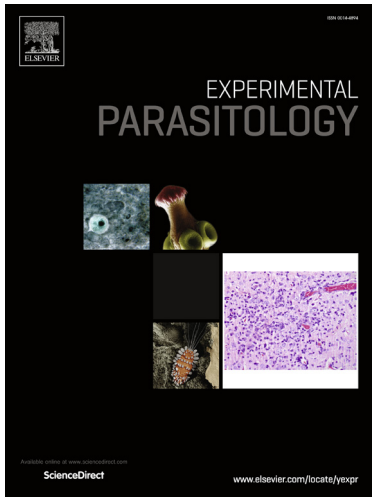

Development of a quantitative PCR (qPCR) for *Giardia* and analysis of the prevalence, cyst shedding and genotypes of *Giardia* present in sheep across four states in Australia

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

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Development of a quantitative PCR (qPCR) for *Giardia* and analysis of the prevalence, cyst shedding and genotypes of *Giardia* present in sheep across four states in Australia.

Rongchang Yang[1], Caroline Jacobson[1], Graham Gardner[1], Ian Carmichael[2], Angus J D Campbell[3] and Una Ryan[1*]

[1]*School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, 6150.*

[2]*South Australian Research and Development Institute, 33 Flemington Street, Glenside, SA 5065, Australia.*

[3]*Faculty of Veterinary Science, University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia*

Corresponding author: Una Ryan

Phone: 08 9360 2482

Fax: 08 9310 4144

E-mail: Una.Ryan@murdoch.edu.au

Abstract

A novel quantitative PCR (qPCR) for *Giardia* at the glutamate dehydrogenase (*gdh*) locus was developed and validated. The qPCR was used to screen a total of 3,412 lamb faecal samples collected from approximately 1,189 lambs at three sampling periods (weaning, post-weaning and pre-slaughter) from eight farms across South Australia (SA), New South Wales (NSW), Victoria (Vic) and Western Australia (WA). The overall prevalence was 20.2% (95% CI 18.9-21.6) and of the 690 positives, 473 were successfully typed. In general, the prevalence of *Giardia* varied widely across the different farms with the highest prevalence in one WA farm (42.1%) at pre-slaughter sampling and the lowest prevalence in one Victorian farm (7.2%) at weaning. The range of cyst shedding at weaning, post-weaning and pre-slaughter overall across all states was 63 – 1.3 x 10^9 cysts g^{-1} (median = 1.7 x 10^4), 63 - 1.1 x 10^9 cysts g^{-1} (median = 9.6 x 10^3), 63 – 4.7 x 10^9 cysts g^{-1} (median = 8.1 x 10^4) respectively. Assemblage specific primers at the triose phosphate isomerase (*tpi)* locus identified assemblage A in 22.4% (106/473) of positive samples typed, assemblage E in 75.9% (359/473) and mixed A and E assemblages in 1.7% (8/473) of samples. A subset of representative samples from the 8 farms (n=32) were typed at both the *gdh* and *beta-giardin* loci and confirmed these results and identified sub-assemblage AII in 16 representative assemblage A isolates across the 8 farms. This demonstrates a prevalence of *Giardia* previously not recognised in Australian sheep, highlighting a need for further research to quantify the production impacts of this protozoan parasite.

Keywords: *Giardia*; lambs; qPCR; *gdh*; *tpi*; *beta-giardin; assemblage A* and *E*

1. Introduction

Giardia duodenalis (syn. Giardia lamblia, Giardia intestinalis) is one of the most common protozoan parasites in humans and animals. It is also one of the most prevalent waterborne parasitic infections producing diarrhea and is responsible for 2.8×10^8 cases yearly (Lane and Lloyd, 2002). In a recent review it has been reported that of the 199 published outbreaks caused by protozoa during the period 2004-2010, 70 (35%) were caused by *Giardia* (Baldursson and Karanis, 2011). There has been considerable interest in identifying animal species which may be hosts for *Giardia* assemblages which have the potential to be transmitted to humans, in order that suitable measures and initiatives can be implemented which limit the possibilities of faecal contamination of drinking-water sources by host animals (Robertson, 2009).

Assessing the zoonotic transmission of the parasite requires molecular characterisation as there is considerable genetic variation within *G. duodenalis*. To date eight major genetic groups (assemblages) have been identified, two of which (A and B) are found in both humans and animals, whereas the remaining six (C to H) are host-specific and do not infect humans (Feng and Xiao, 2011; Ryan and Caccio, 2013).

The public health significance of giardiasis in sheep is currently unclear. *Giardia* is widely distributed in sheep with prevalence's of 1.5% to 55.6% reported (Feng and Xiao, 2011). The host specific assemblage E (livestock genotype) and the zoonotic assemblage A have been predominately reported in sheep (Giangaspero et al., 2005; Ryan et al., 2005; Santín et al., 2007; Geurden et al., 2008, Gómez-Muñoz et al., 2009; Sprong et al., 2009; Yang et al., 2009; Lebbad et al., 2010; Nolan et al., 2010; Robertson et al., 2010; Sweeny et al., 2011; Feng and Xiao, 2011; Gómez-Muñoz et al., 2012; Zhang et al., 2012), although assemblage B has also been reported (Aloisio et al., 2006; Zhang et al., 2012). Previous studies conducted in Australia have examined lambs in Western Australia (WA) and Victoria (Vic) only (Ryan et al., 2005; Yang et al., 2009; Nolan et al., 2010; Sweeny et al., 2011). However, most of these were point prevalence studies and

due to intermittent shedding of *Giardia* cysts, longitudinal studies are important for a more accurate understanding of the prevalence and cyst shedding of *Giardia*.

