



## Review

## Current trends and future directions in koala chlamydial disease research



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## ABSTRACT

Infectious diseases can be key threatening processes for biodiversity conservation. However, establishing the relative importance of disease (among other threatening processes) as a driver of species declines can be challenging. Bias in the directions that a research field may take as it develops – due to factors such as conservation policy, funding, public perception, and available expertise and technology – may exacerbate this difficulty. Chlamydiosis (infection with bacteria in Family *Chlamydiaceae*) is an example of an infectious disease with a poorly understood role in koala (*Phascolarctos cinereus*) population dynamics. The arboreal folivorous koala is an internationally recognized iconic species of high conservation, sociocultural and economic value. To date, no studies have quantitatively examined the breadth and scope of research related to koala chlamydiosis, nor systematically identified the current research gaps. We systematically and quantitatively reviewed a comprehensive database of literature related to koala chlamydiosis, classified and examined the main foci of the research, and evaluated research gaps with the goal of assisting policy planning for funding further koala chlamydiosis research. We examined published literature with regard to journal category, authorship, funding, spatiotemporal scope, study foci and type, chlamydial species examined, methodological design and overall findings. Among the 117 peer-reviewed papers published between 1970 and 2016 that fit our criteria, the most striking finding was the relative lack of population-level disease studies within the last two decades to examine mechanisms of chlamydial infection dynamics. This research gap is of particular concern given the potential role of *Chlamydia* in koala population declines, and the recent dramatic changes in our understanding of pathogen phylogeny and improved diagnostic approaches. Our results demonstrate a pressing need for future *in situ* comprehensive longitudinal population-level studies from diverse geographic regions. These studies must utilize up-to-date diagnostic methods capable of distinguishing chlamydial species and strains to elucidate the role of chlamydial infection in koala population declines and the underlying mechanisms involved. They should also employ rigorous epidemiological methodologies and evaluate co-infection, habitat, climatic and demographic data. Our findings suggest some key research gaps concerning koala chlamydiosis, and are hence important for guiding future research into koala chlamydiosis and conservation.

## 1. Introduction

Infectious diseases are increasingly recognized as key threatening processes for the conservation of biodiversity (Daszak, 2000; Smith et al., 2009; Tompkins et al., 2015). Diseases such as chytridiomycosis in amphibians, West Nile Virus in birds, and white nose syndrome in bats have exerted devastating effects on wildlife species around the globe (George et al., 2015; Skerratt et al., 2007; Thogmartin et al., 2012). Microorganisms and parasites are, however, ubiquitous among hosts and in the environment, and many recognized infectious agents

persist in reservoirs in the absence of clinical disease (Haydon et al., 2002). Thus, determining the specific role of infectious agents in host population declines can be difficult, particularly if their effects are not readily detected in the form of mass mortalities and/or overt disease (Scheele et al., 2016; Smith et al., 2009).

Chlamydiosis (associated with infection by bacteria in Family *Chlamydiaceae*) is an example of an infectious disease thought to be endemic and ubiquitous in koala populations (*Phascolarctos cinereus*, Goldfuss), however, it has a poorly understood role in host population declines (Polkinghorne et al., 2013; Rhodes et al., 2011). The arboreal

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flivorous koala is the sole extant representative of Family *Phascolarctidae*, and an internationally recognized iconic species of high conservation, sociocultural and economic value (Black et al., 2014; Hundloe and Hamilton, 1997; Price, 2012). In recent decades widespread population declines have become apparent, particularly in the northern parts of the koala's range (DERM, 2009; McAlpine et al., 2015; Melzer et al., 2000). Koalas are now listed as 'Vulnerable' since 2012 under Australian Federal Government legislation (Environment Protection and Biodiversity Conservation Act 1999; EPBC Act), across two states and one territory (Queensland, New South Wales and the Australian Capital Territory; McAlpine et al., 2015).

Successfully mitigating disease and managing koala populations to prevent extirpation and reverse declines is a current priority for koala conservation. However, the relative impact of chlamydiosis among other threats (including habitat loss, fragmentation, car collisions and dog attacks) is poorly understood, and decision makers have been unable to effectively guide management policy because there is no consensus regarding the nature and extent of the impact of chlamydial infections on koala populations (McAlpine et al., 2015; Rhodes et al., 2011). Inadequate funding and technological constraints may have limited understanding. Indeed, many fundamental aspects of the disease ecology remain understudied (McCallum et al., under revision). To date, no studies have quantitatively examined the breadth and scope of research related to koala chlamydiosis, nor systematically identified the current research gaps.

With the goal of assisting policy planning for funding future koala chlamydial research, we have used the well-established systematic quantitative review technique (Petticrew, 2001; Pickering and Byrne, 2014; Rochman et al., 2016) to examine the extent of the peer-reviewed literature on koala chlamydiosis. Our review examined general characteristics of papers/chapters included (such as journal category, authorship, funding and spatiotemporal scope), their major foci and study types, the breadth of chlamydial species covered, as well as methodological design (including data types, sampling and analysis details). In the following sections we critically analyse the results, consider study caveats, and identify research gaps that should be addressed in future studies.

