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Longitudinal prevalence and faecal shedding of *Chlamydia pecorum* in sheep

Rongchang Yang a, Caroline Jacobson a, Graham Gardner a, Ian Carmichael b, Angus J.D. Campbell c, Una Ryan a,*

a School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, 6150, Australia
b South Australian Research and Development Institute, 33 Flemington Street, Glenside, South Australia 5065, Australia.
c Faculty of Veterinary Science, University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia

* Corresponding author. Tel.: +61 8 9360 2482.
E-mail address: Una.Ryan@murdoch.edu.au (U. Ryan).
Abstract

The prevalence and faecal shedding of *Chlamydia* spp. in sheep in Australia has not been well described. Two species-specific quantitative PCRs (qPCR) targeting the chlamydial outer membrane protein cell surface antigen gene (*ompA*) were validated and used to determine the prevalence and faecal shedding of *C. abortus* and *C. pecorum* from faecal samples of lambs at three sampling times (weaning, post-weaning and pre-slaughter) from eight farms in South Australia, New South Wales, Victoria and Western Australia. A total of 3412 faecal samples were collected and screened from approximately 1189 lambs across the four states. *C. abortus* was not detected in any of the samples screened. The overall prevalence of *C. pecorum* was 1027/3412 (30.1%) and median bacterial concentrations at weaning, post-weaning and pre-slaughter were $1.8 \times 10^7$, $1.2 \times 10^7$ and $9.6 \times 10^5$/g faeces, respectively. A subset of *C. pecorum* positive samples from each farm, ($n = 48$) were sequenced to confirm their identity. The present study demonstrates that *C. pecorum* is prevalent in Australian sheep, highlighting a need for further research on the impact of this bacterium on production.

Keywords: *Chlamydia pecorum*; Lambs; Quantitative PCR; *ompA*; Prevalence
Introduction

Members of the genus *Chlamydia* cause disease in humans and animals, and most are zoonotic (Everett et al., 1999; Vlahovick and Lasta, 2006). Two species, *Chlamydia abortus* (*Chlamydia psittaci* serotype 1) and *Chlamydia pecorum* are known to infect sheep (Berri et al., 2009; Lenzko et al., 2011). Both species cause abortions in sheep and *C. pecorum* also causes enteritis in sheep (Berri et al., 2009). *Chlamydia abortus*, the causative agent of enzootic abortion of ewes (EAE), is also zoonotic (Rodolakis and Yousef Mohamad, 2010).

Infections caused by *Chlamydia* spp. have long been underestimated due to difficulties in diagnosis of these obligate intracellular pathogens, which require growth in embryonated eggs or tissue culture (Nordentoft et al., 2011). Immunoassays have also been developed, but lack specificity (Jones et al., 1997; McCauley et al., 2010). Currently, *C. abortus* is believed to be absent from Australia based on culture and immunoassays (McCauley et al., 2010; Animal Health Australia, 2012). However, relatively few studies have been conducted on the prevalence of ovine *Chlamydia* infections in Australia (St George, 1971; McCauley et al., 2010; Jelocnik et al., 2013) and no molecular surveys for *C. abortus* have been undertaken.

The aim of the present study was to use species-specific quantitative PCR (qPCR) primarily for *C. pecorum* (but also for *C. abortus*) to determine the prevalence, faecal shedding concentrations and species of *Chlamydia* in lambs over a wide geographical area, representing the major sheep growing regions of Australia, specifically Western Australia (WA), New South Wales (NSW), Victoria (Vic) and South Australia (SA), at three sampling times (weaning, post-weaning and pre-slaughter), and to compare these data between states.
Materials and methods

Animals and collection of faecal samples

Faecal samples were collected from cross-bred lambs from eight different farms across four states of Australia (Table 1). Lambs were born and reared in paddocks and were not housed indoors at any stage. Lambs were sampled on three occasions (i.e. the same animals were sampled on each occasion) at weaning (~12 weeks of age), post-weaning (~19 weeks of age) and pre-slaughter (~29 weeks of age). A total of 3412 faecal samples from ~1189 lambs were collected directly from the rectum. All sample collection methods used were approved by the Murdoch University Animal Ethics Committee (approval number R2352/10).

DNA isolation

Genomic DNA was extracted from 200 mg of each faecal sample using a QIAamp DNA Mini Stool Kit (Qiagen) or from 250 mg of each faecal sample using a Power Soil DNA Kit (MolBio). A negative control (no faecal sample) was used in each extraction group.