Cyst shedding concentration has important implications not just for understanding the intensity of *Giardia* infections in sheep, but also for understanding the potential contribution of sheep to contamination of drinking water catchments. Cyst transport to surface water can occur by deposition of manure directly in the water or by wash-off in surface runoff. Current risk management practices for minimizing pathogen health risks to water supplies require both prevalence and the concentration of pathogens in faeces to establish pathogen source loads in watersheds, enabling the assessment of risk from faecal contamination of source waters (Cox et al., 2005). Accurate quantification of *Giardia* cysts in animal faecal deposits on land is an essential starting point for estimating catchment *Giardia* loads.

The aim of the present study was to determine cyst concentrations directly by qPCR as well as the prevalence and assemblages of *Giardia* in lambs over a wider geographical area representing the major sheep growing regions of Australia, specifically WA, New South Wales (NSW), Vic and South Australia (SA), at three sampling periods (weaning, post-weaning and pre-slaughter) and compare this data between states.

2. Materials and Methods

2.1 Animals and faecal sample collection

Faecal samples were collected from cross-bred lambs from 8 different farms across 4 states (Table 1). Lambs were born and reared in paddocks and were not housed indoors at any stage. Lambs were sampled on 3 occasions (i.e. the same animals were sampled on each occasion) at weaning (approx. 12 weeks of age), post-weaning (approx. 19 weeks) and pre-slaughter (approx. 29 weeks). A total of 3,412 faecal samples approximately 1,189 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).

2.2 DNA isolation

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

2.3 PCR amplification.

All samples were screened at the glutamate dehydrogenase (*gdh*) locus using a quantitative PCR (qPCR) using the forward primer, *gdh*F1 F1 5' GGGCAAGTCCGACAACGA 3', the reverse primer *gdh*R1 5' GCACATCTCCTCCAGGAAGTAGAC 3' and the probe 5'-(Joe 670)-TCATGCGCTTCTGCCAG BHQ2 3' which produces a 261 bp product. An internal amplification control (IAC) consisted of a fragment of a coding region from Jembrana Disease Virus (JDV) cloned into a pGEM-T vector (Promega, USA) was used as previously described (Yang et al., 2013). The IAC primers were JDVF (5'- GGT AGT GCT GAA AGA CAT T) and JDVR (5'- ATG TAG CTT GAC CGG AAG T) and the probe was 5'- (Cy5) TGC CCG CTG CCT CAG TAG TGC (BHQ2). Each 15 µl PCR mixture contained 1× PCR Buffer, 5 mM $MgCl_2$, 1 mM dNTP's, 1.0 U Kapa DNA polymerase (MolBio, Carlsbad, California), 0.2 µM each of forward and reverse primers, 0.2 µM each of forward and reverse IAC primers, 50 nM of the probe, 50 nM of IAC probe, 10 copies of IAC template and 1 µl of sample DNA. The PCR cycling conditions consisted of a pre-melt at 95°C for 3 min and then 45 cycles of 95°C for 30 sec, and a combined annealing and extension step of 60°C for 45 sec. A standard curve for quantifying *Giardia* DNA was generated using known number cysts serially diluted from 100,000 cysts to 100 cysts followed by DNA extraction with a QIAamp DNA Mini Kit (Qiagen, Victoria, Australia).

Positives were also amplified using assemblage specific primers at the triose phosphate isomerase (*tpi*) locus as previously described (Geurden et al., 2008). A subset of positives (n=32), were also amplified using a heminested PCR at the *beta-giardin* locus using primers BGexF: 5'-CCCGACGACCTCACCCGCAGT – 3' and BGRev: 5'- GCTCGGCCTTCTCGCGGTCG - 3 for the primary reaction with a predicted PCR product size of 682bp. The forward primer BGinF: 5' – CCTTGCGGAGATGGGCGACACA – 3' was used with BGRev in the secondary PCR with a predicted PCR product size of 380bp. The following cycling conditions were used for both primary and secondary PCRs: 1 cycle of 94 ºC for 3 min, followed by 45 cycles of 94 ºC for 30 sec, 55 ºC for 30 sec and 72 ºC for 1min with a final extension of 72 ºC for 7 min. The same positives were also sequenced at the *gdh* locus using the *gdh* qPCR primers described above. PCR contamination controls were used including negative controls and separation of preparation and amplification areas.

The amplified DNA fragments from the secondary PCR products were separated by gel electrophoresis and purified using an in house filter tip method and used for sequencing without any further purification as previously described (Yang et al., 2013).

2.4 Specificity and sensitivity testing of the gdh qPCR

The analytical specificity of the qPCR assay was assessed by testing DNA from *Giardia duodenalis* assemblage A (n=2) and E isolates (n=2) from sheep, assemblage B from a human (GH18), assemblage C from a dog (C14), assemblage D from a fox (BP-10), assemblage F from a cat (Cat132) assemblage G from a rat (Phy36) and non-*Giardia* spp; *C. suis, C. bovis, C. ryanae, C. xiaoi, C. ubiquitum, Isospora, Tenebrio, Cyclospora, Campylobacter spp., Salmonella spp., Toxoplasma gondii, Trichostrongylus spp., Teladorsagia circumcincta, Haemonchus contortus, Streptococcus bovis* (ATCC 33317), *Enterococcus durans* (ATCC 11576), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633) and *Eimeria sp.,* as well as human, sheep and cattle DNA.

