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Detection of *Chlamydia pecorum* in joints trimmed from ovine carcases with arthritis at an abattoir in southern Australia

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Highlights

- The prevalence of *C. pecorum* in trimmed abnormal joints was determined by qPCR.
- Nine of 148 synovial samples tested positive for *C. pecorum* (prevalence 6.1%).
- None of the positive carcasses were condemned for systemic involvement.
- The arthritis trim weight of positive and negative carcasses did not differ.

Abstract

*Chlamydiae* are obligate intracellular bacteria that infect a broad range of host species, including sheep. Two species of *Chlamydia* infect sheep, *C. abortus*, which is a major cause of abortion in both sheep and goats, and *C. pecorum*, which causes pneumonia, arthritis/polyarthritis, encephalomyelitis, conjunctivitis, enteritis, abortion and metritis and infertility in domestic ruminants and pigs. The prevalence of faecal shedding of *C. pecorum* is relatively common amongst lambs in Australia. The aim of the work presented here was to use qPCR to determine the prevalence of *C. pecorum* in synovial samples obtained from abnormal joints trimmed from lamb carcasses at one abattoir in southern Australia. The study included 53,131 carcasses screened for arthritis, of which 369 had at least one abnormal joint trimmed. One hundred and forty eight trimmed joints were undamaged and suitable for PCR testing. The prevalence of *C. pecorum* in synovial tissue collected from the abnormal joints was 6.1% and the bacterial concentration ranged from $6 \times 10^3$ to $7.6 \times 10^5$/g of synovial tissue. Five of the positive joint samples were from carcasses that had one joint trimmed for arthritis and four were from carcasses from which two joints had been trimmed. None of the carcasses from which the positive joint samples originated were condemned. The average arthritis trim weight of the carcasses from which synovial tissue tested positive for *C. pecorum* was 1.112 kg (95% confidence interval 0.637-1.586 kg) and this did not differ from the carcasses from which synovial tissue was not positive for *C. pecorum*. (mean 0.997 kg, 95%
Further research is required to determine the on-farm production losses associated with *C. pecorum* infection in Australian lambs.

**Key words**
Sheep; arthritis; *Chlamydia pecorum*; qPCR

**Introduction**

*Chlamydiae* are obligate intracellular bacteria that infect a broad range of host species, including sheep. Two species of *Chlamydia* have principally been reported to infect sheep, *C. abortus* which is a major cause of abortion in both sheep and goats, and *C. pecorum*, which causes pneumonia, arthritis/polyarthritis, encephalomyelitis, conjunctivitis, enteritis, abortion and metritis and infertility in domestic ruminants and pigs (Everett, 2000; Fukushi and Hirai, 1992; Longbottom and Coulter, 2003; Polkinghorne et al., 2009; Rodolakis and Laroucau, 2015; Rodolakis and Mohamad, 2010; Walker et al., 2015). Australia is believed to be free from *C. abortus* (Animal Health Australia, 2016; McCauley et al., 2010; Rodolakis and Laroucau, 2015; Timms, 2009), whereas *C. pecorum* has been associated with arthritis/polyarthritis (Jelocnik et al., 2013a; Jelocnik et al., 2014; Robson, 2003; Robson, 2004; Tammemagi and Simmons, 1968), conjunctivitis (Jelocnik et al., 2013a; Jelocnik et al., 2014; Tighe and Slattery, 2012), and less frequently abortion (Slattery, 2008) in Australian sheep. *C. pecorum* has also been isolated from faecal samples and rectal swabs of healthy Australian sheep without the typical clinical signs of infection (Jelocnik et al., 2014; St George, 1971; Yang et al., 2014).
Bacterial arthritis/polyarthritis is a significant cost to the Australian sheep industry, estimated at A$39m annually (Lane et al., 2015). Globally, *Erysipelothrix rhusiopathiae* is considered to be the most common cause of bacterial arthritis/polyarthritis in lambs (Craig et al., 2015), and previous surveys of Australian slaughter lambs are generally in agreement with this belief (Lloyd et al., 2016; Paton et al., 2003). However, the previous microbiological surveys of bacterial joint infections in Australian lambs have used traditional bacterial culture techniques that would not have detected *C. pecorum*. Despite this, arthritis/polyarthritis due to *C. pecorum* is known to occur in Australia, and has been reported from 13-month old rams in Queensland (Tammemagi and Simmons, 1968) and from 3-6 month old lambs on the Central Tablelands of New South Wales (Jelocnik et al., 2014; Robson, 2003; Robson, 2004).

