress hormone, immunological, and disease/fitness indicators in order to interpret the biological significance of any observed variations; despite suggestions as early as 1988 that stress may trigger overt disease in koalas infected with chlamydia.
(Weigler et al., 1988), we know of no studies directly compare stress and tolerance to chlamydial infection in koalas, nor of comparison of corticosteroids in koalas from disturbed and undisturbed habitats.

Published evidence that prevalence of clinical chlamydial disease in the population drives population dynamics is limited to a single 11-year study of a population of koalas in Queensland (Gordon, McGreevy, & Lawrie, 1990). Gordon et al. recorded population size, number of new recruits, and prevalence of clinically detectable cystitis (which can be associated with sterility in females); although there was a relationship between prevalence of cystitis and number of recruits in the following year, prevalence of cystitis did not influence the finite population growth rate in the following year (McCallum, 2012), suggesting the reduction in recruitment was compensated for by other factors (Gordon et al., 1990).

A deterministic age structured model (Rhodes et al., 2011) identified disease mortality as a significant factor in the decline of a south-east Queensland koala population. However, this conclusion was derived from apportioning estimated annual mortality rates to vehicle collisions, dog attacks, disease, and “natural” causes. The basis on which mortalities were attributed to disease was derived from a dataset in which less than half of the 99 animals recovered were subjected to postmortem (Thompson, 2006). Distinguishing between “dying with disease” and “dying from disease” is not a straightforward issue in wildlife epidemiology.

If a pathogen is endemic in a population (i.e., its prevalence is at or close to equilibrium), then the lower the disease-induced death rate, the higher the prevalence of infection (Anderson, 1979). Given all animals eventually die of something, this means prevalence of infection among the dead and dying animals will also be high. Prevalence and intensity of infection in dead and dying animals similar to that in the general population suggest strongly that disease is not a major causal factor in mortality. If prevalence is substantially higher in morbidity animals than in the general population, then disease may be a substantial contributor to mortality (McCallum & Dobson, 1995), although some third factor (for example, malnutrition) may have contributed to both the mortality and the overt clinical disease. Veterinary pathologists use clinical judgment to determine whether a given infection is likely to have caused mortality, but there may not be a set of clear objective criteria. It is critical to collect information on the prevalence and intensity of infection from an unbiased sample of the total population, in addition to prevalence and intensity in dead and dying animals.

At present, there is limited information that KoRVs influence population dynamics (Denner & Young, 2013). While northern populations, which are declining dramatically, have higher prevalence of infection than those in southern Australia, which appear to be declining more slowly or, in some isolates, increasing (Tarlinton, Meers, & Young, 2006), there are other potential causal factors.

### 2 | Resistance and Tolerance at Individual and Population Levels

The distinction between resistance to infection and tolerance of infection is important (Råberg, Graham, & Read, 2009), but the two concepts are often conflated. An individual is resistant to infection if, when exposed to a pathogen, it does not develop a patent infection, whereas it is tolerant to the pathogen if it develops infection but has few or limited signs of clinical disease.

The same distinction is useful, although less commonly applied, at the population level. Even if individuals are highly susceptible to infection (neither resistant nor tolerant), a population may be resistant to the pathogen if prevalence in the population as a whole remains low and extended transmission chains do not occur. This will be the case if \( R_0 \) is less than or very close to 1. Conventional epidemiological theory suggests that this will occur if the transmission rate is low, or the period for which individuals remain infectious is short (as is the case with very pathogenic diseases, because infected hosts die rapidly). Populations may be tolerant to infection by a pathogen, meaning that there is little effect on population density, although a high proportion of individuals is infected with a pathogen that substantially diminishes the fitness of infected individuals. This can occur if there are compensatory mechanisms. For example, in a series of experiments designed to mimic the effects of a genetically engineered immunosuppressive pathogen on rabbit populations (Williams et al., 2007), up to 80% of females in 12 populations of rabbits in southern Australia were surgically sterilized. Despite sterilization successfully decreasing the abundance of juvenile rabbits, there was no overall effect on population density outside the breeding season, most likely because of increased survival of adult and remaining juvenile rabbits as a response to increased food availability.