In order to determine the sensitivity of the assay, the PCR product amplified from assemblage E from a sheep was cloned into the pGEMT-vector (Promega) and transformed into *E. coli* competent cells. Plasmid DNA was isolated by alkali\SDS lysis followed by column purification using QIAprep Spin Columns (Qiagen) in accordance with the manufacturer's protocol. Plasmid mini-preparations were sequenced using T7 sequencing primer (Stratagene, La Jolla, CA, USA) and clones with the correct sequence then used. The plasmid copy numbers were calculated based on the plasmid size (base pairs) and DNA concentration. 10-fold series dilutions of plasmid were conducted from 10,000 copies down to 1 copy of the plasmid template for sensitivity testing and these were then spiked into faecal samples and the DNA extracted and amplified as described above and mean detection limits, RSQ (R squared) values and % Relative Standard Deviation (RDS) were calculated. Copy numbers detected were converted to cyst numbers on the basis that the *gdh* gene in *Giardia* is a single copy gene (Yee and Denis, 1992) and the fact that there are 4 haploid nuclei per cyst. Therefore, every 4 copies of *gdh* detected by qPCR were equivalent to 1 cyst.

2.5 Investigation of inhibition and efficiency

Inhibition in faecal samples was measured using the IAC as the IAC was added to all faecal DNA samples to detect any PCR inhibitors present in the extracted DNA. If any inhibition is present in a sample, the IAC will not produce a signal. Amplification efficiency (*E*) (which is 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 on faecal samples serial dilutions of individual DNA samples (neat, 1:10, 1:100) were performed and multiple qPCR reactions were conducted on each dilution. The *Ct* values were then

plotted versus the log base 10 of the dilution and a linear regression was performed using the Rotor-Gene 6.0. software.

2.6 Sequence analysis

Purified PCR products were sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer's instructions with the exception that the annealing temperature was raised to 58ºC. Nucleotide sequences were analyzed using Chromas lite version 2.0 (http://www.technelysium.com.au) and aligned with reference sequences from GenBank using Clustal W (http://www.clustalw.genome.jp).

2.7 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-square and non-parametric analyses were performed using SPSS 21.0 (Statistical Package for the Social Sciences) for Windows (SPSS inc. Chicago, USA) to determine if there was any association between the prevalence and concentration of *Giardia* cysts at different sampling times and across states.

3 Results

3.1 Specificity, sensitivity and efficiency testing of the gdh qPCR

Evaluation of specificity of the *gdh* qPCR assay revealed no cross-reactions with other genera and detected all the *Giardia* isolates tested (data not shown). Sensitivity analysis revealed

that the assay could reliably detected 4 copies of the cloned assemblage E amplicon per µl of faecal DNA extract which is equivalent to a sensitivity of 1 *Giardia* cyst per µl of faecal DNA extract. The mean RSQ was 0.98 and the % RDS = 5.5%. In our hands, the incidence of PCR inhibition as determined by the IAC amplification was about 2%. If inhibition was evident, then the sample was diluted and re-amplified. The mean efficiency for the *Giardia* qPCR was 95.4%.

3.2 Prevalence of Giardia for 8 farms across 4 states

The overall prevalence of *Giardia* from 8 farms across 4 states over 3 sampling periods (weaning, post-weaning and pre-slaughter) was 20.2% (95% confidence interval 18.9-21.6) (Tables 2 and 3 and Fig. 1a). There was no correlation between prevalence and the 3 sampling times and also between different farms ($p>0.05$), as the peak prevalence occurred at different sampling times across the farms tested. There was however a significant difference in prevalence between farms ($p=0.003$). The prevalence of *Giardia* at WA1 was significantly higher than all other farms ($p<0.005$) with the exception of WA2. The highest prevalences for *Giardia* were recorded at WA1, which peaked at 42.1% and 35.2% during pre-slaughter and post-weaning respectively. The lowest prevalence for WA farms was 7.9% (2.7-13) for WA3 at weaning (Table 2). At NSW, the peak prevalence was at pre-slaughter (34.7%). In Vic, peak prevalences were detected for Vic 2 (30.5%) at pre-slaughter and Vic1 (23.3%) at post-weaning. In SA, the prevalence peaked at 20.3% at post-weaning at SA1.

The overall prevalence in WA across the 3 farms was 25.4% (252/992). The prevalence in NSW was 23.2% (113/487), 16.9% (117/989) for Vic and 16.7% (158/944) for SA, but these state-wide prevalences were not significant ($p=0.235$)(Fig 1b).

A total of 24 lambs from WA (14 from WA1 and 10 from WA2) were positive across all 3 samplings. Only 4, 1 and 2 samples from SA, Vic and NSW respectively were positive across all 3 samplings.

3.3 Cyst shedding concentrations

Cyst numbers per gram of faeces (g^{-1}) were also determined using qPCR (Tables 2 and 3). The highest median concentration of *Giardia* cysts were shed by lambs at SA1 during post-weaning (8.3×10^5 cysts g^{-1}) and WA3 during pre-slaughter (5.2×10^5 cysts g^{-1}). Across the other farms, median *Giardia* cyst concentration peaked during the pre-slaughter period at Vic 2, WA1 and WA2 (9.0×10^4, 7.5×10^4 and 5.2×10^4 cysts g^{-1} respectively). At SA2, the highest median concentration of cysts shed was 1.1×10^5 cysts g^{-1} at weaning and while the median concentration of cysts shed at post-weaning was low (9.8×10^3 cysts g^{-1}), individual lambs shed up to 2.1×10^9 cysts g^{-1} during this period. This corresponded with a peak prevalence of 19.9% at post-weaning at SA2. The median concentration of cysts shed at Vic1 was relatively low (1.2×10^4-4.2×10^4 cysts g^{-1}), although individual sheep shed up to 1.3×10^9 cysts g^{-1} during weaning and 1.0×10^9 cysts g^{-1} during post-weaning. This corresponded with a peak prevalence of 23.3% at post-weaning at Vic1. At NSW, the median concentration of cysts shed was also low (2.0×10^3-4.3×0^3 cysts g^{-1}) as was the cyst shedding which ranged between 63- 4.5×10^5 cysts g^{-1}. The range of cyst shedding at weaning overall across all states was 63 – 1.3×10^9 and the median was 1.7×10^4. At post-weaning, the range was 63 - 1.1×10^9 and the median was 9.6×10^3. At pre-slaughter, the range was 63 – 4.7×10^9 and the median was 8.1×10^4 (Table 3).