Using a quantitative PCR (qPCR) targeting the chlamydial outer membrane protein of *Chlamydia pecorum*, the prevalence of faecal shedding of *C. pecorum* was found to be relatively common amongst lambs from eight different farms across four states of Australia (New South Wales, South Australia, Victoria and Western Australia), with approximately 30% of lambs shedding the bacteria in their faeces (Yang et al., 2014). Faecal shedding was highest at weaning (~12 weeks of age), fell during the post-weaning period (~19 weeks of age) and was lowest pre-slaughter (~29 weeks of age), similar to the time-course of shedding in naturally infected dairy calves (Jee et al., 2004). Serological testing of Australian sheep for export to demonstrate freedom from *C. abortus*-infection using a complement fixation technique resulted in a 16% false-positive rate in 380 rams tested, with the prevalence of false-positive results not affected by age, breed or state of origin (McCauley et al., 2010). The complement fixation test used is reported to have low specificity with cross reactions to other chlamydia species, including *C. pecorum* (Longbottom and Coulter, 2003).
We recently reported the results of traditional bacterial culture of samples collected from ovine joints trimmed for arthritis at an abattoir in southern Australia by Australian government Department of Agriculture and Water Resources-accredited meat inspectors in accordance with the Australian Standard for the Hygienic Production and Transportation of Meat and Meat Products for Human Consumption (Lloyd et al., 2016; Anon, 2002). *E. rhusiopathiae*, alone or in combination, was the bacterial species isolated most frequently isolated (28 of 175 joints tested, prevalence 16%), followed by *Streptococcus* spp. (9 of 175 joints tested, prevalence 5.1%), as well as a range of bacteria that constitute the normal skin flora of sheep. The aim of the work presented here was to use qPCR to determine the prevalence of *C. pecorum* in synovial samples in a subset of the trimmed abnormal ovine joints collected as part of the previously reported work. In addition, histopathology and immunohistochemistry were used to assess the associated pathology in joint samples that tested positive for *C. pecorum*.

**Materials and Methods**

**Collection of arthritic joint samples**

Arthritic joint samples were collected from ovine carcases at an abattoir in southern Australia that processes animals from New South Wales, Queensland, South Australia and Victoria as described previously (Lloyd et al., 2016). The number of carcases examined, the number with arthritis and the number sampled, as well as regional source, age and breed of the animals, is provided in Table 1. The samples for *C. pecorum* testing were collected in two phases, December 2014 to January 2015 and in March 2015. The sample collection was part of a larger project designed to investigate the association between arthritis and docked tail length (Lloyd et al., 2016).
Briefly, carcases with arthritis were identified by Australian government Department of Agriculture and Water Resources (DAWR) (formerly AQIS) -accredited meat inspectors in accordance with the Australian Standard for the Hygienic Production and Transportation of Meat and Meat Products for Human Consumption (Anon, 2002). The arthritic joints were not opened during inspection; however, some joints had been damaged during processing prior to inspection. Following trimming of affected joints by the meat inspectors, the joints were placed in plastic bags (one bag per carcase) and stored in buckets on the slaughter floor until the next break and then placed on ice. This ensured that all the sampled joints were chilled within two hours of collection. The joints sampled were from the legs and included the carpus, elbow, tarsus and stifle. Metacarpal and metatarsal joints could not be sampled because these had been removed from the carcases prior to the point of inspection. At the end of each five hour shift, all the collected joints and associated trim were re-examined grossly, weighed and the damaged joints then discarded because of the possibility of cross-contamination on the slaughter floor. The undamaged joints were then placed back on ice for up to 18 hours prior to collection of samples for PCR.

