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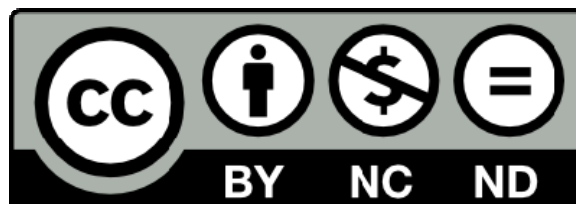
# Accepted Manuscript

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

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1 Original article

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**3 Detection of *Chlamydia pecorum* in joints trimmed from ovine carcases with arthritis at**

**4 an abattoir in southern Australia**

5

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22 Highlights

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

27

28 **Abstract**

29

30 *Chlamydiae* are obligate intracellular bacteria that infect a broad range of host species,

31 including sheep. Two species of *Chlamydia* infect sheep, *C. abortus*, which is a major cause

32 of abortion in both sheep and goats, and *C. pecorum,* which causes pneumonia, arthritis/

33 polyarthritis, encephalomyelitis, conjunctivitis, enteritis, abortion and metritis and infertility

34 in domestic ruminants and pigs. The prevalence of faecal shedding of *C. pecorum* is

35 relatively common amongst lambs in Australia. The aim of the work presented here was to

36 use qPCR to determine the prevalence of *C. pecorum* in synovial samples obtained from

37 abnormal joints trimmed from lamb carcases at one abattoir in southern Australia. The study

38 included 53,131 carcases screened for arthritis, of which 369 had at least one abnormal joint

39 trimmed. One hundred and forty eight trimmed joints were undamaged and suitable for PCR

40 testing. The prevalence of *C. pecorum* in synovial tissue collected from the abnormal joints

41 was 6.1% and the bacterial concentration ranged from 6 x $10^3$ to 7.6 x $10^5$/g of synovial

42 tissue. Five of the positive joint samples were from carcases that had one joint trimmed for

43 arthritis and four were from carcases from which two joints had been trimmed. None of the

44 carcases from which the positive joint samples originated were condemned. The average

45 arthritis trim weight of the carcases from which synovial tissue tested positive for *C. pecorum*

46 was 1.112 kg (95% confidence interval 0.637-1.586 kg) and this did not differ from the

47 carcases from which synovial tissue was not positive for *C. pecorum.* (mean 0.997 kg, 95%

48    confidence interval 0.840-1.154 kg). Further research is required to determine the on-farm

49    production losses associated with *C. pecorum* infection in Australian lambs.

50

51

52    **Key words**

53    Sheep; arthritis; *Chlamydia pecorum*; qPCR

54

55    **Introduction**

56    *Chlamydiae* are obligate intracellular bacteria that infect a broad range of host species,

57    including sheep. Two species of *Chlamydia* have principally been reported to infect sheep, *C.*

58    *abortus* which is a major cause of abortion in both sheep and goats, and *C. pecorum,* which

59    causes pneumonia, arthritis/polyarthritis, encephalomyelitis, conjunctivitis, enteritis, abortion

60    and metritis and infertility in domestic ruminants and pigs (Everett, 2000; Fukushi and Hirai,

61    1992; Longbottom and Coulter, 2003; Polkinghorne et al., 2009; Rodolakis and Laroucau,

62    2015; Rodolakis and Mohamad, 2010; Walker et al., 2015). Australia is believed to be free

63    from *C. abortus* (Animal Health Australia, 2016; McCauley et al., 2010; Rodolakis and

64    Laroucau, 2015; Timms, 2009), whereas *C. pecorum*  has been associated with

65    arthritis/polyarthritis (Jelocnik et al., 2013a; Jelocnik et al., 2014; Robson, 2003; Robson,

66    2004; Tammemagi and Simmons, 1968), conjunctivitis (Jelocnik et al., 2013a; Jelocnik et al.,

67    2014; Tighe and Slattery, 2012), and less frequently abortion (Slattery, 2008) in Australian

68    sheep. *C. pecorum* has also been isolated from faecal samples and rectal swabs of healthy

69    Australian sheep without the typical clinical signs of infection (Jelocnik et al., 2014; St

70    George, 1971; Yang et al., 2014).

71

3

72    Bacterial arthritis/polyarthritis is a significant cost to the Australian sheep industry, estimated

73    at A$39m annually (Lane et al., 2015). Globally, *Erysipelothrix rhusiopathiae* is considered

74    to be the most common cause of bacterial arthritis/polyarthritis in lambs (Craig et al., 2015),

75    and previous surveys of Australian slaughter lambs are generally in agreement with this

76    belief (Lloyd et al., 2016; Paton et al., 2003). However, the previous microbiological surveys

77    of bacterial joint infections in Australian lambs have used traditional bacterial culture

78    techniques that would not have detected *C. pecorum*. Despite this, arthritis/polyarthritis due

79    to *C. pecorum* is known to occur in Australia, and has been reported from 13-month old rams

80    in Queensland (Tammemagi and Simmons, 1968) and from 3-6 month old lambs on the

81    Central Tablelands of New South Wales (Jelocnik et al., 2014; Robson, 2003; Robson, 2004).

82

83    Using a quantitative PCR (qPCR) targeting the chlamydial outer membrane protein of

84    *Chlamydia pecorum*, the prevalence of faecal shedding of *C. pecorum* was found to be

85    relatively common amongst lambs from eight different farms across four states of Australia

86    (New South Wales, South Australia, Victoria and Western Australia), with approximately

87    30% of lambs shedding the bacteria in their faeces (Yang et al., 2014). Faecal shedding was

88    highest at weaning (~12 weeks of age), fell during the post-weaning period (~19 weeks of

89    age) and was lowest pre-slaughter (~29 weeks of age), similar to the time-course of shedding

90    in naturally infected dairy calves (Jee et al., 2004). Serological testing of Australian sheep for

91    export to demonstrate freedom from *C. abortus*-infection using a complement fixation

92    technique resulted in a 16% false-positive rate in 380 rams tested, with the prevalence of

93    false-positive results not affected by age, breed or state of origin (McCauley et al., 2010). The

94    complement fixation test used is reported to have low specificity with cross reactions to other

95    chlamydia species, including *C. pecorum* (Longbottom and Coulter, 2003).