3.4 Giardia assemblages

The 690 positives detected at the *gdh* locus were screened using assemblage specific primers at the *tpi* locus. Of these, 473 were successfully genotyped; assemblage A was identified in 22.4% (106/473) of positive samples typed, assemblage E in 75.9% (359/473) and mixed A and E assemblages in 1.7% (8/473) of samples. Assemblage E was the most prevalent across all states and

peaked at 20.1% for post-weaning in WA (Fig. 2). Assemblage A was most prevalent in NSW and peaked during the pre-slaughter sampling (10.8%). Mixed A and E infections were only identified in WA and were confirmed by sequencing.

Two assemblage A and 2 assemblage E isolates from each of the 8 farms (n=32) were sequenced at both the *gdh* and *beta-giardin* loci. All the 16 assemblage A *gdh* sequences were identical to each other and were identified as sub-assemblage AII by aligning with reference AII *gdh* sequence AY178737. The 16 Assemblage E sequences at the *gdh* locus were also identical to each other and were confirmed as assemblage E by aligning with reference assemblage E *gdh* sequence AY178741. Similarly at the *beta-giardin* locus, all 16 assemblage A sequences were identical to each other and were typed as sub-assemblage AII. The 16 assemblage E sequences at the *beta-giardin* locus were confirmed as assemblage E by aligning with assemblage E *beta-giardin* reference sequence AY072729. Representative sequences were submitted to GenBank under the accession numbers: KF657676 and KF657677.

4. Discussion

The present longitudinal study describes the prevalence, cyst concentration and assemblages of *G. duodenalis* from lamb faecal samples collected at three sampling periods (weaning, post-weaning and pre-slaughter) from eight farms across four states using a novel qPCR at the *gdh* locus.

The qPCR assay was very specific for *Giardia,* as it detected all the *Giardia* species tested and did not cross-react with the non-*Giardia* isolates analysed. The sensitivity of the assay was determined by cloning the *gdh* assemblage E PCR amplicon into a plasmid vector, and then spiking known amounts of plasmid into faecal samples, extracting the DNA and screening by qPCR. The assay could reliably detect 1 *Giardia* cyst per µl of faecal DNA extract, which is similar to or better than sensitivities reported previously for *Giardia* qPCR detection assays (Helmy et al., 2009; Almeida et al., 2010; Baque et al., 2011; Stroup et al., 2012).

The overall prevalence of *Giardia* from 8 farms across 4 states over 3 sampling periods (weaning, post-weaning and pre-slaughter) was 20.2% (690/3412) and ranged from 7.9% to 42.1% (WA). Previous studies have reported prevalences by PCR of 4% - 89.2% in lambs (Ryan et al., 2005; Santín et al., 2007; Geurden et al., 2008; Gómez-Muñoz et al., 2009; Yang et al., 2009; Nolan et al., 2010; Robertson et al., 2010; Sweeny et al., 2011; Gómez-Muñoz et al., 2012). In Australia, previous studies in WA have reported prevalences of 11.1% and 44% in pre and post-weaned lambs (Yang et al., 2009), while in Vic, a prevalence of 15.1% was reported in lambs (<7 weeks) (Nolan et al., 2010).

Cyst numbers per gram of faeces (g^{-1}) were also determined using qPCR. The data showed that although the prevalence for WA3 was the lowest for all farms sampled (7.9-15%), the median cyst shedding concentration was relatively high and peaked at 5.2 $x10^5$ cysts g^{-1} during pre-slaughter. SA1 had a relatively low prevalence (11.9-20.3%) but had the highest median cyst shedding which peaked at 8.3 x 10^5 cysts g^{-1} during post-weaning. There are only limited reports on the concentration and environmental loading of *Giardia* cysts as a result of faecal contamination by sheep. One study reported that the range of cyst shedding for adult sheep in Sydney catchments was 0 – 504 cysts g^{-1} with a median of 26 cysts g^{-1} (Cox et al., 2005). However this was based on immunomagnetic separation (IMS) with recovery rates varying from 13-73%. In contrast, the shedding rates evident in the present study were markedly higher, possibly highlighting the advantage of using a PCR-based detection method that does not require purification of cysts, thereby greatly reducing cyst loses. This method has also been shown to be much more sensitive than microscopy (Ryan et al., 2005).

Understanding the risk of *Giardia* contamination in catchments must also take into account the prevalence of potentially zoonotic assemblages being shed from animal sources. In the present study, the non-zoonotic assemblage E was responsible for the majority (75.9%) of positive isolates typed, whereas the potentially zoonotic Assemblage A was identified in 22.4% of positive isolates typed with mixed A and E infections in 1.7% of samples. Previous studies have also reported that

assemblages E and A are the dominant assemblages infecting sheep and although assemblage E is usually more prevalent (Feng and Xiao, 2011; Caccio and Ryan, 2013), one Australian study in Victoria reported that assemblage A was more prevalent than E in sheep (Nolan et al., 2010) and in Italy, in one study, only assemblage A was found in sheep (Giangaspero et al., 2005).