PCR amplification, quantification and sequencing

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-TGCGACGCGATTAGCTTACGCGTAG-TAMARA-3’, as described previously (Pantchev et al., 2010). A C. abortus species-specific qPCR, also based on the ompA gene, which produces an 86 bp product, was amplified using the forward primer CpaOMP1-F 5’-GCAACTGACACTAAGTCGGCTACA-3’, the reverse primer CpaOMP1-R 5’- ACAAGCATGTCTCATTACGCTGGA-3’ and the probe CpaOMP1-Sb 5’-dFAM-
TAAATACCACGAATGGCAAGTTGGTTTAGCG-BHQ-1-3’, as described previously (Pantchev et al., 2009). In the original studies by Pantchev et al. (2009, 2010), these were single PCRs; however for the present study, both assays were multiplexed into a single reaction with detection in different channels.

An internal amplification control (IAC), consisting of a fragment of a coding region from Jembrana disease virus (JDV) cloned into pGEM-T (Promega), was used as described previously (Yang et al., 2013). The IAC primers were JDVF (5’-GGTAGTGCTGAAAGACATT-3’) and JDVR (5’-ATGTAGCTTGACCAGGAAGT-3’), and the probe was 5’-(Cy5)-TGCCCGCTGCCTCAGTAGTGC-BHQ2-3’. 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 IAC primers, 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 min, followed by 45 cycles of 95 °C for 20 s and 60 °C for 45 s. 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 or C. abortus into pGEMT (Promega) and transforming Escherichia coli competent cells. Plasmid DNA for each pathogen was isolated by alkali sodium dodecyl sulphate lysis, followed by column purification using QIAprep Spin Columns (Qiagen). Plasmid mini-preparations were sequenced using the T7 sequencing primer (Stratagene) and clones with the correct sequence then used as positive controls for generating a standard curve. A subset of two positive samples from each farm (n = 48) were
agarose gel purified using an in-house filter tip method and used for sequencing without any further purification, as described previously (Yang et al., 2013).

Specificity and sensitivity

The analytical specificity of the \textit{C. abortus} and \textit{C. pecorum} species-specific qPCR assays has been described previously (Pantchev et al., 2009, 2010), but was further assessed by testing DNA from a wide range of bacterial and parasitic species. To determine the sensitivity of the assay, 10-fold serial dilutions of plasmids were prepared containing the cloned PCR products amplified from \textit{C. abortus} or \textit{C. pecorum}, these were spiked into faecal samples and the DNA was extracted and amplified as described above. The mean detection limits, R squared (RSQ) values and \% relative standard deviation (RDS) were calculated. Template copy numbers were converted to numbers of organism present on the basis that the targeted gene (\textit{OmpA}) is a single copy gene (Lan and Igo, 1998) and bacterial genomes are haploid. Therefore, the detected plasmid numbers were equivalent to the numbers of \textit{Chlamydia} spp.

Inhibition and efficiency

Inhibition in faecal samples was measured using the IAC, which was added to all faecal DNA samples to detect any PCR inhibitors. If inhibition is present in a sample, the IAC will not produce a signal. Amplification efficiency ($E$), a measure of inhibition, was estimated by using the slope of the standard curve and the formula $E = -1 + 10^{-1/\text{slope}}$. A reaction with 100\% efficiency will generate a slope of -3.32. A PCR efficiency less than or greater than 100\% can indicate the presence of inhibitors in the reaction, but reaction efficiencies between 90 and 110\% are typically acceptable (Nybo, 2011). To estimate amplification efficiency, serial dilutions of individual DNA samples (neat, 1:10, 1:100) were performed and multiple
qPCR reactions were conducted at each dilution. The \( Ct \) values were then plotted vs. the \( \log_{10} \) of the dilution and a linear regression was performed using Rotor-Gene 6.0 software.

**Molecular typing and sequence analysis**

A subset of *C. pecorum* positive samples from each farm \((n = 48)\) were sequenced to confirm their identity. Purified PCR products were sequenced using an ABI Prism Dye Terminator Cycle Sequencing kit (Applied Biosystems) according to the manufacturer’s instructions with the exception that the annealing temperature was raised to 58 °C. Nucleotide sequences were analysed using Chromas lite version 2.0\(^1\) and aligned with reference sequences from GenBank using Clustal W\(^2\).