96

4

97  We recently reported the results of traditional bacterial culture of samples collected from

98  ovine joints trimmed for arthritis at an abattoir in southern Australia by Australian

99  government Department of Agriculture and Water Resources-accredited meat inspectors in

100  accordance with the Australian Standard for the Hygienic Production and Transportation of

101  Meat and Meat Products for Human Consumption (Lloyd et al., 2016; Anon, 2002). *E.*

102  *rhusiopathiae*, alone or in combination, was the bacterial species isolated most frequently

103  isolated (28 of 175 joints tested, prevalence 16%), followed by *Streptococcus* spp. (9 of 175

104  joints tested, prevalence 5.1%), as well as a range of bacteria that constitute the normal skin

105  flora of sheep. The aim of the work presented here was to use qPCR to determine the

106  prevalence of *C. pecorum* in synovial samples in a subset of the trimmed abnormal ovine

107  joints collected as part of the previously reported work. In addition, histopathology and

108  immunohistochemistry were used to assess the associated pathology in joint samples that

109  tested positive for *C. pecorum.*

110

111  **Materials and Methods**

112  *Collection of arthritic joint samples*

113  Arthritic joint samples were collected from ovine carcases at an abattoir in southern Australia

114  that processes animals from New South Wales, Queensland, South Australia and Victoria as

115  described previously (Lloyd et al., 2016). The number of carcases examined, the number with

116  arthritis and the number sampled, as well as regional source, age and breed of the animals, is

117  provided in Table 1. The samples for *C. pecorum* testing were collected in two phases,

118  December 2014 to January 2015 and in March 2015. The sample collection was part of a

119  larger project designed to investigate the association between arthritis and docked tail length

120  (Lloyd et al., 2016).

121

122    Briefly, carcases with arthritis were identified by Australian government Department of

123    Agriculture and Water Resources (DAWR) (formerly AQIS)-accredited meat inspectors in

124    accordance with the Australian Standard for the Hygienic Production and Transportation of

125    Meat and Meat Products for Human Consumption (Anon, 2002). The arthritic joints were not

126    opened during inspection; however, some joints had been damaged during processing prior to

127    inspection. Following trimming of affected joints by the meat inspectors, the joints were

128    placed in plastic bags (one bag per carcase) and stored in buckets on the slaughter floor until

129    the next break and then placed on ice. This ensured that all the sampled joints were chilled

130    within two hours of collection. The joints sampled were from the legs and included the

131    carpus, elbow, tarsus and stifle. Metacarpal and metatarsal joints could not be sampled

132    because these had been removed from the carcases prior to the point of inspection. At the end

133    of each five hour shift, all the collected joints and associated trim were re-examined grossly,

134    weighed and the damaged joints then discarded because of the possibility of cross-

135    contamination on the slaughter floor. The undamaged joints were then placed back on ice for

136    up to 18 hours prior to collection of samples for PCR.

137

138    Faecal samples were not collected from the carcases with arthritis because by the time a

139    carcase reached the point of inspection it had been eviscerated and the associated

140    gastrointestinal tract sealed for hygiene/food safety reasons.

141

142    A sterile set of instruments was used to remove subcutaneous and peri-synovial tissue from

143    around the joint and then a second set of sterile instruments was used to open the joint. At

144    least 1 g of synovial tissue was collected for PCR, as well samples for histopathology and

145    immunohistochemistry (synovial tissue samples in 4% neutral buffered formalin). The

146    synovial samples for PCR were stored at -20 $^{0}$C until further analysis.

6

147

148 During December 2014 and in January 2015 only the most swollen, undamaged joint per

149 carcase was sampled for PCR. In March 2015 all the undamaged abnormal joints from each

150 carcase were sampled PCR. No joints classified as negative by the inspectors were sampled

151 because these joints are not trimmed from the carcases and to do this would have resulted in a

152 financial penalty for farmers.

153

154 All the trim associated with arthritis from each carcase was collected into a clean plastic bag

155 and then weighed using a 30 kg by 5 g electronic crane scale.

156

157 Lamb age, breed and property identification code (PIC) were obtained from abattoir records.

158 Lamb age was determined by abattoir personnel who checked the dentition of the animals at

159 the time of slaughter. A lamb is defined as female, castrate or entire male ovine that has zero

160 permanent incisor teeth and a young lamb as a female, castrate or entire male ovine that has

161 zero permanent incisor teeth and no evidence of eruption of permanent upper molar teeth

162 (AUS-MEAT Limited, 2010). The PIC code was assigned to a region within South Australia

163 using a key provided by Primary Industries and Research South Australia. For lambs sourced

164 from outside South Australia, region was at the level of the State.