Subtyping at the *gdh* and *beta-giardin* loci identified sub-assemblage AII. Within assemblage A, three main sub-assemblages have been identified; AI, AII and AIII. AI and AII have been reported in both humans and animals, while AIII is associated mostly with wild hoofed animals (Feng and Xiao, 2011; Ryan and Caccio, 2013). Relatively few studies have subtyped assemblage A isolates in sheep but both AI and AII have been reported (Feng and Xiao, 2011; Ryan and Caccio, 2013). Sprong et al., (2009) reported that 78% of the assemblage A sequences obtained from sheep and goats were sub-assemblage AI. This sub-assemblage was also frequently found in other studies carried out in sheep (Giangaspero et al., 2005, Lebbad et al., 2010 and Gómez-Muñoz et al., 2009; Sweeny et al., 2011; Gómez-Muñoz et al., 2012; Zhang et al., 2012). In the present study sub-assemblage AII was identified on all 8 farms and was identical to AII sub-assemblages, which have been identified in humans in Australia (Lee et al., 2010), which indicates that sheep are a potential zoonotic reservoir for human *Giardia* infections.

In conclusion, the present study identified that *Giardia* is prevalent in lambs across Australia and that lambs are capable of harboring *Giardia* species that are known to be potentially zoonotic as well as those that appear to be host-specific. In addition, lambs may contribute *Giardia* cysts to catchments, which has important implications for catchment management. Further studies are required to determine the prevalence of assemblage A subtypes in the human population in Australia, and the extent of economic loss associated with *Giardia* in sheep.

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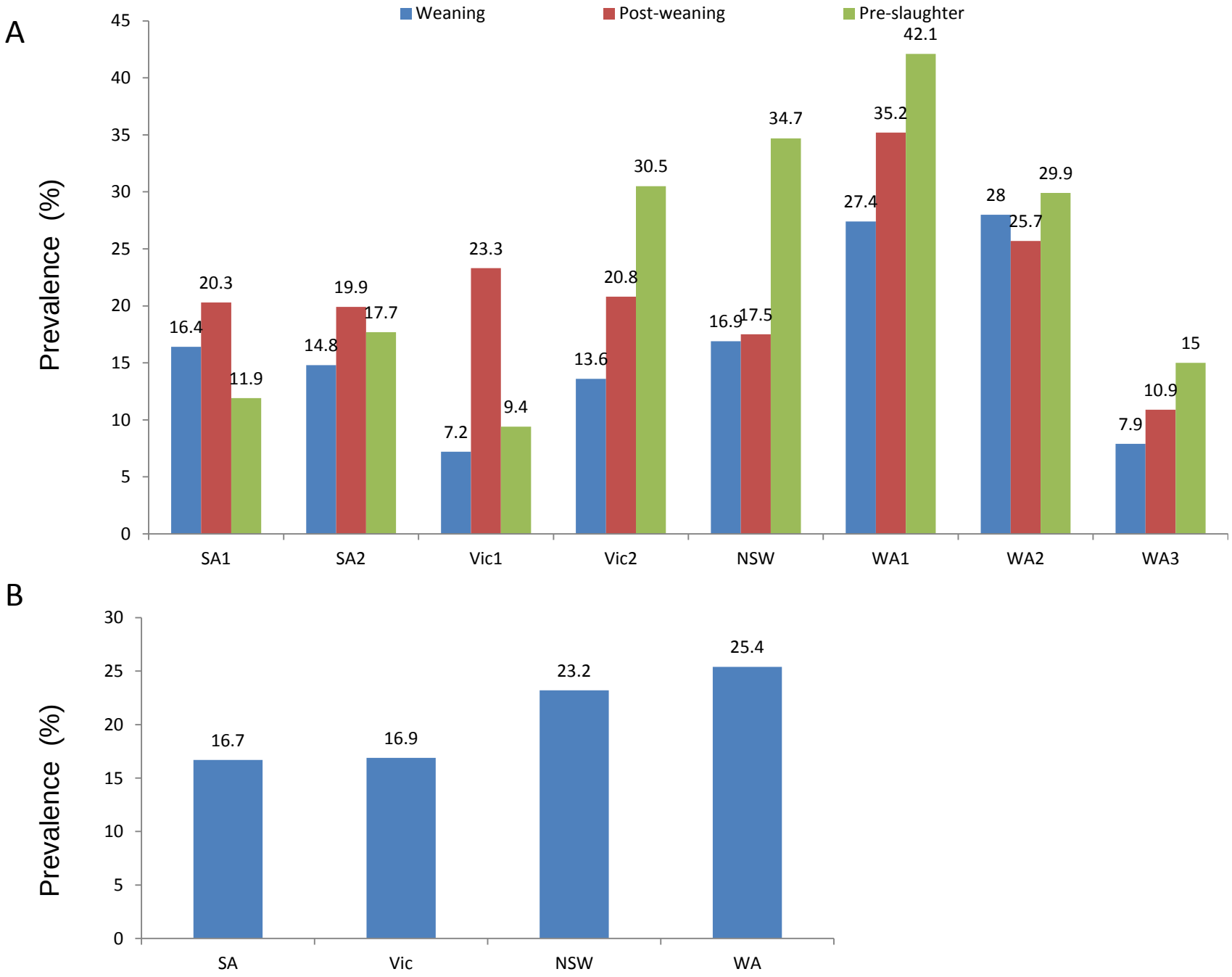
Figure 1A. Prevalence (%) of *Giardia* in sheep faecal samples from 8 farms across 4 states (NSW, SA, Vic and WA) over 3 sampling times (weaning, post-weaning and pre-slaughter) as determined by qPCR. 1B. Overall *Giardia* prevalence per state.

Figure 2. The number of animals with *G. duodenalis* assemblage A or E sequences detected in sheep faecal samples from SA, Vic, WA and NSW (A) and across different sampling times (B).


