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Chlamydia pecorum in Joint Tissue and Synovial Fluid of a Koala (*Phascolarctos cinereus*) with Arthritis

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ABSTRACT: A small number of koalas (*Phascolarctos cinereus*) presented to wildlife hospitals in Queensland, Australia, with signs of arthritis in one or more joints. Molecular analysis identified *Chlamydia pecorum* in the tarsal tissue and synovial fluid of an affected joint of a koala, suggesting that in addition to livestock, *C. pecorum* has the potential to cause arthritis in the koala.

Chlamydia pecorum is an endemic bacterial infection within koala (*Phascolarctos cinereus*) populations, particularly those from Queensland and New South Wales, Australia (Polkinghorne et al. 2013; Patterson et al. 2015). Ocular and urogenital mucosa are the primary sites of infection. To date, the most common clinical presentations of koala chlamydiosis are ocular disease in the form of unilateral or bilateral keratoconjunctivitis that can result in blindness and inflammation of the bladder (cystitis) and reproductive tract organs (Polkinghorne et al. 2013; Johnston et al. 2015). Pathology in the upper reproductive of female koalas is of greatest concern, threatening the fecundity of infected populations (Polkinghorne et al. 2013). Additionally, koala retrovirus (KoRV)-B, a new strain of the earlier KoRV-A (Denner and Young 2013), has recently been associated as a factor influencing the outcome of *C. pecorum* infection in koalas (Waugh et al. 2017).

Polyarthritis is a common but underdiagnosed presentation of *C. pecorum* infection in lambs (*Ovis aries*) that presents as lameness, stiffening of the joints, fever, and weight loss (Walker et al. 2015). Over more than a decade, arthritis in one or more joints has been observed in a small number of wild koalas presenting to wildlife hospitals in Queensland. The etiology of this disease is not known in koalas. We used molecular techniques to identify the causative agent of arthritis in a koala.

A free-ranging male koala from Doonan, Queensland, Australia, presented to Australia Zoo Wildlife Hospital (Beerwah, Queensland, Australia) for evaluation. Veterinary examination revealed the animal to be in poor body condition, apparently blind, with a swollen right tarsal joint, a skull tumor, chronic dermatitis, and exhibiting clinical signs consistent with cystitis. Ultrasound examination revealed thickening of the bladder wall. The koala was euthanized, and a urogenital swab and soft tissue and synovial fluid from the tarsal joint were collected for molecular analysis at the University of the Sunshine Coast (Sippy Downs, Queensland, Australia). Further pathologic and microbiologic investigation (including bacterial culture on blood agar plates and cytology of synovial fluid) was performed at Vepalabs Veterinary Pathology (Woolloongabba, Queensland, Australia). Tissues for histologic examination were collected in 10% buffered formalin for fixation and later were prepared by Vepalabs Veterinary Pathology as paraffin-embedded blocks and sectioned prior to H&E staining. Bony tissue was decalcified prior to paraffin preparation and staining.

Histopathologic examination revealed an osteochondroma of the skull and severe focal chronic granulomatous deep dermatitis with intralesional fungal hyphae. Cytology of the tarsal joint (synovial) fluid consisted of greater than 90% neutrophils, some of which appeared to be degenerate, greater than 10% activated macrophages or synovial lining cells, and the occasional small lymphocyte. Bacteria, fungi, or other microorganisms were not seen. This presentation was deemed to be

neutrophilic arthritis, most likely of bacterial origin. Culture of the synovial fluid did not reveal any bacterial growth.

