



## Short paper

# Treatment of *Chlamydia*-associated ocular disease via a recombinant protein based vaccine in the koala (*Phascolarctos cinereus*)

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## ABSTRACT

Koalas (*Phascolarctos cinereus*) are affected by debilitating chlamydial disease that can lead to blindness, infertility, and death. The causative agent is the intracellular bacterium *Chlamydia pecorum*. While antibiotics can be used to treat koala chlamydial infection, they are often ineffective or cause severe dysbiosis to the animal's unique gut flora. Recent work has progressed on the development of a protective vaccine for *Chlamydia* in the koala. This study demonstrates that the use of a vaccine can have a positive effect in koalas already with clinical signs of ocular disease, suggesting a possible therapeutic effect and an alternative to antibiotic therapy.

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## 1. Introduction

Disease caused by *Chlamydia pecorum* infections contributes significantly to morbidity and mortality in the koala, and is predicted to incite localized extinction events within 5 years [1]. Disease manifests at the ocular (keratoconjunctivitis) and the urogenital (urinary and reproductive tract disease) sites and can lead to blindness, infertility, and death [2]. Current treatment regimens for *Chlamydia* infections in humans and animals are generally with antibiotics [3], but in the era of increasing antibiotic resistance it is important to develop alternative methods [4]. Therapeutic vaccines are a promising new approach to enhance immunogenicity, and reduce pathogen load [5]. There has been some success with temporary improvement of chlamydial disease by therapeutic vaccination with *Chlamydophila abortus* and *C. pecorum* in cattle [6], and early human trials with whole chlamydial organism vaccines showed promise in reducing ocular *Chlamydia trachomatis* infections and trachoma [7]. Development of a chlamydial vaccine for koalas has already shown strong immune responses and reduced infection loads in vaccinated animals e.g. Refs. [8–11]. Here we show, for the first, time that a recombinant

Major Outer Membrane Protein (MOMP) vaccine can not only reduce infectious load but also result in improved clinical signs in koalas with chlamydial ocular disease.

## 2. Materials and methods

Animals included in the study (n = 6) were recruited from the Friends of the Koala Care Center, Lismore, Australia. The vaccinated group (n = 4) received a one-dose regime of the vaccine via the subcutaneous route, consisting of the three rMOMP proteins as the antigens (MOMP-G, MOMP-A, and MOMP-F) and a Tri-adjutant (Tri-Adj). The vaccine is described in detail elsewhere [8]; in brief the Tri-Adj consists of polyphosphazine (PCEP), the host defence peptide HH2 (VQLRIRVAVIRA0-NH<sub>2</sub>) and Poly I:C (Vaccine and Infectious Disease Organisation, Saskatchewan, Canada) and was combined with our rMOMP antigens. Each 500 µl dose consisted of 50 µg of each MOMP and 250 µg each of PCEP, poly I:C and 500 µg of HH2. Veterinary assessments and sampling, while under a short period of anesthesia, were conducted at Keen Street Veterinary Clinic (Lismore, Australia) on each animal at 0, 2, and 3 month time-points. Chlamydial disease scores were assessed using a published disease scoring criteria [2]. Ocular swabs were collected for *Chlamydia* load and stored at –20 °C until DNA was extracted and screened for the presence of *C. pecorum* using a diagnostic quantitative real-time PCR (RT-PCR) targeting a 204 bp fragment of the

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chlamydial 16S rRNA gene [12]. Procedures were performed under: 1) Animal Ethics Committees from University of the Sunshine Coast (AN/A/13/82) and Southern Cross University (ARA 13\_54), and 2) a New South Wales Scientific License (SL101311).

### 3. Results

Four wild-caught koalas with low (Grade 1;  $n = 1$ ) or severe (Grade 3;  $n = 3$ ) *Chlamydia* associated keratoconjunctivitis were vaccinated with a recombinant (r) MOMP based vaccine adjuvanted with Tri-Adj [8]. Two koalas with medium (Grade 2;  $n = 1$ ) or severe (Grade 3;  $n = 1$ ) keratoconjunctivitis were treated with antibiotics for comparison (60 mg/kg subcutaneous injections of chloramphenicol daily [13]). Each koala was sampled at 0, 1, 2 and 3 months post-vaccination. Impressively, all six koalas eliminated their chlamydial infectious loads regardless of treatment (Fig. 1). Two of the four (50%) vaccinated koalas decreased their chlamydial eye disease by 2 months post-vaccination (Fig. 1A and B). The eye scores decreased further by 3 months, however, antibiotic intervention was administered due to veterinary constraints between 2 and 3 months, making it unclear if this trend would have continued regardless. Of the other two vaccinated koalas, one koala's eye score increased at 1 month while the other koalas grade remained stable (Fig. 1C and D). Even after antibiotic intervention in vaccinated koalas, chlamydial disease could not be eliminated, despite clearance of infection, evidencing the inherent difficulty of treating this disease. The eye scores in both antibiotic treated koalas also decreased (Fig. 1E and F), but again did not fully resolve the ocular disease symptoms in the animal with severe (Fig. 1E) disease.