Faecal samples were not collected from the carcases with arthritis because by the time a carcase reached the point of inspection it had been eviscerated and the associated gastrointestinal tract sealed for hygiene/food safety reasons.

A sterile set of instruments was used to remove subcutaneous and peri-synovial tissue from around the joint and then a second set of sterile instruments was used to open the joint. At least 1 g of synovial tissue was collected for PCR, as well samples for histopathology and immunohistochemistry (synovial tissue samples in 4% neutral buffered formalin). The synovial samples for PCR were stored at -20 °C until further analysis.
During December 2014 and in January 2015 only the most swollen, undamaged joint per carcase was sampled for PCR. In March 2015 all the undamaged abnormal joints from each carcase were sampled PCR. No joints classified as negative by the inspectors were sampled because these joints are not trimmed from the carcases and to do this would have resulted in a financial penalty for farmers.

All the trim associated with arthritis from each carcase was collected into a clean plastic bag and then weighed using a 30 kg by 5 g electronic crane scale.

Lamb age, breed and property identification code (PIC) were obtained from abattoir records. Lamb age was determined by abattoir personnel who checked the dentition of the animals at the time of slaughter. A lamb is defined as female, castrate or entire male ovine that has zero permanent incisor teeth and a young lamb as a female, castrate or entire male ovine that has zero permanent incisor teeth and no evidence of eruption of permanent upper molar teeth (AUS-MEAT Limited, 2010). The PIC code was assigned to a region within South Australia using a key provided by Primary Industries and Research South Australia. For lambs sourced from outside South Australia, region was at the level of the State.

**DNA isolation**

DNA was extracted from 50 mg of synovial tissue using a QIAamp® Fast DNA Tissue Kit (QIAGEN). A negative control (no synovium sample) was used in each extraction group.
The testing for *C. pecorum* was conducted using the method of Yang et al. (2014). Briefly, a species-specific 76 base pair (bp) product was amplified from the *C. pecorum* outer membrane protein cell surface antigen gene (ompA) using the forward primer CpecOMP1 F 5’-CCATGTGATCCTTGCGCTACT-3’, the reverse primer CpecOMP1 5’-TGTCGAAAACATAATCTCCGTAAAAT-3’ and the probe CpecOMP1-S 5’-CAL-Fluor Orange-560-TGCCACGCTTAGCTTAGTAMARA-3’. Each 15 μL PCR mixture contained 1x PCR buffer, 4 mM MgCl₂, 1 mM each deoxynucleotide triphosphate, 1.0 U KAPA DNA polymerase (MolBio), 0.2 μM each of forward and reverse primers, 0.2 μM each of forward and reverse internal amplification control (IAC) primers targeting a plasmid containing a fragment of a coding region from Jembrana disease virus (JDV), 50 nM specific probe, 50 nM IAC probe, 10 copies of IAC template and 1 μL sample DNA. The PCR cycling conditions consisted of 95 °C for 3 minutes, followed by 45 cycles of 95 °C for 20 seconds and 60 °C for 45 seconds. A standard curve was generated using 10-fold serial dilutions of plasmids containing the cloned ompA gene amplified from *C. pecorum*. PCR contamination controls were used, including negative controls and separation of preparation and amplification areas.

