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Understanding the health and production impacts of endemic *Chlamydia pecorum* infections in lambs

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ABSTRACT

Lamydia pecorum is a globally recognised livestock pathogen that is capable of causing severe and economically significant diseases such as arthritis in sheep and cattle. Relatively little information is available on the clinical progression of disease and the long-term effects of asymptomatic and symptomatic chlamydiosis in sheep. Recent studies in calves indicate that endemic *C. pecorum* infections may reduce growth rates. To investigate the clinical health parameters and production impacts of endemic *C. pecorum* infection in an Australian commercial lamb flock, we performed bimonthly sampling and clinical health assessments on 105 Border Leicester lambs from two to ten months of age. Chlamydial status was investigated via serology and species-specific quantitative PCR. Throughout the study period, conjunctivitis remained a persistent clinical feature while signs of arthritis (e.g. palpable synovial joint effusions) resolved in a subset of lambs while persisting in others. Clinical disease and *C. pecorum* infection were highest at six months of age (weaning). As previously reported, peak seroconversion tends to occur two months after the onset of clinical symptoms (6 months of age), with lambs clearing chlamydial infection by 10 months of age, despite ongoing disease still being present at this time. Notably, the presence of chlamydial infection did not affect lamb mass or growth rates throughout the study. At necropsy, *C. pecorum* was not detected within the joints of lambs with chronic arthritis. Molecular analysis of the strains in this flock suggest that the infecting strains circulating in this flock are clonal *C. pecorum* pathotypes, denoted ST 23, commonly associated with conjunctivitis and polyarthritis in Australian sheep. This study provides a platform for further research in the epidemiology and disease transmission dynamics of *C. pecorum* infections in sheep.

1. Introduction

Of the 11 species of the genus *Chlamydia* in the family *Chlamydiaceae* (Sachse et al., 2014), *Chlamydia pecorum* (*C. pecorum*) is one of the most globally distributed. This intracellular bacterial pathogen is capable of infecting a range of hosts including sheep, goats, pigs, cattle and wildlife (Polkinghorne et al., 2013; Walker et al., 2015). Owing to its highly adapted intracellular lifecycle requiring eukaryotic cells for replication, *Chlamydia* is able to successfully evade host immunity and cause persistent, asymptomatic and symptomatic infections in its hosts (Everett, 2000; Hogan et al., 2004). Typical clinical presentations in livestock include arthritis and conjunctivitis in sheep and cattle and encephalomyelitis in cattle (Walker et al., 2015). Molecular epidemiological studies using multi-locus sequence typing (MLST) suggest that multiple genetically distinct *C. pecorum* sequence types are present in both clinically healthy and diseased sheep and cattle (Jelocnik et al., 2013, 2014a,b). Sheep with conjunctival and joint disease for example, had unique sequence types (e.g. ST23) in comparison to rectally shed *C. pecorum* strains (e.g. ST62, ST63, ST81; Jelocnik et al., 2014a).

While overt disease caused by *C. pecorum* infections is predicted to have an impact for producers (Walker et al., 2015), studies in cattle have suggested that asymptomatic infections may be common. For example, *C. pecorum* appears to be endemic to the gastrointestinal tract of dairy cattle (Li et al., 2016). While these infections are generally thought to be harmless, several studies over the last 10 years have emerged to suggest that these asymptomatic chlamydial infections in dairy calves may contribute to a variety of pathoclinical features including clinically silent respiratory infections (Jaeger et al., 2007), the presence of co-infections (Reinhold et al., 2008), reduced body weight

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gains (Reinhold et al., 2008; Poudel et al., 2012) and changes in biochemistry (Poudel et al., 2012) and haematology (Reinhold et al., 2008) parameters.

The Australian national prevalence of *C. pecorum* shed in faeces is estimated to be 30% in lamb flocks (Yang et al., 2014). Similar to calves (Jee et al., 2004), lambs are likely born free of chlamydiae but are thought to rapidly acquire infection by three months of age (Clarkson and Philips, 1997), presumably by the faecal-oral route. It is unknown whether physiological and health impacts occur in asymptomatically infected lambs as they appear to do in calves. Potential economic implications such as weight loss, poor lamb survival and losses associated with on farm culling due to chronic chlamydial infection are yet to be investigated. Alongside these unknown impacts, the detection of *C. pecorum* in the gastrointestinal tract is not well correlated with symptomology as demonstrated in recently described presumptive cases of chlamydial arthritis (Walker et al., 2016). This therefore poses diagnostic conundrums and treatment dilemmas for field veterinarians dealing with subclinical and clinical infections.

In this current study, we examine the impacts of symptomatic and asymptomatic *C. pecorum* infection in an endemically infected lamb flock by characterising disease progression, genetic identity of the infecting strains, detection rates over time and the subsequent acute and long term effects *C. pecorum* exerts on the health status of infected lambs.

2. Materials and methods

2.1. Animals sampled in this study

This study analysed samples collected from longitudinal sampling of homebred lambs on a commercial sheep farm in Nevertire, NSW, Australia, as previously described (Bommana et al., 2018) to investigate the relationship between infection and disease in these animals This farm had a known history of *C. pecorum* infections and associated clinical disease (e.g. arthritis, conjunctivitis and ill-thrift) with some years worse than others. Long-acting oxytetracycline antimicrobials have been used in the past on this farm with some success in individual animals, but the owner reported ongoing cases every year with and without treatment.