**Statistical analysis**

Prevalences were expressed as the percentage of samples positive by PCR, with 95% confidence intervals calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rózsa et al., 2000). \( \chi^2 \) and non-parametric analyses were performed using SPSS 21.0 for Windows (SPSS/IBM) to determine if there was any association between the prevalence and concentration of *C. pecorum* at different sampling times and across states.

**Results**

**Specificity, sensitivity and efficiency**

Evaluation of specificity of the multiplex *C. abortus* and *C. pecorum* qPCR assay revealed no cross-reactions with other genera and only amplified the relevant bacterial species (data not shown). There was no cross-detection of *C. pecorum* with the *C. abortus* primers


and probe, and vice versa. Sensitivity analysis revealed that the mean limits of detection for
*C. abortus* and *C. pecorum* were 5 and 5 organisms/μL, respectively, which equates to 1250
bacteria/g faeces. The mean RSQ values for *C. abortus* and *C. pecorum* qPCRs were 0.98 and
0.98, respectively. The RDS for *C. abortus* and *C. pecorum* were 4.7% and 3.9%,
respectively. The frequency of PCR inhibition, as determined by the IAC amplification, was
~2%. If inhibition was evident, then the sample was diluted and re-amplified. The mean
efficiencies for *C. abortus* and *C. pecorum* were 96.5% and 94.4%, respectively.

*Prevalence of Chlamydia spp.*

*C. abortus* was not detected in any samples. The overall prevalence of *C. pecorum* on
eight farms across four states over three sampling times (weaning, post-weaning and pre-
slaughter) was 1027/3412 (30.1%) (Fig. 1). Overall, there were significant differences in the
prevalence of *C. pecorum* between states (*P* < 0.01); for example, the prevalence of *C.
pecorum* was lower in WA than in the eastern states (Fig. 1). The *C. pecorum* prevalence was
highest (94.2%) in SA2 during the post-weaning period, followed by 80.8% in NSW during
the pre-slaughter period and 77% in SA1 during the post-weaning period. The highest
prevalence for *C. pecorum* in WA was 48.6% at WA2 during the pre-slaughter period. There
was no relationship between prevalence and the three sampling times (*P* > 0.05), since the
peak prevalence occurred at different sampling times across the farms tested. A total of 422,
114, 309 and 152 lambs were positive for *C. pecorum* across all three samplings at SA, Vic,
NSW and WA, respectively.

*Concentration of Chlamydia spp. in faeces*

*Chlamydia* spp. concentrations in faeces were also determined using the multiplex
qPCR (Table 2; see Appendix A: Supplementary Table 1). The highest median number of *C.
*pecorum* organisms/g was detected in NSW at post-weaning, Vic1 at weaning and WA2 at pre-slaughter (3.1 x 10^9, 2.3 x 10^9 and 1.4 x 10^9 organisms/g, respectively), with a maximum pathogen load of 4.4 x 10^{12} organisms/g detected in one NSW sample at post-weaning. This corresponded to *C. pecorum* prevalences of 72.5% in NSW at post-weaning and 80.8% in NSW at pre-slaughter. There were also peaks in the median numbers of organisms for the weaning and post-weaning periods at SA2 (7.4 x 10^7 and 3.8 x 10^7 organisms/g, respectively) and the post-weaning period at Vic 2 (6.9 x 10^7 organisms/g). The range of *C. pecorum* shedding at weaning overall across all states was 2.5 x 10^2 to 3.8 x 10^{11} organisms/g and the median was 1.8 x 10^7 organisms/g. At post-weaning, the range was 2.5 x 10^2 to 4.4 x 10^{12} and the median was 1.2 x 10^7. At pre-slaughter, the range was 2.5 x 10^2 to 1.4 x 10^{11} and the median was 9.6 x 10^5 (see Appendix A: Supplementary Table 1).

**Sequencing**

In subset of 48 positive samples (two from each sampling period from each farm), all were confirmed by sequencing to be *C. pecorum* (data not shown).

**Discussion**

In this longitudinal study, the prevalence, concentration and species of *Chlamydia* were determined in faecal samples collected from lambs at three sampling times (weaning, post-weaning and pre-slaughter) from eight farms across four Australian states using species-specific qPCR primers. Two species-specific qPCRs for *C. abortus* and *C. pecorum* (Pantchev et al., 2009, 2010) were multiplexed into a single assay for rapid detection of both *Chlamydia* spp. The multiplex PCR was specific for *C. abortus* and *C. pecorum*, since it only detected the relevant species and did not cross-react with non-*Chlamydia* spp. isolates, in agreement with the previous extensive specificity testing of these primers and probes (Pantchev et al., 2009,
In previous studies, the specificity was validated against 25 *Chlamydia* spp. isolates and 14 non-*Chlamydia* bacterial species (Pantchev et al., 2009, 2010). In the present study, the sensitivity of the species-specific qPCR assays was determined by cloning the PCR amplicons from *C. abortus* and *C. pecorum* sp. into a plasmid vector, then spiking known amounts of plasmid into faecal samples, extracting the DNA and screening by qPCR.