A standard curve for quantifying *Chlamydia* spp. DNA was generated by cloning the PCR products amplified from *C. pecorum* into pGEM-T (Promega) and transforming into *Escherichia coli* competent cells. Plasmid copy number was calculated based on the plasmid size (base pairs) and DNA concentration. 10-fold series dilutions of plasmids were conducted from 10,000 copies down to 1 copy of the genomic template for sensitivity testing. The limit of quantitation (LOQ) for this qPCR assay was 8 gene copies per μl of DNA (Yang et al., 2014). Template copy numbers were converted to numbers of organism present on the basis that the targeted gene (*OmpA*) is a single copy gene (Lan and Igo, 1998) and bacterial
genomes are haploid. Therefore, the number of plasmids detected was equivalent to the number of *Chlamydia* spp. per µl of DNA (total elution volume = 100µl/50mg of tissue). This was then converted to number of *Chlamydia* per gram of tissue.

**Histopathology**

Tissue was fixed in 4% phosphate buffered formalin and trimmed as required after fixation. Following routine processing, 4µm sections were stained with Mayer’s haematoxylin and Young’s eosin, and examined microscopically.

Synovial proliferation and inflammatory infiltrate results were allocated to seven categories, from nil, minimal, mild, mild to moderate, moderate, moderate to marked or marked, with the cell type or types comprising the inflammatory infiltrate described. Other changes (i.e. haemorrhage, fibrosis) were also noted. Synovial proliferation and inflammatory infiltrate were converted to a numerical score, with nil equal to zero and marked equal to six. The scores for synovial proliferation and inflammatory infiltrate were then added to give a total score for each sample.

**Immunohistochemistry**

Sections were cut on silane slides at 4 µ, deparaffinized to water and placed into a DAKO® Autostainer Plus. Slides were exposed to 3% hydrogen peroxide, *Chlamydia* antibody (1:200, anti-*Chlamydia* mouse monoclonal, R-Biopharm), DAKO® REAL ™Envision, and DAKO® DAB+ substrate-chromogen solution with Tris Buffered Saline rinses in between. Sections were then removed from the Autostainer and counterstained with haematoxylin before being dehydrated and mounted. For each sample the negative control was a section exposed to 3%
hydrogen peroxide, DAKO® REAL™ Envision, and DAKO® DAB+ substrate-chromogen, but not Chlamydia antibody.

Statistical analysis

Prevalence was expressed as the percentage of the samples that were positive for C. pecorum on PCR. The correlation between the total histopathological score for each sample and the concentration of C. pecorum detected in the sample was calculated (Microsoft Excel). A 95% confidence interval was used to determine significant differences in trim weight between carcases from which synovial tissue tested positive for C. pecorum and those that were negative.

Results

Prevalence of C. pecorum

Nine of the 148 synovial samples were positive for C. pecorum (prevalence 6.1%). Two of the positive samples were from carcases sourced from New South Wales, three were from carcases sourced from Victoria and four were from carcases sourced from South Australia (Table 2). The prevalence of infection was too low to determine if there were associations between infection and source of the carcases or the breed or age of the lambs.

Five of the positive joint samples were from carcases that had one joint trimmed for arthritis and four were from carcases from which two joints had been trimmed. The most common joint trimmed was the tarsus followed by the stifle and carpus. None of the carcases from which the positive joint samples originated were condemned. There was no significant difference in the trim weight of the carcases from which synovial tissue tested positive for C. pecorum.
peccorum and those that did not (positive 1.112 kg, 95% confidence interval 0.637-1.586 kg; negative 0.997 kg, 95% confidence interval 0.840-1.154 kg).

Histopathological changes

The majority of the q-PCR positive joint samples had no evidence of synovial proliferation (Table 3). One sample had minimal synovial proliferation and two had mild proliferation. Inflammatory infiltrates ranged from nil to marked and were predominantly plasmacytic or lymphoplasmacytic, although in some samples there was a significant component of neutrophilic infiltration. Total histopathology scores ranged from zero to eight (Table 3).

On immunohistochemistry, C. pecorum was detected either peri-vascularly or associated with lymphocytic-plasmacytic infiltrates, neutrophils or hypertrophied synovium (Figure 1).