165

166 *DNA isolation*

167 DNA was extracted from 50 mg of synovial tissue using a QIAamp® Fast DNA Tissue Kit

168 (QIAGEN). A negative control (no synovium sample) was used in each extraction group.

169

170 *PCR*

171    The testing for *C. pecorum* was conducted using the method of Yang et al. (2014). Briefly, a

172    species-specific 76 base pair (bp) product was amplified from the *C. pecorum* outer

173    membrane protein cell surface antigen gene (ompA) using the forward primer CpecOMP1 F

174    5′-CCATGTGATCCTTGCGCTACT-3′, the reverse primer CpecOMP1 5′-

175    TGTCGAAAACATAATCTCCGTAAAT-3′ and the probe CpecOMP1-S 5′-CAL-Fluor

176    Orange-560-TGCGACGCGATTAGCTTACGCGTAG-TAMARA-3′.  Each 15 µL PCR

177    mixture contained 1x PCR buffer, 4 mM MgCl$_2$, 1 mM each deoxynucleotide triphosphate,

178    1.0 U KAPA DNA polymerase (MolBio), 0.2 µM each of forward and reverse primers, 0.2

179    µM each of forward and reverse internal amplification control (IAC) primers targeting a

180    plasmid containing a fragment of a coding region from Jembrana disease virus (JDV), 50 nM

181    specific probe, 50 nM IAC probe, 10 copies of IAC template and 1 µL sample DNA. The

182    PCR cycling conditions consisted of 95 °C for 3 minutes, followed by 45 cycles of 95 °C for

183    20 seconds and 60 °C for 45 seconds. A standard curve was generated using 10-fold serial

184    dilutions of plasmids containing the cloned ompA gene amplified from *C. pecorum*.  PCR

185    contamination controls were used, including negative controls and separation of preparation

186    and amplification areas.

187

188    A standard curve for quantifying *Chlamydia* spp. DNA was generated by cloning the PCR

189    products amplified from *C. pecorum* into pGEM-T (Promega) and transforming into

190    *Escherichia coli* competent cells. Plasmid copy number was calculated based on the plasmid

191    size (base pairs) and DNA concentration.  10-fold series dilutions of plasmids were

192    conducted from 10,000 copies down to 1 copy of the genomic template for sensitivity testing.

193    The limit of quantitation (LOQ) for this qPCR assay was 8 gene copies per µl of DNA (Yang

194    et al., 2014). Template copy numbers were converted to numbers of organism present on the

195    basis that the targeted gene (*OmpA*) is a single copy gene (Lan and Igo, 1998) and bacterial

8

196    genomes are haploid. Therefore, the number of plasmids detected was equivalent to the

197    number of *Chlamydia* spp. per µl of DNA (total elution volume = 100µl/50mg of tissue).

198    This was then converted to number of *Chlamydia* per gram of tissue.

199

200    *Histopathology*

201

202    Tissue was fixed in 4% phosphate buffered formalin and trimmed as required after fixation.

203    Following routine processing, 4µm sections were stained with Mayer's haematoxylin and

204    Young's eosin, and examined microscopically.

205

206    Synovial proliferation and inflammatory infiltrate results were allocated to seven categories,

207    from nil, minimal, mild, mild to moderate, moderate, moderate to marked or marked, with the

208    cell type or types comprising the inflammatory infiltrate described. Other changes (i.e.

209    haemorrhage, fibrosis) were also noted. Synovial proliferation and inflammatory infiltrate

210    were converted to a numerical score, with nil equal to zero and marked equal to six. The

211    scores for synovial proliferation and inflammatory infiltrate were then added to give a total

212    score for each sample.

213

214    *Immunohistochemistry*

215    Sections were cut on silane slides at 4 µ, deparaffinized to water and placed into a DAKO®

216    Autostainer Plus. Slides were exposed to 3% hydrogen peroxide, *Chlamydia* antibody (1:200,

217    anti-*Chlamydia* mouse monoclonal, R-Biopharm), DAKO® REAL ™Envision, and DAKO®

218    DAB+ substrate-chromogen solution with Tris Buffered Saline rinses in between. Sections

219    were then removed from the Autostainer and counterstained with haematoxylin before being

220    dehydrated and mounted. For each sample the negative control was a section exposed to 3%

221     hydrogen peroxide, DAKO® REAL ™Envision, and DAKO® DAB+ substrate-chromogen,

222     but not *Chlamydia* antibody.

223

224     *Statistical analysis*

225     Prevalence was expressed as the percentage of the samples that were positive for *C. pecorum*

226     on PCR. The correlation between the total histopathological score for each sample and the

227     concentration of *C. pecorum* detected in the sample was calculated (Microsoft Excel). A 95%

228     confidence interval was used to determine significant differences in trim weight between

229     carcases from which synovial tissue tested positive for *C. pecorum* and those that were

230     negative.

231

232     **Results**

233     *Prevalence of C. pecorum*

234     Nine of the 148 synovial samples were positive for *C. pecorum* (prevalence 6.1%). Two of

235     the positive samples were from carcases sourced from New South Wales, three were from

236     carcases sourced from Victoria and four were from carcases sourced from South Australia

237     (Table 2).  The prevalence of infection was too low to determine if there were associations

238     between infection and source of the carcases or the breed or age of the lambs.

239

240     Five of the positive joint samples were from carcases that had one joint trimmed for arthritis

241     and four were from carcases from which two joints had been trimmed. The most common

242     joint trimmed was the tarsus followed by the stifle and carpus. None of the carcases from

243     which the positive joint samples originated were condemned. There was no significant

244     difference in the trim weight of the carcases from which synovial tissue tested positive for *C.*

245    *pecorum* and those that did not (positive 1.112 kg, 95% confidence interval 0.637-1.586 kg;

246    negative 0.997 kg, 95% confidence interval 0.840-1.154 kg).

247

248    *Histopathological changes*

249    The majority of the q-PCR positive joint samples had no evidence of synovial proliferation

250    (Table 3). One sample had minimal synovial proliferation and two had mild proliferation.

251    Inflammatory infiltrates ranged from nil to marked and were predominantly plasmacytic or

252    lymphoplasmacytic, although in some samples there was a significant component of

253    neutrophilic infiltration. Total histopathology scores ranged from zero to eight (Table 3).

254

255    On immunohistochemistry, *C. pecorum* was detected either peri-vascularly or associated with

256    lymphocytic-plasmacytic infiltrates, neutrophils or hypertrophied synovium (Figure 1).

257

258    *Concentration of C. pecorum*

259    The concentration of *C. pecorum* ranged from $6.0 \times 10^3$ to $7.6 \times 10^5$ organisms per gram of

260    tissue. There was no correlation ($P<0.05$) between the concentration of *C. pecorum* and the

261    total histopathological score for each sample (Correlation coefficient = 0.24).

262

263    **Discussion**

264    This study used a qPCR targeting the chlamydial outer membrane protein of *C. pecorum*

265    (Yang et al., 2014) to determine the prevalence of *C. pecorum* in abnormal joints trimmed

266    from lamb carcases at a commercial abattoir in southern Australia. Of the 148 synovial tissue

267    samples tested, including samples collected from carcases from animals sourced from three

268    states in Australia (New South Wales, South Australia and Victoria), nine were positive for

269    *C. pecorum* (prevalence 6.1%). The148 samples tested for *C. pecorum* were a subset of 175

11

270     samples tested using traditional microbiological culture methods techniques in which we

271     detected 16% prevalence of *E. rhusiopathiae* and 5.1% prevalence of *Streptococcus* spp.

272     (Lloyd et al., 2016).Together these results suggest that *C. pecorum,* although present across

273     the sheep-raising regions of south-eastern Australia, may be less common than *E.*

274     *rhusiopathiae* but more common than *Streptococcus* spp., as a cause of bacterial

275     arthritis/polyarthritis in Australian slaughter lambs. However, false negative results on culture

276     of synovial fluid of animals with septic arthritis are common (Craig et al., 2015). In addition,

277     the cell walls of *E. rhusiopathiae* and *Streptococcus* spp. are relatively resistant to breakdown

278     by mammalian lysosomal enzymes and subsequent removal by macrophages and, as a result,

279     persistent inflammation can develop, even when infection appears to have resolved or

280     bacteria cannot be isolated (Craig, et al.,). PCR is likely to be much more sensitive at

281     detecting bacteria in ovine joint infections than traditional bacterial culture methods, which

282     suggests that these rankings of relative importance should be interpreted with caution. Indeed,

283     PCR is reported to be more sensitive at detecting *E. rhusiopathiae* in abattoir samples,

284     including ovine joints, than bacterial culture (Wang et al., 2002).

285

286     Compared to a recent longitudinal survey of the prevalence of faecal shedding of *C. pecorum*

287     by Australian lambs using the same qPCR, in which the prevalence of *C. pecorum* was found

288     to be 30% overall (Yang et al., 2014), the prevalence of *C. pecorum* in trimmed joint samples

289     in the current study was much less (6.1%). A possible explanation for this discrepancy could

290     be differences in study design, with the study by (Yang et al., 2014) assessing longitudinal

291     prevalence as determined by faecal shedding at three time points (weaning, post-weaning and

292     pre-slaughter) on only eight sheep farms in southern Australia. In contrast, the current study

293     assessed prevalence as determined by detection in abnormal joints trimmed at slaughter in

294     animals from a much larger number of sheep farms. Collecting paired faecal samples from

295     the carcases in this study was not possible because by the time a carcase reached the point of

296     inspection in the abattoir it had been eviscerated and the associated gastrointestinal tract

297     sealed for hygiene/food safety reasons. We also could not sample the eyes because in

298     Australia, the head of the carcase is removed prior to the point of inspection. The higher

299     detection rate of *C.* pecorum in faecal swabs compared to other sites (i.e. vaginal swabs,

300     pharyngeal swabs) has been reported previously in both cattle and sheep (Lenzko et al., 2011;

301     Li et al., 2016; Reinhold et al., 2008). Historically, enteric isolates of *C. pecorum* were not

302     thought to be normally invasive in sheep (Clarkson and Philips, 1997; Philips and Clarkson,

303     1998; St George, 1971). More recently it has been realized that many *Chlamydia* spp.,

304     including *C. pecorum*, exist in an asymptomatic state within the gastrointestinal tract of the

305     host (Everett, 2000), with the potential for persistent shedding, reinfection or recrudescence

306     into systemic infection (Rank and Yeruva, 2014; Walker et al., 2015).In addition, there is

307     evidence to suggest that only some strains of *C. pecorum* cause clinical disease in sheep

308     (Jelocnik et al., 2014). *Chlamydiae* can only be isolated from affected joints of lambs for 3-21

309     days after parenteral infection, despite inflammatory changes persisting for up to 56 days

310     (Storz and Spears, 1979), which could also explain the lower than expected prevalence rate.

311     The subclinical impact of latent infections, at least in cattle, has also been recognized

312     (Reinhold et al., 2008).

313

314     Another possible explanation for the relatively low rate of *C. pecorum*-positive samples

315     compared to the previously reported prevalence in Australian lambs based on screening

316     faecal samples is the age of the animals in the current study, with a predominance of lambs

317     compared to young lambs (92.4% lambs compared to 7.6% young lambs). In their study,

318     Yang et al. (2014) reported that faecal shedding of *C. pecorum* was highest at weaning, fell

319     during the post-weaning period and was lowest pre-slaughter, with weaning defined as ~12

320    weeks of age, post-weaning as ~19 weeks of age and pre-slaughter as ~29 weeks of age. The

321    majority of the carcases sampled in our study are consistent with the pre-slaughter age

322    category (79.1%). At this age Yang et al. (2014) reported the faecal shedding rate of *C.*

323    *pecorum* was 14.3% in South Australian lambs, 4.7% in Victorian lambs, 80.8% in New

324    South Wales lambs and 10.1% in Western Australian lambs. The estimate for New South

325    Wales was based on sampling lambs from one property in a high rainfall region of New

326    South Wales. In contrast, the current study assessed arthritic joints from lambs sourced

327    largely from eight properties in the drier, western regions of New South Wales where the

328    animals would have been held at much lower stocking rates. Stocking density has been

329    suggested as a possible risk factor for *C. pecorum* infection in both cattle and sheep (Jee et

330    al., 2004; Lenzko et al., 2011). This difference in regional source could also explain the lower

331    rate of *C. pecorum*-positive samples in our study compared to previous estimates based on

332    faecal shedding. In New South Wales disease due to *C. pecorum* is thought to be most

333    prevalent in the productive central tablelands region of the state, another region where

334    animals are held at high stocking rates (Jelocnik et al., 2013b). However, due to training and

335    continual practice of the Australian government Department of Agriculture and Water

336    Resources-accredited meat inspectors who are constantly scrutinised under Hazard Analysis

337    and Critical Control Points, along with the large number of carcases examined, we believe

338    that our results are an accurate representation of the prevalence of active *C. pecorum*-

339    associated arthritis in slaughter lambs in South Australia, Victoria and western New South

340    Wales.

341

342    The qPCR method we used had low analytical sensitivity, with a LOQ of just 8 gene copies

343    per µl of DNA. However, in the absence of an accepted gold standard for the diagnosis of *C.*

344    *pecorum* infection in sheep, it is difficult to determine the sensitivity of the qPCR method.

345 Culture for *C. pecorum* is technically demanding, not conducted routinely in veterinary

346 diagnostic laboratories and less sensitive than PCR, and many *C. pecorum* strains are difficult

347 to grow (Degraves et al., 2003; Longbottom, 2004; Timms, 2009). Because of this, it was not

348 possible to determine the negative predictive value of the qPCR method we used. In contrast,

349 the high analytical specificity of the *C. pecorum* qPCR method we used has been described

350 previously (Pantchev et al., 2010; Yang et al., 2014).In the current study we did not observe

351 cross-reactivity in synovial tissue samples that were positive for other bacteria using

352 traditional culture techniques and in which bacteria were observed on histopathology (i.e.

353 *Corynebacterium* spp., *E. rhusiopathiae*, *Staphylococcus* spp., *Streptococcus* spp., data not

354 shown). PCR contamination controls were used, including negative controls, as well as

355 separation of preparation and amplification areas. For these reasons, we believe the

356 specificity and positive predictive value of the PCR method we used approached 100%. The

357 immunohistochemistry results support this belief.

358

359 Histopathological examination of synovial tissue from *C. pecorum*-positive joints revealed a

360 varying inflammatory infiltrate. Most synovia were infiltrated with variable numbers of

361 lymphocytes and plasma cells, but in some joints this was accompanied by neutrophils, with

362 five of the nine synovial samples containing fibrin and/or neutrophils in significant numbers.

363 *Chlamydia* spp. are highly cell associated and their usual habitat is epithelial cells lining

364 mucosa. Invasion of virulent strains of *C. pecorum* via portal blood and lymphatics results in

365 initial localisation within the liver and mesenteric lymph nodes, spread to the spleen, lungs

366 and kidney, and a secondary wave of more widespread bacteraemia that may involve the

367 synovia (Craig et al., 2015). This method of systemic spread is consistent with our choice of

368 synovial tissue samples for detecting *C. pecorum* in abnormal joints, rather than synovial

369 fluid as would be sampled in a live animal. The mixed inflammatory responses seen in these

370 joints suggests an initial acute suppurative and fibrinous inflammatory response to

371 chlamydial synovial invasion, followed by a predominantly lymphocytic-plasmacytic cell

372 infiltrate when the infection became more subacute, as described by Storz and Spear (1979).

373 On immunohistochemistry we observed *C. pecorum* peri-vascularly, as well as associated

374 with hypertrophied synovium, neutrophils and within lymphocytic-plasmacytic infiltrates,

375 consistent with haematogenous spread of the organism and the inflammatory responses

376 within the joints.

377

378 **Conclusions**

379 This study has revealed 6.1% prevalence of *C. pecorum* in 148 abnormal joints trimmed from

380 lamb carcases at one abattoir in southern Australia. The average arthritis trim weight of the

381 carcases from which synovial tissue tested positive for *C. pecorum* was 1.112 kg (95%

382 confidence interval 0.637-1.586 kg) and this did not differ from the carcases from which

383 synovial tissue was not positive for *C. pecorum*.   None of the carcases from which the

384 positive joint samples originated were. Further studies are required to determine the on-farm

385 production losses associated with *C. pecorum* infection in Australian lambs.
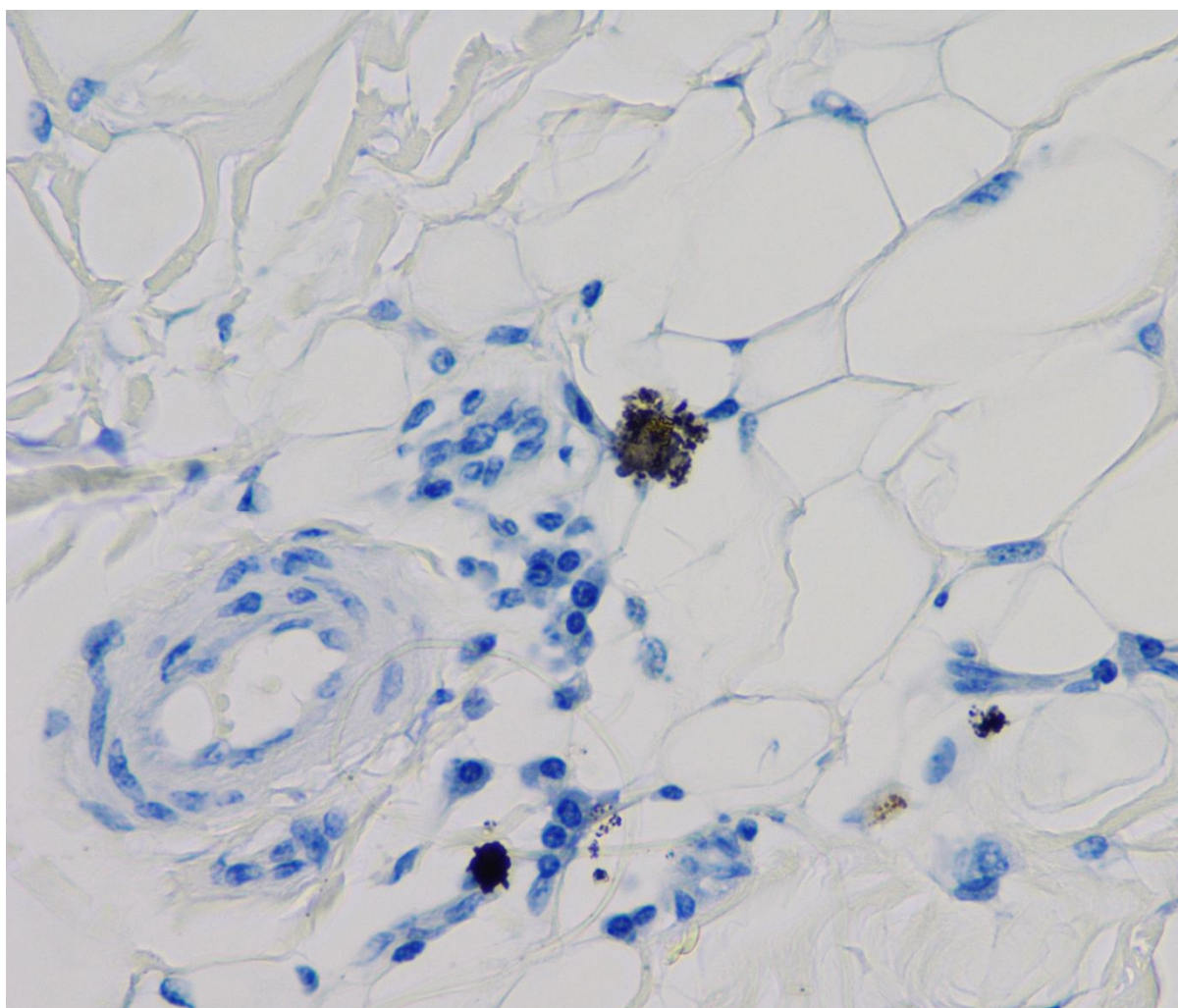
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387 **Conflict of interest statement**

392

393 **Acknowledgements**

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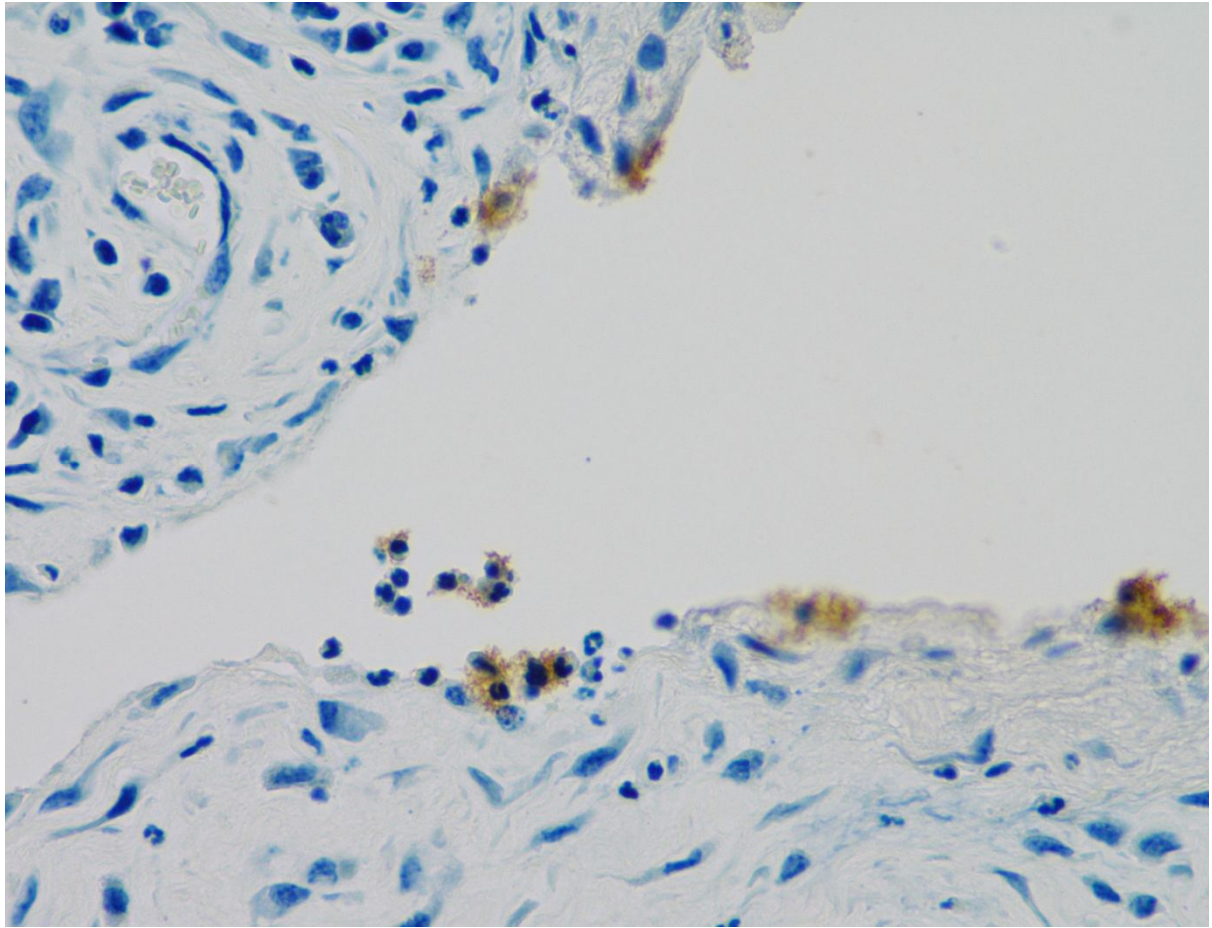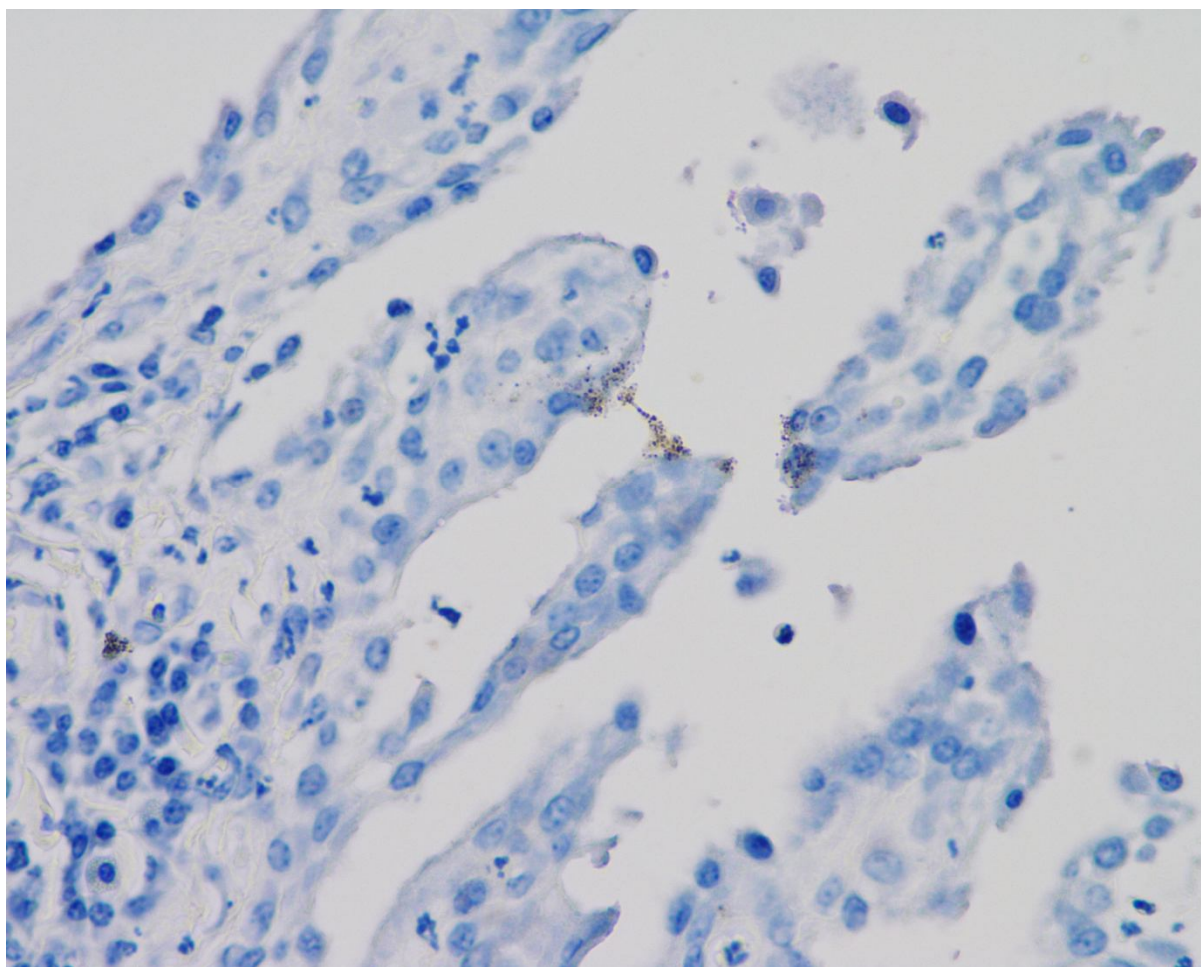404