A
Weaning
Post-weaning
Pre-slaughter
Prevalence (%)
45
40
35
30
25
20
15
10
5
0
16.4
20.3
11.9
14.8
19.9
17.7
7.2
23.3
9.4
13.6
20.8
30.5
16.9
17.5
34.7
27.4
35.2
42.1
28
25.7
29.9
7.9
10.9
15
SA1
SA2
Vic1
Vic2
NSW
WA1
WA2
WA3
B
Prevalence (%)
30
25
20
15
10
5
0
16.7
16.9
23.2
25.4
SA
Vic
NSW
WA

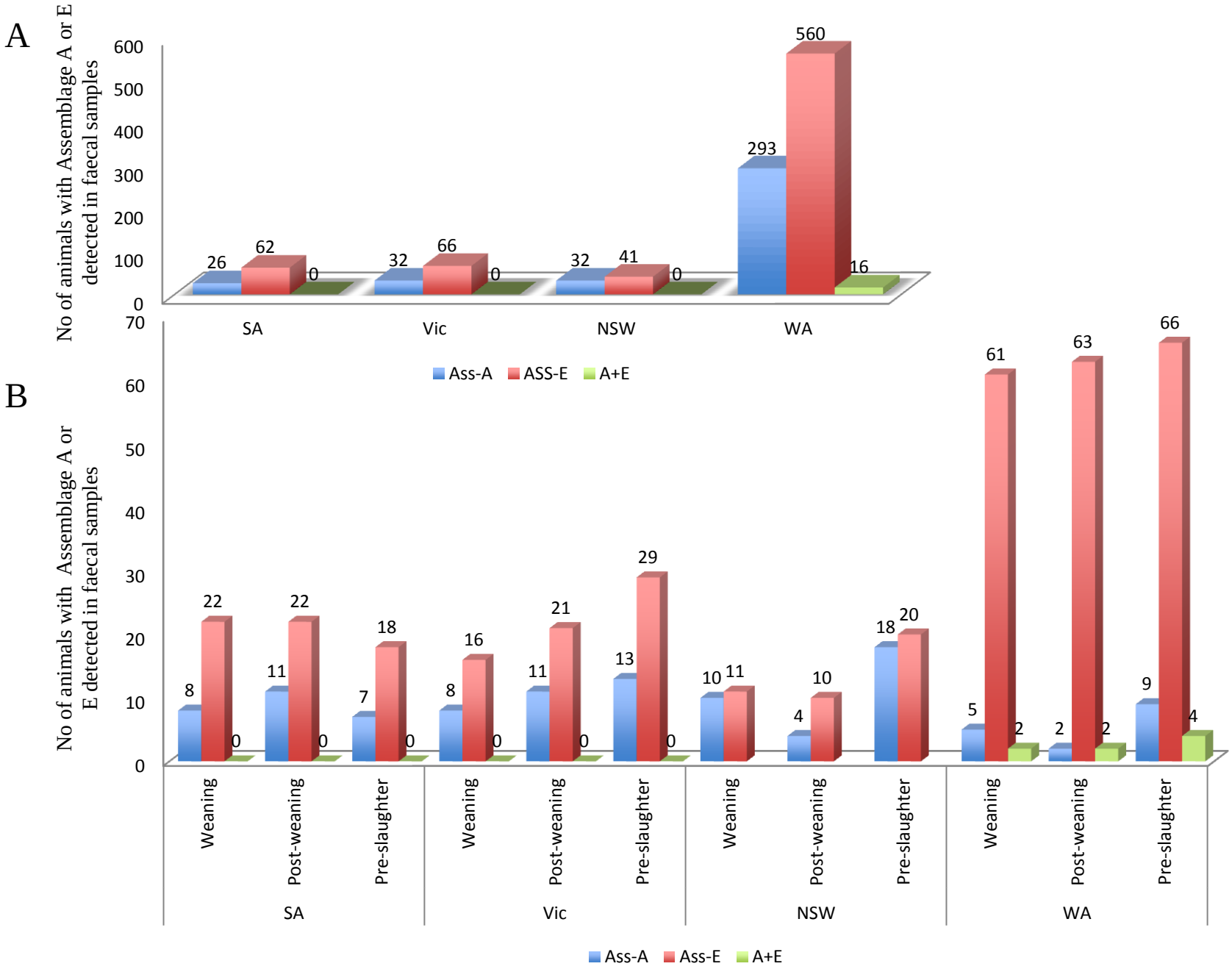

A
No of animals with Assemblage A or E detected in faecal samples
600
500
400
300
200
100
0
26
62
0
32
66
0
32
41
0
293
560
16
SA
Vic
NSW
WA
B
No of animals with Assemblage A or E detected in faecal samples
70
60
50
40
30
20
10
0
Ass-A
ASS-E
A+E
8
22
0
11
22
0
7
18
0
8
16
0
11
21
0
13
29
0
10
11
4
10
18
20
5
61
2
2
63
2
9
66
4
Weaning
Post-weaning
Pre-slaughter
SA
Vic
NSW
WA
Ass-A
Ass-E
A+E

Table 1. Sheep farms sampled during the present study.