All animals used in this study were Border Leicester lambs (n = 105) born and reared in typical paddock conditions for Central West, New South Wales. Border Leicesters, originally from Britain, were first introduced into Australia in the 1870's. This introduction revolutionised the Australian sheep industry in the production of prime lamb mothers (first cross Border Merino ewes) by combining the meat and maternal abilities of the Border Leicester with the traditional wool producing Merino (ASSBA, 2017). 105 Border Leicester lambs were initially enrolled in this study, but on farm losses (death from predation, husbandry procedures such as marking and other causes of loss) and miss-musters of lambs occurring on a working sheep farm meant that additional lambs were subsequently enrolled at the second sampling time point to account for these losses. The only time lambs were housed indoors (e.g. the shearing shed) was for sample collection and routine on-farm shearing. Otherwise, lambs were managed outdoors under normal on farm practices including marking, feeding, weaning and vaccinations. Lambs are routinely marked at two months of age, weaned at six months of age and finished at ten months of age. Lambs receive routine clostridial vaccinations at marking and weaning and are drenched biannually (two times in the summer) for internal parasites. No antimicrobials nor veterinary prescription drugs were administered to any lambs at any point during this study. Ethics approval for this study was granted by the University of the Sunshine Coast Animal Ethics Committee (AN/S/14/31).

2.2. Clinical examination, sample collection and necropsy

Lambs in this study were uniquely ear tagged, individually examined and sampled at bi-monthly intervals over a 10 month period. Clinical assessment and sample collection were performed by District Veterinarians. Assessment of individual lambs consisted of an conjunctival, gait and joint score. The conjunctival assessment consisted of a scoring system based on the presence of conjunctival inflammation and conjunctival discharge. Conjunctival redness was expressed as a nominal score from 0 to 2 (0 = normal pink, 1 = pale pink, 2 = red). Type of conjunctival discharge was expressed as a score from 0 to 3 (0 = nil discharge, 1 = dry, 2 = serous, 3 = purulent). In this study, a dry conjunctival discharge was defined as a discharge that was dry (non-wet) and crusty as opposed to wet and sticky. For the purpose of this study, animals were defined as having conjunctivitis if any signs of conjunctival discharge were present regardless of individual subjective colour assessment. Individual lambs were also given a gait score from 0 to 3 (0 = normal gait; 1 = mild stiffness and weight bearing; 2 = moderate shifting lameness; 3 = severe non-weight bearing). Gait scoring was assessed at the time of sample collection and post-sample collection (1 h later) by observing lamb locomotion in the sheep yards. All joints of the leg, with particular attention to the carpal and hock joints were palpated for joint effusions and assigned a score as follows: 0 = normal joints; 1 = one joint affected; 2 = two joints affected; 3 = three or more joints affected). Body weight was also recorded just prior to sample collection at each time point.

Samples collected in this study consisted of conjunctival, vaginal and rectal cytological brush swabs and venous jugular blood. Methods used for serological and *C. pecorum* PCR testing of these animals has been recently described in Bommana et al. (2018). At the conclusion of the study, the owner elected for euthanasia of two male lambs with ongoing joint lesions (lamb ids B46 and Or53) which would normally be culled on farm due to not meeting owner and/or market specifications. Following euthanasia, a complete necropsy of both lambs was performed on farm. All visceral organs, airways, gastrointestinal tract sections and affected joints were swabbed for *C. pecorum* qPCR analysis. Affected joints were also submitted whole to the State Veterinary Diagnostic Laboratory for histopathological analysis.

2.3. Chlamydia screening

Chlamydia screening results from the animals analysed in this study were previously reported (Bommana et al., 2018). Briefly, *C. pecorum* infections in the sampled animals were assessed for the presence of chlamydial DNA shedding from different anatomical sites (e.g. vaginal, rectal and conjunctival) using a *C. pecorum*-species-specific qPCR. For each of the samples collected, qPCR was performed in duplicate. Anti-*Chlamydia* antibodies using the *Chlamydia* complement fixation test (CFT) was performed on serum samples recently described in Bommana et al. (2018).

2.4. C. pecorum molecular typing

C. pecorum-specific multi-locus sequence typing (MLST) was applied on a subset of four conjunctival and five rectal *C. pecorum* positive samples from six randomly selected lambs from this study (Supplementary Table 1) as previously described (Jelocnik et al., 2013). The sequence type (ST) for each of the nine typed *C. pecorum* strains from this study was determined from the *Chlamydiales* MLST database (http://pubmlst.org/chlamydiales/). In order to assess the phylogenetic relationships of these newly typed strains to the previously described 10 *C. pecorum* STs found infecting Australian sheep (Jelocnik et al., 2014a), we constructed a Maximum Likelihood (ML) phylogenetic tree. The *C. pecorum* ST 48, found in ovine hosts from Europe and USA, was used as an outgroup. A mid-point rooted ML phylogenetic tree was constructed from the alignment of the concatenated MLST sequences