The mean limit of detection for *C. abortus* and *C. pecorum* was 5 and 5 organisms/μL faecal DNA extract, respectively. These detection limits are similar to or better than other studies on qPCR detection of *Chlamydia* spp. (Jee et al., 2004; Pelletier et al., 2006; Yang et al., 2006; Berri et al., 2009; Pantchev et al., 2009, 2010). PCRs can be inhibited in faecal specimens by factors such as bile acids, bilirubin, haem and complex carbohydrates (Wilson, 1997). In the present study, PCR inhibition (as determined by the IAC amplification) occurred in ~2% of samples.

Whilst numerous studies have conducted single point prevalence analysis by sampling a random selection of sheep within a flock at a specific time, few longitudinal studies have been conducted. The prevalence at one time of sampling may not provide a true indication of the overall prevalence in flocks over an extended period of time. In the present study, the overall prevalence of *C. pecorum* was 30.1%. However, the prevalence varied widely among states and at different sampling times. For example, the prevalence of *C. pecorum* was highest (94.2%) in SA2 during the post-weaning period, but was only 12.2% during the pre-slaughter period ~10 weeks later. Differences in prevalence could be related to a wide range of factors, including environment, stocking density and potential for faecal contamination of feed or water.
C. pecorum was the only Chlamydia spp. identified in Australian sheep in the present study. This is supported by a serological survey of 891 sheep from 109 properties across southern Australia (McCauley et al., 2010). An obvious limitation of the present study is that vaginal swabs were not screened and, therefore, conclusive evidence for the absence of C. abortus in Australian sheep is lacking. However, C. abortus has been detected in faeces, as well as the genital tract, of sheep (Tsakos et al., 2001; Lenzko et al., 2011).

PCR is more sensitive than traditional microscopy, culture and immunoassays for detecting Chlamydia spp. (Amin, 2003; Nordentoft et al., 2011; Hazlett et al., 2013). In the present study, the prevalence of C. pecorum across all states was highest in NSW (42.5-80.8%) and lowest in Vic using qPCR (4.9-18.3%). A previous study using culture identified C. pecorum in 4.8% of sheep in Egypt (Osman et al., 2011). A study in Germany using a Chlamydiaceae-specific qPCR or conventional PCR identified C. pecorum in 13/32 (40.6%) flocks, with in-flock prevalences of 9.1-27.3% (Lenzko et al., 2011). Chlamydia (thought to be C. pecorum) has been detected in sheep faeces in Australia (St George, 1971) and, in the most recent study conducted in Australia, C. pecorum was detected by PCR in 17-50% of rectal swabs in sheep in New South Wales (Jelocnik et al., 2013).

The source of C. pecorum infections in Australian sheep is unclear. The organism is also prevalent amongst koala populations (Jackson et al., 1999) and there is potential for C. pecorum ‘spillover’ or ‘spillback’ between infected livestock and/or wildlife infections. However, a recent C. pecorum-specific multilocus sequence analysis (MLSA) of koala-derived and Australian sheep-derived isolates showed that the koala isolates formed a distinct clade, with limited clustering with C. pecorum isolates from Australian sheep (Jelocnik et al., 2013).
Across all states, *C. pecorum* had median bacterial concentrations at weaning, post-weaning and pre-slaughter of $1.8 \times 10^7$, $1.2 \times 10^7$ and $9.6 \times 10^5$ organisms/g, respectively. However, individual sheep shed up to $4.4 \times 10^{12}$ organisms/g (one lamb sampled post-weaning in NSW). The reasons for the higher bacterial shedding during weaning and post-weaning compared to pre-slaughter in the present study are unknown, but may be due to stress or to polyparasitism reducing host immunity, since these sheep were co-infected with *Giardia*, *Cryptosporidium* and *Eimeria* spp., as well as strongyles (Yang et al., 2014a and b; R. Yang unpublished).