#### 2.1 Implications for management

The koala is an iconic and charismatic species; as a result, there is considerable pressure for policy responses and management intervention by both organized agencies and informal networks of wildlife carers. Chlamydial disease is one of a number of factors potentially driving koala population declines. In coastal areas of New South Wales and Queensland, habitat destruction associated with urbanization and concomitant increases in dog attacks and roadkill are substantial contributing factors to decline (McAlpine et al., 2015). It could be argued that identifying chlamydial disease as the...
Isolation of uninfected populations

Chlamydia has not yet been detected in some island populations of koalas (Masters, Duka, Berris, & Moss, 2004) (though this may be a result of insufficiently sensitive testing) and it would be sensible to prevent the potential spread of chlamydial infection to these isolated populations. KoRVs are present in almost all koala populations that have been extensively sampled (Simmons et al., 2012), although they were not detected in 11 individuals sampled from an introduced Koala population on Phillip Island in Victoria (Simmons et al., 2012). Obvious requirements are for proper quarantine standards to be applied when veterinary care is required (especially for presumptive uninfected koalas) and that patients are not housed with koalas from other populations during treatment and recovery. This applies especially to island or other geographically isolated populations where chlamydial infections are endemic, but disease prevalence is very low (e.g., North Stradbroke Island)—such populations must be protected from more virulent strains of chlamydiae or variant strains to which they are not tolerant.

Whilst limiting translocations of koalas between isolated subpopulations might mitigate the risk of transferring novel strains of either pathogen, some such populations have low genetic diversity, which threatens their long-term viability (Lee et al., 2012). The risk of loss of genetic diversity and inbreeding might need to be balanced against the possible threat posed by introduction of pathogens.

Vaccination or treatment

Considerable resources have been dedicated to the development of a vaccine against chlamydiae in koalas; however, at present, there is no statistically significant evidence that current putative vaccines will protect against live challenge (Polkinghorne et al., 2013). A recently published field trial did not significantly reduce the incidence of new infections in vaccinated koalas, but is reported to have reduced chlamydial burdens in vaccinated as compared with nonvaccinated koalas that were Chlamydia positive at the time of vaccination (Waugh et al., 2016). A recent study by Stary et al. (2015) highlights the difficulties involved in developing a vaccine for chlamydia; the authors argue earlier chlamydial vaccines for human and other animal use have been at best ineffective and worse sometimes harmful, because vaccines have been developed using: (1) wrong antigen, (2) wrong adjuvant, and (3) wrong mode of administration (nonmucosal). Although a report by Waugh et al. (2015) has made preliminary observations on a mucosal vaccination route in koalas, it did not demonstrate a protective immune response.

A modeling study has suggested that a vaccine with efficacy of 75% administered to 10% of koalas annually could be capable of reversing population declines in koalas within 5 years (Craig et al., 2014). The model was parameterized using a calibration process based on Latin hypercube sampling of the parameter space, retaining parameter sets capable of generating a doubling time of 2.7 years in the absence of disease, but in which the population would halve in between 5 and 10 years in the presence of disease. This latter assumption is based on the observation that populations in the Koala Coast of south-east Queensland are declining with a halving time of approximately 3 years. The model calibration process therefore presumes that the agent of decline is chlamydial infection and that, in the absence of chlamydial infection, populations will recover irrespective of other sources of mortality—this presumption does not appear to be robustly based in light of other significant threats (habitat loss, car strike, and dog attack) facing koalas in this region.

Vaccination will have some negative consequences for koalas, if not from the vaccine itself, then from the handling, capture, and associated stress involved with administering a vaccine to in excess of 10% of the total population on an annual basis. Directly administered vaccines have aroused considerable controversy in other wildlife species, for example, African wild dogs (Prager, Woodroffe, Cameron, & Haydon, 2011). Further, the substantial resources necessary to undertake a large-scale vaccination program would necessarily limit the resources available for other conservation actions. It is therefore important to employ an evidence-based investigation to determine whether chlamydial infection is, in fact, a major agent of decline across a number of populations, and if vaccination of wild koalas would be an appropriate response, given a safe and effective vaccine. A reported association between chlamydia and KoRV-B coinfection and disease (Waugh et al., 2017) may indicate that vaccine efforts could have greater impacts if directed at exogenous KoRV subtypes.

Antibiotics are used extensively in koala care facilities to treat chlamydial infection. Intensive daily treatment over many days is necessary both to reduce clinical signs (Osawa & Carrick, 1990) and to lead to cessation of shedding of chlamydiae (Markey, Wan, Hanger, Phillips, & Timms, 2007). Appropriate management is required to avoid side effects of antimicrobial treatments, including potentially fatal effects due to dysbiosis of the microflora of
the koala gastrointestinal tract (Osawa & Carrick, 1990). Some individuals can have asymptomatic infections, and thus may constitute an undetected and untreated source of new infections.