We obtained DNA extractions on the tarsal synovial tissue, urogenital tract, and synovial fluid swabs with a QIAamp DNA Mini Kit (Qiagen, Chadstone, Victoria, Australia) per the manufacturer's protocol on DNA purification from tissues and buccal swabs (spin protocol). We used tarsal synovial tissue DNA extracts to test for KoRV-A and KoRV-B DNA, targeting the viral envelope gene, as previously described (Waugh et al. 2017). All extracts were screened for *C. pecorum*, targeting a fragment of the 16S ribosomal RNA gene, as previously described (Wan et al. 2011). *Chlamydia pecorum*–positive samples were subjected to *C. pecorum* multilocus sequence typing (MLST), amplifying seven conserved housekeeping genes (Jelocnik et al. 2013). The *C. pecorum* MLST amplicons were purified by using the Roche High Pure PCR Product Purification Kit (Roche, North Ryde, New South Wales, Australia) and sequenced by Macrogen Inc. (Seoul, South Korea). Sequence analysis was performed in Geneious R9.1.3 (Kearse et al. 2012). We trimmed and identified the sequences by using the PubMLST database (Jolley and Maiden 2010). Concatenated sequences (3,095 base pairs) were aligned with other *C. pecorum* MLST sequences from koalas (n=5), livestock (n=22), and a strain detected in both (n=1), to generate a Bayesian phylogeny by using the MrBayes plugin (Huelsenbeck and Ronquist 2001) under the HKY85 substitution model.

We detected *C. pecorum* in DNA extracts of tarsal synovial tissue and urogenital tract and synovial fluid samples by the *C. pecorum*–specific quantitative PCR. Amplicons could be obtained for all seven genes within the *C. pecorum* MLST scheme for the urogenital tract sample only (GenBank accession numbers: MF993359-MF993365). Phylogenetic analysis of the resulting concatenated MLST gene fragments revealed that this *C. pecorum* is a novel strain clustering in a subclade of *C. pecorum* strains of koala and livestock origins (Fig. 1). We amplified *FumC* from the DNA extract of the tarsal synovial tissue, however, amplification of the remaining six *C. pecorum* MLST housekeeping genes failed, likely due to low *C. pecorum* DNA levels present. The DNA extract of the right tarsal tissue was also positive for KoRV-A and KoRV-B DNA.

To our knowledge, this is the first case report describing arthritis in a *C. pecorum*–infected koala, suggesting that *C. pecorum*–induced presentations of arthritis are not limited to livestock; infected koalas may also exhibit this clinical presentation. Moreover, the detection of *C. pecorum* within tissue and joint fluid from an affected joint suggests that *C. pecorum* is not limited to mucosal epithelia and has the potential to disseminate systemically in the koala. The presence of *C. pecorum* in a joint is surprising but not completely unexpected, as it can be sporadically detected following cell culture or, more easily, by PCR from the affected joints of sheep with *C. pecorum* polyarthritis (Walker et al. 2015). Although the *C. pecorum* MLST suggests that this particular *C. pecorum* strain is novel, it is too early to speculate whether certain *C. pecorum* strains in koalas may be predisposed to joint dissemination, as occurs in sheep and cattle (Jelocnik et al. 2014). Unfortunately, little is known regarding the potential of *C. pecorum* strains to disseminate from a primary site of infection in koalas or in other species, with the mechanism of this proposed dissemination also unclear.

The confirmation of KoRV-A and particularly KoRV-B raises questions over whether retroviral infection may have had a causal role in the noted osteochrondroma, fungal dermatitis, poor body condition, and more importantly, the severity and dissemination of the *C. pecorum* infection. Interestingly, both osteochrondroma and dermatitis previously have been linked to KoRV-positive Queensland koalas (Hanger and Loader 2014). Neoplasia and opportunistic infections are commonly associated and exacerbated in immunocompromised retroviral hosts (Denner and Young 2013). Cytokine and lymphocyte modulation