Concentration of C. pecorum

The concentration of C. pecorum ranged from $6.0 \times 10^3$ to $7.6 \times 10^5$ organisms per gram of tissue. There was no correlation ($P<0.05$) between the concentration of C. pecorum and the total histopathological score for each sample (Correlation coefficient = 0.24).

Discussion

This study used a qPCR targeting the chlamydial outer membrane protein of C. pecorum (Yang et al., 2014) to determine the prevalence of C. pecorum in abnormal joints trimmed from lamb carcases at a commercial abattoir in southern Australia. Of the 148 synovial tissue samples tested, including samples collected from carcases from animals sourced from three states in Australia (New South Wales, South Australia and Victoria), nine were positive for C. pecorum (prevalence 6.1%). The 148 samples tested for C. pecorum were a subset of 175
samples tested using traditional microbiological culture methods in which we detected 16% prevalence of *E. rhusiopathiae* and 5.1% prevalence of *Streptococcus* spp. (Lloyd et al., 2016). Together these results suggest that *C. pecorum*, although present across the sheep-raising regions of south-eastern Australia, may be less common than *E. rhusiopathiae* but more common than *Streptococcus* spp., as a cause of bacterial arthritis/polyarthritis in Australian slaughter lambs. However, false negative results on culture of synovial fluid of animals with septic arthritis are common (Craig et al., 2015). In addition, the cell walls of *E. rhusiopathiae* and *Streptococcus* spp. are relatively resistant to breakdown by mammalian lysosomal enzymes and subsequent removal by macrophages and, as a result, persistent inflammation can develop, even when infection appears to have resolved or bacteria cannot be isolated (Craig, et al.,). PCR is likely to be much more sensitive at detecting bacteria in ovine joint infections than traditional bacterial culture methods, which suggests that these rankings of relative importance should be interpreted with caution. Indeed, PCR is reported to be more sensitive at detecting *E. rhusiopathiae* in abattoir samples, including ovine joints, than bacterial culture (Wang et al., 2002).

Compared to a recent longitudinal survey of the prevalence of faecal shedding of *C. pecorum* by Australian lambs using the same qPCR, in which the prevalence of *C. pecorum* was found to be 30% overall (Yang et al., 2014), the prevalence of *C. pecorum* in trimmed joint samples in the current study was much less (6.1%). A possible explanation for this discrepancy could be differences in study design, with the study by (Yang et al., 2014) assessing longitudinal prevalence as determined by faecal shedding at three time points (weaning, post-weaning and pre-slaughter) on only eight sheep farms in southern Australia. In contrast, the current study assessed prevalence as determined by detection in abnormal joints trimmed at slaughter in animals from a much larger number of sheep farms. Collecting paired faecal samples from
the carcases in this study was not possible because by the time a carcase reached the point of inspection in the abattoir it had been eviscerated and the associated gastrointestinal tract sealed for hygiene/food safety reasons. We also could not sample the eyes because in Australia, the head of the carcase is removed prior to the point of inspection. The higher detection rate of *C. pecorum* in faecal swabs compared to other sites (i.e. vaginal swabs, pharyngeal swabs) has been reported previously in both cattle and sheep (Lenzko et al., 2011; Li et al., 2016; Reinhold et al., 2008). Historically, enteric isolates of *C. pecorum* were not thought to be normally invasive in sheep (Clarkson and Philips, 1997; Philips and Clarkson, 1998; St George, 1971). More recently it has been realized that many *Chlamydia* spp., including *C. pecorum*, exist in an asymptomatic state within the gastrointestinal tract of the host (Everett, 2000), with the potential for persistent shedding, reinfection or recrudescence into systemic infection (Rank and Yeruva, 2014; Walker et al., 2015). In addition, there is evidence to suggest that only some strains of *C. pecorum* cause clinical disease in sheep (Jelocnik et al., 2014). *Chlamydiae* can only be isolated from affected joints of lambs for 3-21 days after parenteral infection, despite inflammatory changes persisting for up to 56 days (Storz and Spears, 1979), which could also explain the lower than expected prevalence rate. The subclinical impact of latent infections, at least in cattle, has also been recognized (Reinhold et al., 2008).