## 2. Methods

We used systematic search and compilation methodology common to quantitative systematic literature reviews to provide a comprehensive and reproducible overview of the current status of literature on koala chlamydiosis (Pickering and Byrne, 2014; Rochman et al., 2016). Our purpose was to evaluate the breadth and scope of the peer-reviewed literature available since 1970 (encompassing the first paper reporting aetiological diagnosis of chlamydiosis in koalas), including identifying the presence of research trends and weaknesses, in order to highlight gaps to inform policy and funding for future research agendas.

We searched *Web of Science: All Databases* (WoS; Thomson Reuters) and *Scopus* (Elsevier) literature databases using 'title' or 'title, abstract, keyword' searches respectively, with date range from 1 January 1970 to 28 April 2016. We initially examined all English-language literature pertaining to the host species of interest, using the phrase "(koala\* OR *Phascolarct*\*)" (808 articles from WoS, 1151 articles from Scopus), then narrowed these lists by including pathogen or disease-associated terms ("diseas\* or chlam\* or cystit\* or wet bottom" or "dirty tail" or conjunctiv\* or keratoconjunc\*") (103 articles from WoS, 350 articles from Scopus). We assessed each of these entries individually based on title and abstract (and where necessary, a more detailed assessment of the paper) for relevance and inclusion in our database. We then modified this combined list with (i) the addition of any relevant literature cited by the existing articles that was not originally included in the database results, and (ii) specific inclusion and exclusion criteria (see Table 1).

In this study we define koala chlamydiosis as any infection (or co-infection) of the host species *P. cinereus* with bacteria of Family

*Chlamydiaceae*. Importantly, this definition includes infection with both the main species of culturable *Chlamydia* bacteria that have been identified as infecting koalas to date (*Chlamydia pecorum* and *C. pneumoniae*), despite their putatively differing host pathogenicity, as well as phylogenetic and geographic origins (Polkinghorne et al., 2013). It also includes the potential infection with a suite of as yet uncharacterized and potentially unculturable chlamydial species or strains that have been detected via genetic sequencing methodologies (Burach et al., 2014; Devereaux et al., 2003). We have been deliberately inclusive and have not distinguished between infections causing subclinical and clinical disease because a thorough recognition of the transmission and infection dynamics with various chlamydial species will likely be crucial for understanding the expression of clinical disease in koalas, and subsequent population-level impacts.

In addition, we recognize four broadly inclusive disease syndromes related to the clinical signs and gross pathology of chlamydiosis in the koala, including ocular keratoconjunctivitis, urinary tract disease, reproductive tract disease, and respiratory disease (including rhinitis-pneumonia complex) as described by Vogelnest et al. (2008). Development and widespread implementation of appropriate diagnostic tests for atypical intracellular pathogens such as those in Family *Chlamydiaceae* was a gradual process (for example, see Hammerschlag, 1996). This constitutes a bottleneck in the study of atypical emerging infectious diseases and poses challenges for defining sufficiently comprehensive inclusion criteria when specific diagnostic testing is not yet consistently performed. We thus chose to include the many relevant studies with a major focus on investigating clinical signs and pathology that were highly consistent with our chlamydiosis disease syndromes (see above), despite the absence of a clear aetiological diagnosis or pathogen isolation and characterization. Our broad definition allows us to capture the variety of studies that have been performed on chlamydial infections in koalas.

We were particularly interested in peer-reviewed original empirical data papers describing *in situ* population-level chlamydial infection or disease dynamics in koalas. In general, we excluded population-level demographic studies that made no specific mention of chlamydial infection or disease signs in the results. Despite this focus, we recognize that key understanding has been developed through studies predominantly focused on the pathogen in terms of phylogeny and characterization, so these were included. We excluded both general review and modelling papers from the database to eliminate overlapping data reporting, and 'unpublished data' from which their results sometimes derive. Data were extracted from all publications included in the systematic database by a single investigator for consistency, and were cross-checked and evaluated by all co-authors.

For each paper that met our inclusion criteria we thoroughly reviewed their content over several occasions and constructed a database, recording data under the following headings: (i) literature details, (ii) study focus, (iii) chlamydial species, (iv) study type, (v) koala sampling details, (vi) koala source location, (vii) diagnostic methods, (viii) data type, and (ix) funding details. We summarized and analysed the resulting database to detect patterns and also examined trends within the groups defined by major study focus where this was relevant. However, we did not explicitly apply weighting criteria to the various studies due to the breadth of research covered and lack of an appropriately broad and inclusive rating scheme.

Under the 'Study focus' heading, we assessed papers for their main focus of either 'Koala' or 'Pathogen', and after examining the complete database, categorized studies into the following sub-foci that best described their major purpose or aim. Under the 'Koala' category: (i) population-level, (ii) pathology, (iii) immunology, (iv) pharmacology, (v) other pathogen, (vi) diagnosis, and (vii) experimental infection; and under the 'Pathogen' category: (i) *in vitro* response, and (ii) phylogeny. Several of these categories had to be relatively broad to encompass the diversity in the koala chlamydial literature, and it is important to note that papers often reported data that were somewhat peripheral to their

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