Farm	Farm location	Mean annual rainfall (mm)	Farm size	Number of sheep	Breed of sheep	Commencement of lambing	Goats and/or cattle on property ?	Winter stocking rate	Weaning sample collection	Post-weaning sample collection	Pre-slaughter sample collection
SA1	Wirrega, SA	430	1040 ha	1800	Suffolk	mid April	No	10DSE/ha	24th Aug 2011	18th Oct 2011	8th Nov 2011
SA2	Struan, SA	550	1500 ha	5500	BL/Merino x Suffolk	June	Yes	15DSE/ha	1st Nov 2011	20th Dec 2011	19th Feb 2011
Vic1	Rosedale, Victoria	620	300 ha (winter)	300 ewes1	BL/Merino x Dorset & Southdown	mid July	No	10DSE/ha	20th Jan 2012	24th Feb 2012	15th May 2012
Vic2	Ballarat, Victoria	750	1960 ha	7000	Merino x Suffolk	early August	Yes	13DSE/ha	20th Jan 2012	8th Mar 2012	17th May 2012
NSW	Armidale, NSW	495	2958 ha	1000	BL/Merino	May - August	No	20 DSE/ha	23th Jan 2012	3rd Apr 2012	2nd May 2012
WA1	Pingelly, WA	450	1500 ha	1350	Merino x Suffolk	mid July	No	12DSE/ha	8th Nov 2009	24th Feb 2010	3rd Mar 2010
WA2	West Arthur, WA River	500	1250 ha	1750	Merino x Suffolk	Early August	No	10DSE/ha	1st Jan 2009	18th Feb 2010	23rd Mar 2010
WA3	Frankland, WA	550	560Ha	3300	Merino x Suffolk	Mid July	No	21 DSE/Ha	5th Oct 2011	19th Dec 2011	19th Jan 2012

Note: DSE = dry sheep equivalent, is a standard unit frequently used to compare animal carrying capacity and potential productivity of a given farm or area of grazing land. Samples from Western Australia were previously analysed using different primers and in some cases different loci as described in Sweeny et al., (2011) and Sweeny (2012).

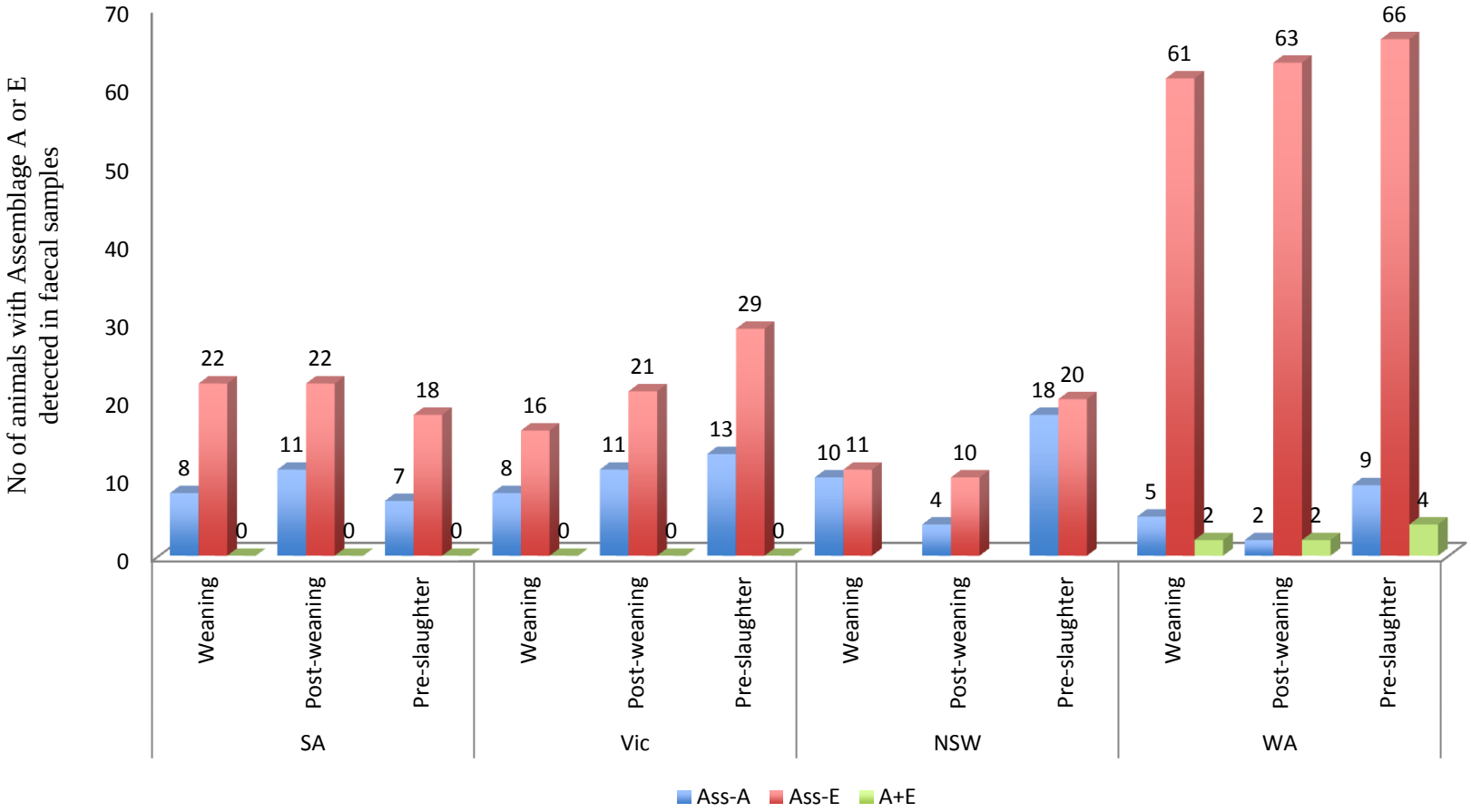
Table 2. Prevalence and number of *Giardia* cysts per gram of sheep faeces (g^{-1})(range and median) in samples collected from 8 farms in 4 states over 3 sampling periods. 95% confidence intervals are given in parenthesis.