Another possible explanation for the relatively low rate of *C. pecorum*-positive samples compared to the previously reported prevalence in Australian lambs based on screening faecal samples is the age of the animals in the current study, with a predominance of lambs compared to young lambs (92.4% lambs compared to 7.6% young lambs). In their study, Yang et al. (2014) reported that faecal shedding of *C. pecorum* was highest at weaning, fell during the post-weaning period and was lowest pre-slaughter, with weaning defined as ~12
weeks of age, post-weaning as ~19 weeks of age and pre-slaughter as ~29 weeks of age. The majority of the carcases sampled in our study are consistent with the pre-slaughter age category (79.1%). At this age Yang et al. (2014) reported the faecal shedding rate of *C. pecorum* was 14.3% in South Australian lambs, 4.7% in Victorian lambs, 80.8% in New South Wales lambs and 10.1% in Western Australian lambs. The estimate for New South Wales was based on sampling lambs from one property in a high rainfall region of New South Wales. In contrast, the current study assessed arthritic joints from lambs sourced largely from eight properties in the drier, western regions of New South Wales where the animals would have been held at much lower stocking rates. Stocking density has been suggested as a possible risk factor for *C. pecorum* infection in both cattle and sheep (Jee et al., 2004; Lenzko et al., 2011). This difference in regional source could also explain the lower rate of *C. pecorum*-positive samples in our study compared to previous estimates based on faecal shedding. In New South Wales disease due to *C. pecorum* is thought to be most prevalent in the productive central tablelands region of the state, another region where animals are held at high stocking rates (Jelocnik et al., 2013b). However, due to training and continual practice of the Australian government Department of Agriculture and Water Resources-accredited meat inspectors who are constantly scrutinised under Hazard Analysis and Critical Control Points, along with the large number of carcases examined, we believe that our results are an accurate representation of the prevalence of active *C. pecorum*-associated arthritis in slaughter lambs in South Australia, Victoria and western New South Wales.

The qPCR method we used had low analytical sensitivity, with a LOQ of just 8 gene copies per µl of DNA. However, in the absence of an accepted gold standard for the diagnosis of *C. pecorum* infection in sheep, it is difficult to determine the sensitivity of the qPCR method.
Culture for *C. pecorum* is technically demanding, not conducted routinely in veterinary diagnostic laboratories and less sensitive than PCR, and many *C. pecorum* strains are difficult to grow (Degraves et al., 2003; Longbottom, 2004; Timms, 2009). Because of this, it was not possible to determine the negative predictive value of the qPCR method we used. In contrast, the high analytical specificity of the *C. pecorum* qPCR method we used has been described previously (Pantchev et al., 2010; Yang et al., 2014). In the current study we did not observe cross-reactivity in synovial tissue samples that were positive for other bacteria using traditional culture techniques and in which bacteria were observed on histopathology (i.e. *Corynebacterium* spp., *E. rhusiopathiae*, *Staphylococcus* spp., *Streptococcus* spp., data not shown). PCR contamination controls were used, including negative controls, as well as separation of preparation and amplification areas. For these reasons, we believe the specificity and positive predictive value of the PCR method we used approached 100%. The immunohistochemistry results support this belief.