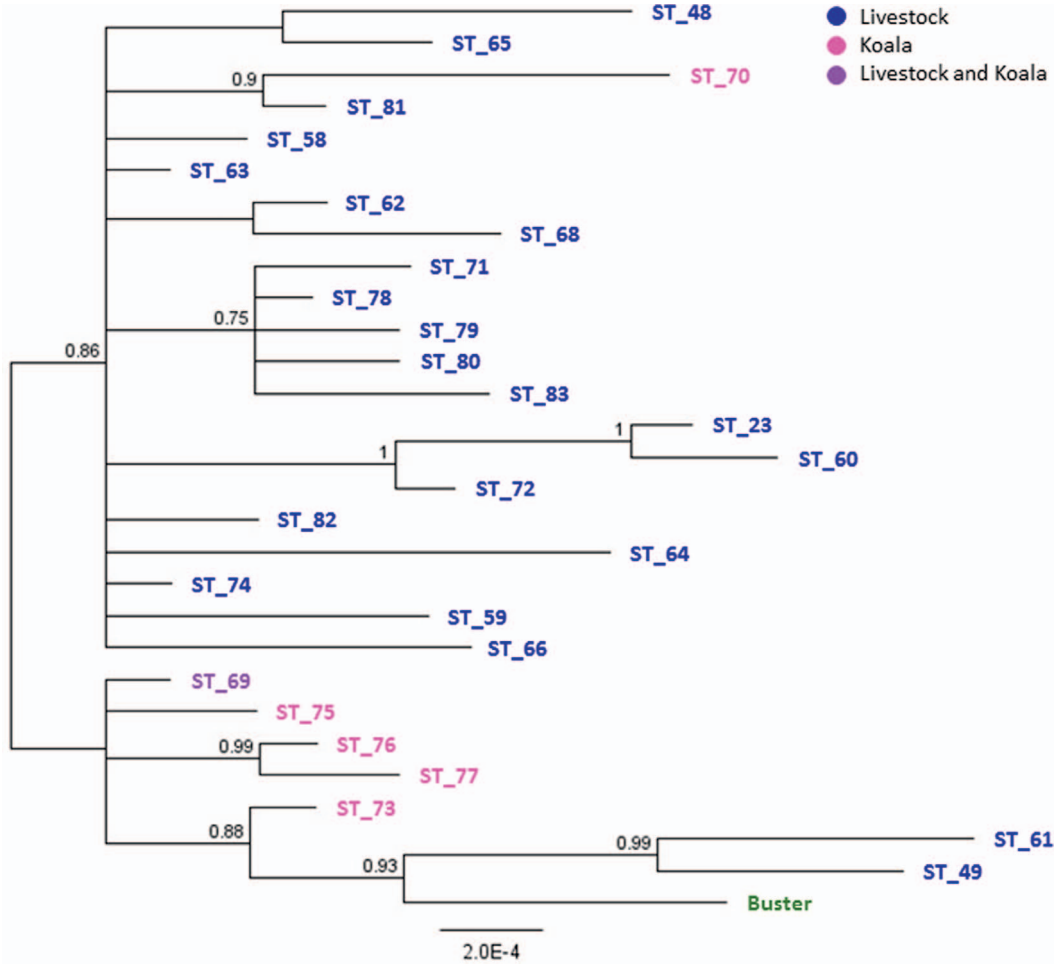

FIGURE 1. Bayesian phylogeny of *Chlamydia pecorum* multilocus sequence types identified in livestock and koalas from the PubMLST database (Jolley and Maiden 2010). The phylogenetic tree was built by using 29 sequences of 3,095 base pairs length under the HKY85 evolutionary model. Posterior probabilities exceeding 0.75 are shown at internal nodes.

has also been observed in koalas with KoRV-B (Denner and Young 2013; Maher and Higgins 2016). Although a recent study found that KoRV-B coinfection may predispose koalas to chlamydiosis (Waugh et al. 2017), this is not the case for KoRV-A infection (Legione et al. 2017). It is currently unclear if and how viral coinfection and potential immunosuppression may support the dissemination of *C. pecorum* infection from a primary site of infection, such as the genital tract, to the joint. Our case study provides insight into the diverse range of pathologies that *C. pecorum* can cause in the koala and highlights how little we still know about interaction between this pathogen, other coinfections, and their iconic marsupial host.

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