**Conclusions**

This study identified a high prevalence of faecal shedding of *C. pecorum* by sheep in four states in Australia. *C. abortus* was not detected in sheep faecal samples, but further screening of vaginal swabs is required to confirm the absence of *C. abortus* in sheep in Australia. Further studies are required to determine production losses associated with *C. pecorum* infection in sheep.

**Conflict of interest statement**

The study was financed by Meat and Livestock Australia (MLA), Australian Wool Innovation Limited (AWI) and the Australian Government, which had no influence on study design, data evaluation or manuscript preparation. None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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References


Jones, G.E., Low, J.C., Machell, J., Armstrong, K., 1997. Comparison of five tests for the
detection of antibodies against chlamydial (enzootic) abortion of ewes. Veterinary
Record 141, 164-168.

*Escherichia coli* K-12 depends upon the level of active OmpR. Journal of Bacteriology
180, 171-174.

Lenzko, H., Moog, U., Henning, K., Lederbach, R., Diller, R., Menge, C., Sachse, K.,
Sprague, L.D., 2011. High frequency of chlamydial co-infections in clinically healthy
sheep flocks. BMC Veterinary Research 7, 29.

of *Chlamydomphila abortus* in Australian sheep and implications for the rejection of

Nordentoft, S., Kabell, S., Pedersen, K., 2011. Real-time detection and identification of
*Chlamydomphila* species in veterinary specimens by using SYBR green-based PCR
assays. Applied and Environmental Microbiology 77, 6323-6330.


*Chlamydomphila pecorum* infections in goats and sheep in Egypt. Revue Scientifique et
Technique (Office International des Epizooties) 30, 939-948.

for species-specific detection of *Chlamydomphila psittaci* and *Chlamydomphila abortus*
from tissue samples. The Veterinary Journal 181, 145-150.

*Chlamydomphila* and *Chlamydia* spp. of veterinary interest using species-specific real-
time PCR assays. Comparative Immunology, Microbiology and Infectious Diseases 33,
473-484.

Pelletier, C., Chartier, S., Berthillier, J., Dégletagne, E., Rigaud, C., Berthet, H., Valognes, A.,
Reynaud, A., Véry, P., 2006. Validation of an internal method for the diagnosis of
infections with *Chlamydomphila abortus* and *Coxiella burnetii* by real-time multiplex

Microbiology 140, 382-391.

of Parasitology 86, 228-232.

Australian Veterinary Journal 47, 74.


Table 1
Sheep farms sampled during the present study.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Farm location</th>
<th>Mean annual rainfall (mm)</th>
<th>Farm size (Ha)</th>
<th>Number of sheep</th>
<th>Breed</th>
<th>Commencement of lambing</th>
<th>Goats and/or cattle on property?</th>
<th>Winter stocking rate (DSE/Ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA1</td>
<td>Wirrega, SA</td>
<td>430</td>
<td>1040</td>
<td>1800</td>
<td>Suffolk</td>
<td>Mid-April</td>
<td>No</td>
<td>10</td>
</tr>
<tr>
<td>SA2</td>
<td>Struan, SA</td>
<td>550</td>
<td>1500</td>
<td>5500</td>
<td>BL/Merino x Suffolk</td>
<td>June</td>
<td>Yes</td>
<td>15</td>
</tr>
<tr>
<td>Vic1</td>
<td>Rosedale, Vic</td>
<td>620</td>
<td>300</td>
<td>300</td>
<td>BL/Merino x Dorset and Southdown ewes</td>
<td>Mid-July</td>
<td>No</td>
<td>10</td>
</tr>
<tr>
<td>Vic2</td>
<td>Ballarat, Vic</td>
<td>750</td>
<td>1960</td>
<td>7000</td>
<td>Merino x Suffolk</td>
<td>Early August</td>
<td>Yes</td>
<td>13</td>
</tr>
<tr>
<td>NSW</td>
<td>Armidale, NSW</td>
<td>495</td>
<td>2958</td>
<td>1000</td>
<td>BL/Merino</td>
<td>May-August</td>
<td>No</td>
<td>20</td>
</tr>
<tr>
<td>WA1</td>
<td>Pingelly, WA</td>
<td>450</td>
<td>1500</td>
<td>1350</td>
<td>Merino x Suffolk</td>
<td>Mid-July</td>
<td>No</td>
<td>12</td>
</tr>
<tr>
<td>WA2</td>
<td>West Arthur, WA</td>
<td>500</td>
<td>1250</td>
<td>1750</td>
<td>Merino x Suffolk</td>
<td>Early August</td>
<td>No</td>
<td>10</td>
</tr>
<tr>
<td>WA3</td>
<td>Frankland, WA</td>
<td>550</td>
<td>560</td>
<td>3300</td>
<td>Merino x Suffolk</td>
<td>Mid-July</td>
<td>No</td>
<td>21</td>
</tr>
</tbody>
</table>

DSE, dry sheep equivalent; BL, Border Leicester; SA, South Australia; Vic, Victoria; NSW, New South Wales, WA, Western Australia.