2.1.3 Culling

Culling of all animals in an infected population is clearly inappropriate for a widespread potential pathogen present in most populations at high prevalence. A recent paper (Wilson, Craig, Hanger, & Timms, 2015) has suggested that culling of koalas with chlamydial disease would enable rapid population recovery and elimination of the disease. As with the paper on vaccination from the same group of authors (Craig et al., 2014), the model presumes that chlamydial infection is the primary factor in population declines and that in its absence koala populations will increase, discounting the impact of other threatening processes. The modeling assumes that between 130 and 140 infected koalas could be culled each year, which means that essentially, all infected animals could be removed once the population has declined sufficiently and that there is no covert infection. Additionally, the modeling supposes all infected koalas will clear chlamydial infection after 10–18 months in the absence of veterinary intervention, an assumption not supported by any evidence to date.

Current management actions are a de facto application of a strategy of culling infected animals. Animals with clinical disease are regularly brought in to koala care facilities (Griffith, Dhand, Krockenberger, & Higgins, 2013). If urogenital chlamydiosis has rendered them sterile, or their condition is sufficiently grave that the koala is sterilized, current practice in Queensland is to place animals into permanent care or they are euthanased. There has been some dispute between the animal welfare sector and ecologists about the ethics of releasing sterile animals, as the release of sterilized animals may have negative effects on the viability of the recipient populations. Conversely, in some populations, animals are routinely captured and tested for chlamydial infection and if positive may be euthanased, whether or not they display significant clinical signs of disease (for instance, older females with detectable cysts but no other clinical issues are often euthanased; Loader, 2010).

2.1.4 Genetic management

An option for managing wildlife disease is to select for hosts that are either resistant or tolerant to infection and thus accelerate the evolution of resistance and tolerance observed when novel pathogens are introduced into naive populations (Fenner & Fantini, 1999). Given Chlamydial disease is generally the result of a hypersensitive immune response to infection (Darville & Hiltke, 2010), it might be expected that koalas will adapt over time to reduce their immune response. However, as the association between Chlamydia and koalas has existed for at least a century (if not much longer), any such evolution in koala populations may have occurred already. Indeed, evidence of populations with high prevalence of infection but very low prevalence of disease suggests that some coevolution is already underway; the main hazard is the introduction of chlamydial strains into a particular population that has not been previously exposed and thus lacks tolerance.

Few studies to date have typed chlamydial infections to the strain level, and then examined any association with disease. However, in principle, a second possibility would be to select for or even genetically engineer lower pathogenicity strains of Chlamydia and to promote their dispersal through wild populations. If there is significant cross immunity between such lower pathogenicity strains and high pathogenicity strains, this may act as a proliferating partial vaccine. The perceived risks to promoting the spread of a pathogen, particularly one genetically engineered, in an iconic wildlife species would likely make such a strategy politically unacceptable, even if it were biologically feasible.

As an endogenous retrovirus with an extensive association with koalas (Ishida et al., 2015), coevolution of koalas and KoRV-A is likely. However, exogenous KoRV variants are expected to have entered or arisen in koala populations more recently, and adaption or the development of tolerance to these more novel disease agents is less likely; further work is obviously required to determine if there are any options for genetic management of these retroviruses.

2.1.5 Habitat modification

This strategy is most widely used for control of vector transmitted infections, but has potential for managing wildlife diseases, provided that the ecology of transmission is well understood (Wobeser, 2002). For koalas, the possibility of managing habitat to reduce opportunities for pathogen transmission or disease transmission is limited. However, habitat destruction and fragmentation are major threatening processes for koala populations. Koalas forced to move across roads and through suburbia are exposed to trauma and dog attack, reducing the capacity of koala populations to compensate for a degree of disease induced mortality or reduction in fecundity. Strategies to mitigate the impact of anthropogenic habitat modification, particularly roads, are available and were recommended by an Australian Senate committee (Australian Senate Environment and Communications References Committee 2011; Rhodes, Lunney, Callaghan, & McAlpine, 2014).