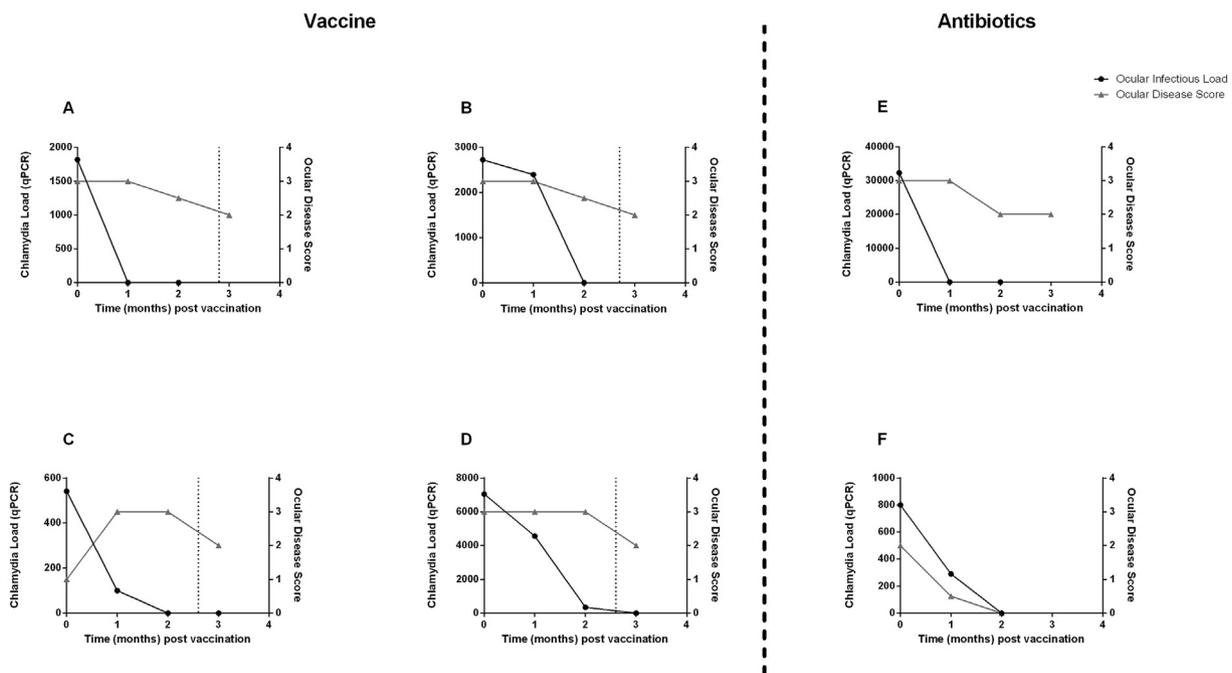
### 4. Discussion

While it remains uncertain what drives the process of pathology seen with chlamydial infection in humans or animals, it is assumed to be an immunopathological process. To date, previous attempts at

vaccination have focussed on reduction of infection load in naïve animals or preventing healthy animals from progressing to disease. Few studies have attempted to reverse the chlamydial immunopathology via vaccination. We have shown here that a rMOMP vaccine can clearly reduce and even eliminate infection, and may also have the potential to reduce, or halt, chlamydial ocular disease in the koala. In comparison, the currently utilized antibiotic treatment was only effective for low (Grade 1) disease signs. The progression of ocular chlamydial disease in an infected koala with no intervention is not currently known. It is assumed that koalas progress from grade 1 through to grade 3 as described in Wan et al. [2]; the timing of this process is also unclear. Studies are required to address this lack of knowledge. It is thus promising that vaccinated animals did not increase their chlamydial ocular disease, as we might expect in naturally diseased animals with no intervention.

Antibiotics do seem to have some beneficial effect in the treatment of koala *C. pecorum*, though they have been reported to be relatively ineffective in the koala with more severe disease e.g. Refs. [13,14]. Further, they have major detrimental side effects to the individual (gut microbiome shifts e.g. Ref. [15]) and the environment (promotion of antibiotic resistance genes e.g. Ref. [16]). In comparison our *C. pecorum* MOMP based vaccine has proved safe to use and associated with increased life span [17]. Thus, therapeutic vaccines could become key tools for disease management and protection in animals and humans e.g. Ref. [5].

The mechanism driving this decreasing infectious load and the positive effect on disease progression is unknown, yet is likely due to the particular immune response beget by vaccination of the koala with this formulation (rMOMP–TriAdj). The specific immune response induced by rMOMP–TriAdj includes a unique set of specific epitope-directed antibodies, divergent from that induced by natural infections [9]. These epitopes have been shown to be directly responsible for the neutralisation activity seen *in vitro* in the plasma and mucosal secretions of vaccinated koalas; thereby potentially allowing the vaccine to take a therapeutic role. The



**Fig. 1.** *Chlamydia* infectious load (black) and disease score (grey) of vaccinated (A, B, C, D) and antibiotic treated (E, F). Vertical dotted line indicates veterinary intervention with antibiotics in vaccinated group. All vaccinated koalas received at 3 rMOMP + TriAdj vaccine at Time 0. All antibiotic treated animals received daily injections of 60 mg/kg chloramphenicol for 45 days.

current study is the first to suggest that the continued development and optimisation of a rMOMP vaccine may result in not only elimination of infection but also reduction and control of chlamydial disease in the koala.

### Contributions

PT and CW designed the study. PT supervised the study. RA conducted koala health assessments and took samples. CW analysed the samples. PT, CW, and RA interpreted the data. PT and CW wrote the manuscript. RA and AP revised the manuscript. PT and AP provided funding.

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