Histopathological examination of synovial tissue from *C. pecorum*-positive joints revealed a varying inflammatory infiltrate. Most synovia were infiltrated with variable numbers of lymphocytes and plasma cells, but in some joints this was accompanied by neutrophils, with five of the nine synovial samples containing fibrin and/or neutrophils in significant numbers. *Chlamydia* spp. are highly cell associated and their usual habitat is epithelial cells lining mucosa. Invasion of virulent strains of *C. pecorum* via portal blood and lymphatics results in initial localisation within the liver and mesenteric lymph nodes, spread to the spleen, lungs and kidney, and a secondary wave of more widespread bacteraemia that may involve the synovia (Craig et al., 2015). This method of systemic spread is consistent with our choice of synovial tissue samples for detecting *C. pecorum* in abnormal joints, rather than synovial fluid as would be sampled in a live animal. The mixed inflammatory responses seen in these
joints suggests an initial acute suppurative and fibrinous inflammatory response to chlamydial synovial invasion, followed by a predominantly lymphocytic-plasmacytic cell infiltrate when the infection became more subacute, as described by Storz and Spear (1979). On immunohistochemistry we observed *C. pecorum* peri-vascularly, as well as associated with hypertrophied synovium, neutrophils and within lymphocytic-plasmacytic infiltrates, consistent with haematogenous spread of the organism and the inflammatory responses within the joints.

Conclusions

This study has revealed 6.1% prevalence of *C. pecorum* in 148 abnormal joints trimmed from lamb carcases at one abattoir in southern Australia. The average arthritis trim weight of the carcases from which synovial tissue tested positive for *C. pecorum* was 1.112 kg (95% confidence interval 0.637-1.586 kg) and this did not differ from the carcases from which synovial tissue was not positive for *C. pecorum*. None of the carcases from which the positive joint samples originated were. Further studies are required to determine the on-farm production losses associated with *C. pecorum* infection in Australian lambs.

Conflict of interest statement

The study was funded by Australian sheep producers and the Australian Government through Meat & Livestock Australia (MLA) Limited, who had no influence on study design, data evaluation or manuscript preparation. None of the authors had financial or personal relationships that could inappropriately influence or bias the content of this paper.

Acknowledgements
This study was funded by Australian sheep producers and the Australian Government through Meat & Livestock Australia (MLA) Limited. We thank Gerald Martin, Thomas Foods International and the abattoir meat inspectors for assistance with sample collection and Tina Sizer-Taylor for coordination of the laboratory support for the study. Haematoxylin and eosin staining was performed by Gribbles Veterinary Pathology, Adelaide and the immunohistochemistry staining by Elaine Chew, University of Sydney. The project would also not have been possible without the assistance of Biosecurity SA, the managers of the South Australian Enhanced Abattoir Surveillance Program, in particular Drs Elise Matthews and Celia Dickason. The Enhanced Abattoir Surveillance Program is funded by the South Australian Sheep Industry Fund and Animal Health Australia.
Figure 1. Location of *Chlamydia pecorum* in synovial samples that tested positive on qPCR as demonstrated by immunohistochemistry, magnification x 400; (a) perivascular; (b) associated with lymphocytic-plasmacytic infiltrates; (c) associated with neutrophils; (d) associated with hypertrophied synovium.
## Table 1. Source of the arthritic joint samples

<table>
<thead>
<tr>
<th>Category</th>
<th>Descriptor</th>
<th>Number of carcasses examined</th>
<th>Number of carcasses with arthritis</th>
<th>Number of arthritic joints sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>New South Wales</td>
<td>6,554</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>South Australia</td>
<td>35,608</td>
<td>301</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>Queensland</td>
<td>768</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Victoria</td>
<td>9,292</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Not recorded(^a)</td>
<td>909</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Breed</td>
<td>Dorper</td>
<td>7,914</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Merino</td>
<td>13,478</td>
<td>135</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Unspecified crossbred</td>
<td>31,739</td>
<td>206</td>
<td>89</td>
</tr>
<tr>
<td>Age</td>
<td>Young lamb(^b)</td>
<td>3,994</td>
<td>49</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Lamb(^c)</td>
<td>49,137</td>
<td>320</td>
<td>118</td>
</tr>
</tbody>
</table>

\(^a\)Boxed lots from saleyards

\(^b\)Female, castrate or entire male ovine that has zero permanent incisor teeth and no evidence of eruption of permanent upper molar teeth\(^2\)

\(^c\)Female, castrate or entire male ovine that has zero permanent incisor teeth

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Table 2. Number of joints trimmed for arthritis that were positive for *Chlamydia pecorum* as determined by qPCR by regional source, age and breed of the animals

<table>
<thead>
<tr>
<th>Regional source</th>
<th>Age</th>
<th>Breed</th>
<th>Number tested</th>
<th>Number positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>New South Wales</td>
<td>Young lamb</td>
<td>Dorper</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Merino</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unspecified crossbred</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lamb</td>
<td>Dorper</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Merino</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unspecified crossbred</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>South Australia</td>
<td>Young lamb</td>
<td>Dorper</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Merino</td>
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<td>0</td>
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<td>Dorper</td>
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<td>Dorper</td>
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<td>Young lamb</td>
<td>Dorper</td>
<td>0</td>
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</tr>
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<td></td>
<td>Merino</td>
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<td>0</td>
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<td></td>
<td></td>
<td>Merino</td>
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<td>-</td>
</tr>
<tr>
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<td></td>
<td>Unspecified crossbred</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>148</td>
<td>9</td>
</tr>
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</table>
Table 3. Histopathological scores and changes in synovial tissue from ovine joints trimmed for arthritis at an abattoir in southern Australia, concentration of *Chlamydia pecorum* as determined by qPCR and results of immunohistochemistry for *Chlamydia* spp.

<table>
<thead>
<tr>
<th>Total histopathological score\textsuperscript{a}</th>
<th><em>Chlamydia pecorum</em> (number per gram of synovial tissue)</th>
<th>Histopathological changes</th>
<th>Immunohistochemistry for <em>Chlamydia</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.8 x 10\textsuperscript{4}</td>
<td>Normal</td>
<td>Positive</td>
</tr>
<tr>
<td>1</td>
<td>1.4 x 10\textsuperscript{4}</td>
<td>Minimal synovial proliferation.</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>4.1 x 10\textsuperscript{5}</td>
<td>Mild perivascular plasmacytic synovitis.</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>6 x 10\textsuperscript{3}</td>
<td>Moderate suppurative and fibrinous synovitis, mild to moderate lymphoplasmacytic synovitis, mild subsynovial fibroplasia.</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>2.2 x 10\textsuperscript{4}</td>
<td>Moderate lymphoplasmacytic synovitis, moderate fibrinous and neutrophilic synovitis with oedema, focal granulomatous</td>
<td>Positive</td>
</tr>
</tbody>
</table>
synovitis associated with intralesional foreign body.

5  3.0 x 10^4  Moderate lymphoplasmacytic synovitis, mild neutrophilic synovitis, mild synovial hyperplasia.

5  7.6 x 10^5  Moderate suppurative synovitis, moderate to marked lymphoplasmacytic synovitis.

5  3.2 x 10^4  Marked suppurative and fibrinous synovitis, moderate to marked lymphoplasmacytic synovitis, mild to moderate fibroplasia.

8  8.3 x 10^4  Marked suppurative synovitis, mild synovial proliferation.

^Total score for synovial proliferation and inflammatory infiltrate, with synovial proliferation and inflammatory infiltrate allocated to seven categories (nil, minimal, mild, mild to moderate, moderate, moderate to marked or marked) which was converted to a numerical score, with nil equal to zero and marked equal to six.
References


Li, J., Guo, W., Kaltenboeck, B., Sachse, K., Yang, Y., Lu, G., Zhang, J., Luan, L., You, J., Huang, K., Qui, H., Wang, Y., Li, M., Uang, Z., Wang, C., 2016. Chlamydia pecorum is the
endemic intestinal species in cattle while *C. gallinacea*, *C. psittaci* and *C. pneumoniae* associate with sporadic systemic infection. Vet Microbiol 193, 93-99.