2.2 Filling research gaps

It is likely that there are multiple causes of koala population declines: habitat loss, drought, predation, mortality associated with vehicle strikes, as well as disease impacts on mortality and fecundity. These will interact and the relative importance
of each factor will depend on the characteristics of the local population. With respect to disease impacts, the two important questions for conserving koala populations are whether it is possible to reduce the impact of disease in wild populations in a cost-effective manner and second, whether feasible reductions of disease impact would improve the viability of enough populations to arrest the decline of koala populations or to permit their recovery.

The best strategy for conclusively assessing the role of chlamydiae and retroviruses in driving koala population dynamics would be a large scale randomized and replicated trial, where some populations were either cleared of infection or treated with a vaccine, with monitoring to determine differences in the demography of disease-free and infected populations. Such an experiment, even given the existence of appropriate means to remove or reduce infection in experimental populations, would be expensive, time-consuming, and difficult to undertake on a sufficiently large spatial scale with adequate replication. Experiments in which grouse in Scotland were treated with anthelmintics, resulting in suppression of population cycles (Hudson, Dobson, & Newborn, 1998), are one of the few examples where this approach has been successfully used.

A less satisfactory but more practical approach is to treat some individuals within populations and compare survival and fecundity with control individuals from the same populations. This approach has been applied to a number of other wildlife diseases (Gulland, 1992). The implications of these individual-level effects would then need to be explored at the population level by appropriate models. A fundamental limitation in building such models is a poor understanding of the extent to which populations may be able to compensate for disease-induced reductions in fecundity or survival, which entails understanding density-dependent constraints on koala populations. Analysis of existing long-term datasets on koala population dynamics may shed light on the nature of density-dependent factors in koala populations.

Once field experiments are complete and suitable models are constructed, parameterized, and analyzed, larger-scale adaptive management approaches could be used. But, first, there needs to be a demonstrably effective vaccine. While progress has undoubtedly been made toward a vaccine against chlamydia in koalas (Waugh et al., 2015, 2016), the effectiveness of this vaccine has not yet been established with double blind challenge trials. It is salutary to note here that despite more than 50 years research, a safe and effective vaccine against human strains of chlamydia has yet to be developed (Mabey, Hu, Bailey, Burton, & Holland, 2014; Stary et al., 2015).

One further option is gaining better quality epidemiological data from existing field studies, allowing comparative analysis and modeling between different populations.

Elucidating the relationship between stress and either or both of resistance to chlamydial infection, or tolerance of infection without substantial clinical disease, is a critical research gap. Whether a causal relationship exists in either direction can only be adequately resolved by following individual koalas in the wild through time and regularly monitoring stress levels, infection status, and clinical disease. If stress precedes infection or clinical disease, this would be strong evidence for a causal relationship. Cross-sectional surveys in which individuals are not individually followed can only determine correlation. In other wildlife disease studies, following individual animals through time have been crucial in understanding the dynamics of disease (Gulland, 1992; Hamede, McCallum, & Jones, 2013).

### 2.3 Implications for developing policy to manage endemic disease in other species

The koala is an iconic species in which disease has been identified as a potential threat for decades, and yet it is still unclear whether managing disease is an effective strategy to prevent species decline.

If endemic disease is an important threatening process, it is likely to be one of a number of multiple stressors, and, as with chlamydial disease in koalas, disease may interact with these stressors. Perhaps, the single most important lesson to be learned from this case study is that it is difficult to determine unequivocally the impact of an endemic infectious disease on free ranging wildlife populations. Where a well-established and cost-effective treatment for disease is available, then adaptive management, by experimentally intervening in some populations, may be an appropriate strategy (Joseph et al., 2013). However, when such a treatment is not available (as is the case with chlamydia in koalas), at what point does it become good policy to allocate substantial resources to developing a treatment, vaccine, or disease management strategy?

A major barrier to understanding the impact of chlamydial infection in koalas is the lack of long-term monitoring of infected populations, with the only time series exceeding a decade being that of Gordon et al. (1990). Monitoring is costly and not always optimal for conservation management (McDonald-Madden et al., 2010), but applying the decision tree in McDonald-Madden et al. (2010), monitoring is needed to assess and manage endemic disease threats. Where endemic disease is recognized as a potential threatening process, it is critical to undertake long-term monitoring both of population size and structure and of disease burden. Ideally, this would involve longitudinal sampling of the same individuals so that multistate mark recapture methods can be applied to estimate both disease effects on survival and fecundity and to estimate the epidemiological parameters necessary to build appropriate models to evaluate management strategies (McCallum, 2016).
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