Farm	Sampling period	Total no of samples	No of positives	Prevalence %	Cysts g^{-1} Range	Cysts g^{-1} Median
SA1	Weaning	165	27	16.4 (10.7-22.0)	63-3.7 $x10^5$	3.9 x 10^3
	Post-weaning	148	30	20.3 (13.8-26.7)	390-1.9 $x10^8$	8.3 x 10^5
	Pre-slaughter	159	19	11.9 (6.9-17.0)	1.4 $x10^3$-1.7 $x10^5$	1.5 x 10^5
SA2	Weaning	169	25	14.8 (9.4-20.1)	1.1 $x10^3$-1.2 $x10^5$	1.1 x 10^5
	Post-weaning	156	31	19.9 (13.6-26.1)	188-2.1 x 10^9	9.8 x 10^3
	Pre-slaughter	147	26	17.7 (11.5-23.9)	438-9.5 x 10^5	8.2 $x10^4$
Vic1	Weaning	180	13	7.2 (3.4-11.0)	390-1.3 x 10^9	2.3 x 10^4
	Post-weaning	172	40	23.3 (16.9-29.6)	63-1.0 $x10^9$	4.2 x 10^4
	Pre-slaughter	160	15	9.4 (4.9-13.9)	1.6 $x10^3$-4.8 $x10^5$	1.2 x 10^4
Vic2	Weaning	176	24	13.6 (8.6-18.7)	63-1.8 x 10^5	9.8 $x10^3$
	Post-weaning	173	36	20.8 (14.8-26.9)	313-2.0 x 10^8	1.8 x 10^3
	Pre-slaughter	128	39	30.5 (22.5-28.4)	125-4.7 x 10^5	9.0 $x10^4$
NSW	Weaning	160	27	16.9 (11.1-22.7)	63-4.5 x 10^5	2.0 x 10^3
	Post-weaning	160	28	17.5 (11.6-23.4)	313-2.2 x 10^5	3.9 x 10^3
	Pre-slaughter	167	58	34.7 (27.5-42.0)	125-2.0 x 10^5	4.3 x 10^3
WA1-AR	Weaning	124	34	27.4 (19.6-35.3)	313-5.0 x 10^6	2.6 $x10^4$
	Post-weaning	122	43	35.2 (26.8-43.7)	63-1.2 x 10^5	7.6 x 10^3
	Pre-slaughter	121	51	42.1 (33.4-50.9)	813-9.5 x 10^3	7.5 $x10^4$
WA2-PL	Weaning	107	30	28.0 (19.5-36.5)	63-6.9 x 10^5	4.2 x 10^3
	Post-weaning	109	28	25.7 (17.5-33.9)	63-1.1 x 10^7	2.8 x 10^4
	Pre-slaughter	107	32	29.9 (21.1-38.6)	125-2.4 $x10^6$	5.2 x 10^4
WA3-FL	Weaning	101	8	7.9 (2.7-13.2)	1.9 $x10^3$-1.5 $x10^6$	3.7 x 10^5
	Post-weaning	101	11	10.9 (4.8-17.0)	63-7.4 $x10^8$	3.1 x 10^4
	Pre-slaughter	100	15	15.0 (8.0-22.1)	63-4.7 $x10^9$	5.2 x 10^5
Total		**3412**	**690**	**20.2 (18.9-21.6)**	**63-4.7 x 10^9**	**1.6 x 10^4**

Table 3. *Giardia* cyst load g^{-1} and prevalence across four states. 95% confidence intervals are given in parenthesis.

States	Sampling periods	Cysts g^{-1} Range	Cysts g^{-1} Median	Prevalence %
SA	Weaning	63-3.7 $x10^5$	3.8 $x10^4$	15.6 (11.9-19.9)
	Post-weaning	188-2.1 $x10^9$	3.1 $x10^4$	19.7 (15.4-24.5)
	Pre-slaughter	438-9.5 $x10^5$	1.4 $x10^5$	14.7 (10.9-19.2)
Vic	Weaning	125- 1.3 $x10^9$	1.2 $x10^4$	10.4 (7.4-14.0)
	Post-weaning	63-1.0 $x10^9$	1.0 $x10^4$	22.0 (17.8-26.8)
	Pre-slaughter	125-4.8 $x10^5$	7.5 $x10^4$	18.8 (14.4-23.7)
NSW	Weaning	63-4.5 $x10^5$	1.9 $x10^3$	16.9 (11.4-23.6)
	Post-weaning	313-2.2 $x10^5$	3.9 $x10^3$	17.5 (11.6-23.4)
	Pre-slaughter	63-2.0 $x10^5$	4.3 $x10^3$	34.7 (27.5-42.0)
WA	Weaning	63-5.0 $x10^6$	2.3 $x10^4$	21.7 (17.4-26.5)
	Post-weaning	63-1.1 $x10^7$	9.0 $x10^3$	24.7 (20.2-29.7)
	Pre-slaughter	63-2.4 $x10^6$	8.1 $x10^4$	29.9 (25.0-35.2)
All states	Weaning	63-1.3 $x10^9$	1.7 $x10^4$	15.9 (13.9-18.1)
	Post-weaning	63-1.1 $x10^9$	9.6 $x10^3$	21.53 (19.2-24.0)
	Pre-slaughter	63-4.7 $x10^9$	8.1 $x10^4$	23.4 (20.9-26.0)

Development of a quantitative PCR (qPCR) for *Giardia* and analysis of the prevalence, cyst shedding and genotypes of *Giardia* present in sheep across four states in Australia.



Highlights

- First comprehensive study of *Giardia* in sheep across Australia
- Novel qPCR developed
- Prevalence, cyst shedding and assemblages analysed
- Longitudinal study of 3,412 samples over 3 sampling times
- Identification of zoonotic and non-zoonotic assemblages
- High levels of cyst shedding detected