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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.

413

21

414    Table 1. Source of the arthritic joint samples

| Category | Descriptor | Number of carcases examined | Number of carcases with arthritis | Number of arthritic joints sampled |
|---|---|---|---|---|
| Location | New South Wales | 6,554 | 20 | 15 |
| | South Australia | 35,608 | 301 | 115 |
| | Queensland | 768 | 2 | 1 |
| | Victoria | 9,292 | 40 | 14 |
| | Not recorded[a] | 909 | 6 | 3 |
| Breed | Dorper | 7,914 | 28 | 18 |
| | Merino | 13,478 | 135 | 41 |
| | Unspecified crossbred | 31,739 | 206 | 89 |
| Age | Young lamb[b] | 3,994 | 49 | 30 |
| | Lamb[c] | 49,137 | 320 | 118 |

415    [a]Boxed lots from saleyards

416    [b]Female, castrate or entire male ovine that has zero permanent incisor teeth and no evidence

417    of eruption of permanent upper molar teeth[2]

418    [c]Female, castrate or entire male ovine that has zero permanent incisor teeth

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419     Table 2. Number of joints trimmed for arthritis that were positive for *Chlamydia pecorum* as

420     determined by qPCR by regional source, age and breed of the animals

| Regional source | Age | Breed | Number tested | Number positive |
|---|---|---|---|---|
| New South Wales | Young lamb | Dorper | 0 | - |
| | | Merino | 0 | - |
| | | Unspecified crossbred | 0 | - |
| | Lamb | Dorper | 13 | 1 |
| | | Merino | 0 | - |
| | | Unspecified crossbred | 2 | 1 |
| South Australia | Young lamb | Dorper | 1 | 0 |
| | | Merino | 2 | 0 |
| | | Unspecified crossbred | 14 | 0 |
| | Lamb | Dorper | 1 | 0 |
| | | Merino | 35 | 3 |
| | | Unspecified crossbred | 41 | 1 |
| Queensland | Lamb | Dorper | 1 | 0 |
| Victoria | Young lamb | Dorper | 0 | - |
| | | Merino | 0 | - |
| | | Unspecified crossbred | 5 | 1 |
| | Lamb | Dorper | 2 | 0 |
| | | Merino | 0 | - |
| | | Unspecified crossbred | 7 | 2 |
| | | Total | 148 | 9 |

421

23

422    Table 3. Histopathological scores and changes in synovial tissue from ovine joints trimmed

423    for arthritis at an abattoir in southern Australia, concentration of *Chlamydia pecorum* as

424    determined by qPCR and results of immunohistochemistry for *Chlamydia* spp.

| Total histopathological score[a] | *Chlamydia pecorum* (number per gram of synovial tissue) | Histopathological changes | Immunohistochemistry for *Chlamydia* spp. |
|---|---|---|---|
| 0 | $1.8 \times 10^4$ | Normal | Positive |
| 1 | $1.4 \times 10^4$ | Minimal synovial proliferation. | Positive |
| 2 | $4.1 \times 10^5$ | Mild perivascular plasmacytic synovitis. | Positive |
| 3 | $6 \times 10^3$ | Moderate suppurative and fibrinous synovitis, mild to moderate lymphoplasmacytic synovitis, mild subsynovial fibroplasia. | Positive |
| 4 | $2.2 \times 10^4$ | Moderate lymphoplasmacytic synovitis, moderate fibrinous and neutrophilic synovitis with oedema, focal granulomatous | Positive |

| | | | |
|---|---|---|---|
| | | synovitis associated with intra-lesional foreign body. | |
| 5 | $3.0 \times 10^4$ | Moderate lymphoplasmacytic synovitis, mild neutrophilic synovitis, mild synovial hyperplasia. | Positive |
| 5 | $7.6 \times 10^5$ | Moderate suppurative synovitis, moderate to marked lymphoplasmacytic synovitis. | Positive |
| 5 | $3.2 \times 10^4$ | Marked suppurative and fibrinous synovitis, moderate to marked lymphoplasmacytic synovitis, mild to moderate fibroplasia. | Positive |
| 8 | $8.3 \times 10^4$ | Marked suppurative synovitis, mild synovial proliferation. | Positive |

425 [a] Total score for synovial proliferation and inflammatory infiltrate, with synovial proliferation

426 and inflammatory infiltrate allocated to seven categories (nil, minimal, mild, mild to

427 moderate, moderate, moderate to marked or marked) which was converted to a numerical

428 score, with nil equal to zero and marked equal to six.

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