DNA from samples from Western Australia was extracted as described in Sweeney et al., (2011).
<table>
<thead>
<tr>
<th>Location</th>
<th>Age of sheep</th>
<th>Median Number/g sheep faeces</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA1</td>
<td>Weaning</td>
<td>$1.9 \times 10^4$</td>
<td>500 to $9.0 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td>Post-weaning</td>
<td>$1.3 \times 10^4$</td>
<td>250 to $6.6 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>$1.7 \times 10^3$</td>
<td>250 to $2.4 \times 10^6$</td>
</tr>
<tr>
<td>SA2</td>
<td>Weaning</td>
<td>$7.4 \times 10^4$</td>
<td>$1.7 \times 10^4$ to $1.6 \times 10^{11}$</td>
</tr>
<tr>
<td></td>
<td>Post-weaning</td>
<td>$3.8 \times 10^3$</td>
<td>$1.3 \times 10^4$ to $3.0 \times 10^{10}$</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>$8.4 \times 10^4$</td>
<td>$4.5 \times 10^3$ to $1.0 \times 10^6$</td>
</tr>
<tr>
<td>Vic1</td>
<td>Weaning</td>
<td>$2.3 \times 10^4$</td>
<td>$3.0 \times 10^3$ to $2.3 \times 10^{11}$</td>
</tr>
<tr>
<td></td>
<td>Post-weaning</td>
<td>$1.7 \times 10^3$</td>
<td>250 to $1.6 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>$1.9 \times 10^4$</td>
<td>250 to $2.8 \times 10^3$</td>
</tr>
<tr>
<td>Vic2</td>
<td>Weaning</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Post-weaning</td>
<td>$6.9 \times 10^7$</td>
<td>$7.9 \times 10^3$ to $8.2 \times 10^{10}$</td>
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<td>Pre-slaughter</td>
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<td>500 to $6.3 \times 10^3$</td>
</tr>
<tr>
<td>NSW</td>
<td>Weaning</td>
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<td>250 to $3.8 \times 10^{11}$</td>
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<tr>
<td></td>
<td>Post-weaning</td>
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<td>$4.5 \times 10^4$ to $4.4 \times 10^{12}$</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>$8.9 \times 10^5$</td>
<td>250 to $4.0 \times 10^9$</td>
</tr>
<tr>
<td>WA1-AR</td>
<td>Weaning</td>
<td>$3.5 \times 10^6$</td>
<td>250 to $1.8 \times 10^9$</td>
</tr>
<tr>
<td></td>
<td>Post-weaning</td>
<td>$9.3 \times 10^5$</td>
<td>$1.3 \times 10^4$ to $1.7 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>$9.4 \times 10^4$</td>
<td>$3.0 \times 10^4$ to $1.1 \times 10^6$</td>
</tr>
<tr>
<td>WA2-PL</td>
<td>Weaning</td>
<td>$1.5 \times 10^6$</td>
<td>$1.5 \times 10^3$ to $2.9 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td>Post-weaning</td>
<td>$1.0 \times 10^6$</td>
<td>250 to $1.7 \times 10^8$</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>$1.4 \times 10^6$</td>
<td>$1.1 \times 10^6$ to $1.4 \times 10^{11}$</td>
</tr>
<tr>
<td>WA3-FL</td>
<td>Weaning</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Post-weaning</td>
<td>$5.2 \times 10^8$</td>
<td>$1.5 \times 10^3$ to $1.6 \times 10^{11}$</td>
</tr>
<tr>
<td></td>
<td>Pre-slaughter</td>
<td>$1.3 \times 10^3$</td>
<td>250 to $8.9 \times 10^7$</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>$8.1 \times 10^5$</td>
<td>0 to $4.4 \times 10^{12}$</td>
</tr>
</tbody>
</table>
Fig. 1. Prevalence (%) of *Chlamydia pecorum* in ovine faecal samples from eight farms across four states (NSW, SA, Vic and WA) over three sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR.