Prevalence and pathogen load of *Campylobacter* spp., *Salmonella enterica* and *Escherichia coli* O157/O145 serogroup in sheep faeces collected at sale yards and in abattoir effluent in Western Australia

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Abstract

Objective

Develop a multiplex quantitative PCR assay to investigate the prevalence and shedding of *Escherichia coli* O157/O145, *Salmonella* spp. and *Campylobacter* spp. in sheep at sale yards and abattoirs.

Methods

A qPCR for *E. coli* O157/O145 was developed, validated and multiplexed with an existing qPCR for *Campylobacter* and *Salmonella enterica*. The absolute numbers of *E. coli* O157/O145, *Campylobacter* and *Salmonella* in control samples was determined using droplet digital PCR. These were then used as the controls in the multiplex qPCR on a total of 474 sheep faecal samples collected from two saleyards over a 4-month period (April–July 2014) and 96 effluent samples from an abattoir.

Results

The mutiplex qPCR was specific with a sensitivity of 5 organisms/µL faecal DNA extract for *Campylobacter*, *S. enterica* and *E. coli*O157/O145. The overall prevalence of *Campylobacter*, *S. enterica* and *E. coli* O157/O145 in faecal samples was 5.7%, 3.6% and 8.4%

and in effluent samples was 18.8%, 6.3% and 5.2%, respectively. The pathogen loads of *Campylobacter*, *S. enterica* and *E. coli*O157/O145 in faecal and effluent samples was also determined via mutiplex qPCR.

Conclusions

The overall prevalences of *Campylobacter*, *S. enterica* and *E. coli* O157/O145 were generally low (<6%), but point prevalences ranged considerably in healthy sheep (up to 26% for *E. coli* O157/O145). Further work to determine risk factors for shedding of bacterial organisms in meat sheep in the pre-slaughter period (on-farm, sale yards and lairage at abattoirs) could further reduce the risk of contamination of meat products.

Keywords: abattoir effluent; *Campylobacter* spp; *E. coli* O157, O145; lambs; prevalence; quantitative PCR; saleyards; *Salmonella enterica*

Abbreviations

ddPCR, droplet digital PCR; DG, droplet generator; IAC, internal amplification control; qPCR, quantitative PCR; STEC, Shiga toxigenic *Escherichia coli*

Shiga toxigenic *Escherichia coli* (STEC), *Salmonella* spp. and *Campylobacter* spp. are important aetiological agents of gastroenteritis in many developed countries in both human beings and livestock, and are the leading causes of bacterial foodborne gastroenteritis worldwide.[1-7]

STEC causes serious gastroenteritis, which in some cases progresses to life-threatening complications such as haemolytic uraemic syndrome.[2, 8] Only a small number of STEC are required to cause serious illness in humans and children, with the elderly particularly susceptible.[2, 8, 9] Although *E. coli* O157:H7 is the most common STEC serogroup, other serotypes can cause similar disease in humans,[9]with the United States Department of Agriculture naming six other STEC as adulterants, including *E. coli* O26, O45, O103, O111, O121 and O145.[10] The detection of STEC in

livestock in often performed by labour- and time-intensive culture and serogroup/PCR methods.[9, 10] It is therefore important to identify a reliable culture-independent method for the detection of these STEC serogroups and other zoonotic agents from livestock.

Sheep are considered significant reservoirs for these zoonotic pathogens[9, 11-15] and direct contact with sheep faeces has been implicated as a cause of gastroenteritis in humans related to STEC and *Salmonella*.[16, 17] Furthermore, STEC, *Salmonella* and *Campylobacter* have been isolated from both sheep carcases and retail lamb products in numerous countries, including the US, UK, Australia, China and New Zealand,[8, 17-24] highlighting the potential for human exposure.

Sheep are often consigned for slaughter via sale yards where multiple groups (lines) are purchased at sales and mixed during the period prior to slaughter. These sheep are subjected to management practices, including deprivation of feed and water during curfew (on farm), at the saleyard and in lairage (at abattoir), as well as stressors related to transport and mixing of groups, that have an effect on the shedding of pathogens. Understanding the prevalence of specific pathogens in these animals is important because if sheep carry the pathogens in their faeces, then hides and meat products can become contaminated. Contamination of abattoir effluent with potential zoonotic pathogens is also a public health risk, particularly in those circumstances where this effluent is treated and re-used in abattoirs.[25]

In a previous study, we developed and validated a quantitative multiplex PCR (qPCR) for detecting and enumerating *Salmonella* and *Campylobacter* in sheep faecal samples.[26] In the present study, we expanded this multiplex qPCR to include *E. coli* O157/O147 and then used this qPCR assay to assess the prevalence and shedding of *E. coli* O157/O145, *Salmonella* and *Campylobacter* in sheep in sale yards and abattoir effluent in Western Australia.

Materials and methods

Faecal and effluent sample collection

A total of 474 faecal samples were collected from healthy sheep (fit for transport and sale) at two saleyards in Western Australia, saleyard A (n = 238) and saleyard B (n = 236), on four occasions from April to July 2014 (Table 1). On each occasion, faecal samples were collected from randomly selected sheep in six separate consigned lines at each saleyard (≈ 10 samples per consigned line). All sample collection methods used were approved by the Murdoch University Animal Ethics Committee (Approval no. R2352/10). Effluent sampling was conducted at an abattoir in Western Australia daily over a 4-day period per month for 4 months, with effluent samples collected in triplicate from two sampling points (inlets and outlets) over the 4 days (24 samples/month; 96 effluent samples in total). All samples were immediately stored at 4°C before being transferred to the laboratory.

DNA isolation

Genomic DNA was extracted from 250 mg of each faecal and effluent sample using a PowerSoil® DNA extraction kit (MoBio, Carlsbad, CA, USA). A negative control (no faecal sample) was used in each extraction group. The positive control used in the present study was the *E. coli*O157 broth supplied in the *E. coli* O157 Latex Test kit (Thermo Scientific, VIC, Aust). DNA was extracted from 100 μL of the concentrated bacterial broth using the PowerSoil DNA extraction kit according to the manufacturer's instructions

PCR amplification, quantification and sequencing

Primers and probes targeting the *Campylobacter* spp. purine biosynthesis gene (*purA*) and *S. enterica* outer membrane protein (*ompF*) were as previously described.[26] Primers and probes for *E. coli* O157/O145 were designed using software supplied by Biosearch Technologies (Petaluma, CA, USA). A 65-base pair product was amplified from a putative chaperone (IpgB2) protein in *E. coli* O157 (GenBank accession CP008957, protein ID AIG71093) using the forward primer *E. coli* O157F 5′-TGCATTGCAGCTCTGGTAA-3′, the reverse primer *E. coli* O157R 5′-

TGGCAGGCAGATCGTTAGTTC-3′ and the probe 5′-CAL-Fluor eRed-610–CGCAGGTTTAAGCGTCTGTGCA-3′ BHQ1.

An internal amplification control (IAC) consisting of a fragment of a coding region from Jembrana disease virus cloned into a pGEM®-T vector (Promega) and IAC primers were used as described previously.[27] The absolute numbers of *E. coli* O157/O145, *Campylobacter* and *Salmonella* positive controls were determined using droplet digital PCR (ddPCR) on serial dilutions of bacterial DNA from 1 : 10 to 1 : 10,000. The same primers and probes described above were used except that the probe for *E. coli* O157 was labelled with fluorescein amidite at the 5′ end as only FAM and Joe channels are available in the QX100™ ddPCR system (BioRad, NSW, Aust). These data were then used to calibrate the standard curve for qPCR. The ddPCR assays were conducted in triplicate using a QX100™ droplet digital PCR system (BioRad) according to the manufacturer's instructions. Briefly, the ddPCR reaction mixture consisted of 12.5 μL of a 2 × ddPCR master mix (BioRad), 2 μL of primer/probe mix (12.5 mmol/L each of primers and probe), 1 μL of DNA (adjusted to 50 ng/μL) and 9.5 μL of H_2O to make a final volume of 25 μL. Droplets were generated using the droplet generator (DG) with 70 μL DG Oil per well with a DG8 cartridge and cartridge holder, 25 μL PCR reaction mix, and DG8 gasket. Droplets were dispensed into the 96-well PCR plate by aspirating 40 μL from the DG8 cartridge into each well. The PCR plate was then heat-sealed with a foil seal and the sealed plate was placed in the PCR thermocycler. The plates were incubated at 95°C for 10 min, then through 45 cycles of 94°C for 30 s and 58°C for 45 s, then 1 cycle of 98°C for 10 min and then held at 12°C. After the reaction, the droplets were analysed using the droplet reader and QuantaSoft software converted the data into the number of template copies per μL of PCR mixture. The number of template copies in 1 μL of DNA solution was then calculated.

For the multiplex PCR, each 15 μL PCR mixture contained 1 × PCR buffer (10 mmol/L Tris-HCl, 50 mmol/L KCl), 4 mmol/L $MgCl_2$, 1 mmol/L of each of the deoxynucleotide triphosphates (dNTPs), 1.0 U KAPA DNA polymerase (MolBio), 0.2 μmol/L of forward and reverse primers, 0.2 μmol/L of each forward and reverse IAC primers, 50 nmol/L probe, 50 nmol/L IAC probe, 10 copies IAC

template and 1 μL sample DNA. PCR reactions were incubated at 95°C for 3 min, then through 45 cycles of 95°C for 20 s and 60°C for 45 s.

Specificity and sensitivity

The analytical specificity of the multiplex qPCR assays was assessed by testing DNA from a range of *E. coli* strains, including one ATCC strain (ATCC 25922) and 28 other strains kindly supplied by Dr Abraham (Murdoch University) (Table S1) and a range of other bacterial strains (Table S2). The serogroups of the non-ATCC *E. coli* strains have previously been identified.[28-30]

To determine the sensitivity of the assay, 10-fold serial dilutions of plasmids containing the cloned PCR products amplified from each of the three bacteria (*E. coli* O157/O145, *Campylobacter* spp. and *S. enterica*) were prepared from 1×10^6 copies to 10 copies. These were then 'spiked' into faecal samples and the DNA was extracted and amplified as described above. Mean detection limits, the coefficient of determination-R squared values and percentage relative standard deviation were calculated. Template copy numbers were converted to numbers of organisms present on the basis that *purA* (*Campylobacter* spp.), *ompF* (*S. enterica*) and the hypothetical protein in *E. coli*O157/O145 (GenBank accession CP008957) were single copy genes[31-34] and that bacterial genomes are haploid. Therefore, the detected plasmid numbers were equivalent to the numbers of *Campylobacter* spp., *S. enterica* and *E. coli* O157/O145.

Inhibition and efficiency

Inhibition of DNA polymerase used in the PCR of faecal samples was monitored using the IAC, as equal amounts of the IAC template (10 copies) were added to all faecal DNA samples to detect any PCR inhibitors present in the extracted DNA. Amplification efficiency (*E*, a measure of inhibition) was estimated by using the slope of the standard curve and the formula $E = -1 + 10^{(-1/\text{slope})}$.[35] To estimate amplification efficiency on faecal samples, serial dilutions of five individual DNA samples (undiluted, 1 : 10, 1 : 100) were performed and multiple qPCR reactions were conducted on each dilution. The C_t values were then plotted against the log base 10 of the dilution and a linear regression was performed using the Rotor-Gene 6.0 software.

Statistical analysis

Prevalence is 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.[36]

Results

Specificity, sensitivity and efficiency

Specificity, sensitivity and lack of inhibition are important parameters when analysing a diagnostic assay. Evaluation of specificity of the multiplex qPCR against a range of *E. coli* strains, as well as other bacteria, protozoa and nematodes, revealed no cross-reaction with other genera; the qPCR amplified DNA from *E. coli* O157 and O145 (Table S2). Sensitivity analysis revealed that the mean minimum detection for *Campylobacter* spp., *S. enterica* and *E. coli* O157/O145 was 5 organisms/μL for each pathogen, which equates to approximately 1.25×10^3organisms per gram of faeces. The mean determination-R squared value for *Campylobacter* spp., *S. enterica* and *E. coli* O157/O145 was 0.99 for each pathogen. The percentage relative standard deviation for *Campylobacter* spp., *S. enterica* and *E. coli* O157 was 3.5%, 4.5% and 3.2%, respectively. The frequency of PCR inhibition, as determined by IAC amplification, was approximately 2.5%. If inhibition was evident, then the sample was diluted and re-amplified, which reduced inhibition to zero. The mean efficiencies for *Campylobacter* spp., *S. enterica* and *E. coli* O157 were 103.8%, 97.4% and 98.5%, respectively.

Prevalence and pathogen load in faecal samples

The prevalence and pathogen load of the bacteria in sheep faecal samples from sale yards are presented in Table 1. The overall prevalence of *S. enterica* and *E. coli* O157/O145 was higher in lines of lambs from saleyard B compared with saleyard A (Table 1). The prevalence of *E. coli* O157/O145 in saleyard A peaked at 5% during April and was much lower than in saleyard B, which peaked at 25.9% during the same period. The prevalence of *Campylobacter* peaked during June at both saleyard A (12.1%) and saleyard B (5.2%). *Campylobacter* was not detected in the July sample at saleyard

B. *Salmonella enterica* peaked at 6.7% in July at saleyard A, but was not detected during the May sampling. At saleyard B, April and May had the highest prevalence of *S. enterica* at 5.2%.

The number of pathogens (per gram of faeces) was also determined using qPCR (Table 1). *Campylobacter* was the most commonly shed organism in faecal samples, with the highest numbers shed during May at saleyard A (median, 5.4×10^5 organisms/g) and June at saleyard B (median, 2.2×10^4 organisms/g). The highest numbers of *S. enterica* were shed during June (median, 4.5×10^3 organisms/g) and July (median, 7.9×10^3 organisms/g) at saleyards A and B, respectively. The highest numbers of *E. coli* 0157/O145 were shed by lambs at saleyard A during April (median, 1.6×10^4 organisms/g), while in saleyard B, the highest numbers of *E. coli* 0157/O145 were shed during May (median, 5.4×10^3 organisms/g).

Prevalence and pathogen load in abattoir effluent

The prevalence and pathogen load of bacteria in abattoir effluent are presented in Table 2. As with faecal samples, *Campylobacter* was the most prevalent pathogen (18.8%) in abattoir effluent and ranged in prevalence from 8.3% to 58.3% (Table 2). The overall prevalence of *S. enterica* and *E. coli* O157/O145 in effluent was 6.3% and 5.2%, respectively. The highest number of *E. coli* O157/O145 in effluent was detected in outlet samples during July (Table 2). For *Campylobacter*, the highest number of organisms in effluent was detected in the inlet during June, while the highest numbers of *S. enterica* in effluent were detected in the inlet during May. *Salmonella* was not detected in the April inlet, June inlet and outlet, and July inlet samples.

Discussion

This study describes a multiplex qPCR for detecting and enumerating *S. enterica, Campylobacter* and enteropathogenic *E. coli* in sheep faeces with standard curves for qPCR calibrated using ddPCR to provide reference standards. In ddPCR, a fluorescent probe-based PCR assay is partitioned into highly uniform 1-nL reverse-micelles (water-in-oil), such that each droplet in the emulsion is an independent nano-PCR, containing 0, 1, or > 1 copies of the target nucleic acid, assorted in a random fashion.

[37] After PCR amplification, the fluorescence of each droplet is individually measured and defined as positive (presence of PCR product) or negative (absence of PCR product). The absolute number of target nucleic acid molecules contained in the original sample before partitioning can be calculated directly from the ratio of positive events to total partitions, using binomial Poisson statistics.[37] The advantage of this approach over conventional qPCR is superior accuracy for quantitation of target gene copies. In the present study, ddPCR was used to precisely quantify standard dilutions for qPCR calibration curves and the multiplex assay was conducted using qPCR, as this is a more cost-effective approach for large-scale screening.[38]

The multiplex qPCR assay was specific for *Campylobacter*, *S. enterica* and *E. coli* O157 and *E. coli* 0145, with a detection limit of 5 organisms/μL faecal DNA for each pathogen. The gene target for *E. coli* O157/O145 was a putative chaperone (IpgB2) protein in *E. coli* O157 (GenBank accession CP008957, protein ID AIG71093) from the recent whole-genome sequence of *E. coli* O157:H7 (strain EDL933).[34] In-silico analysis revealed that this assay may also detect the closely related *E. coli* serogroup O55, which is an ancestral serogroup of O157 and also carries the *IpgB2* gene. However, *E. coli* O55 is not a predominant STEC in Australian livestock and particularly not in Australian sheep (Robert Barlow, CSIRO, pers. comm.).[8]

The overall prevalence of *E. coli* O157/O145 in sheep at sale yards in this study (8.4%, point prevalence 0–26%) was comparable with a previous study in which *E. coli* O157 was isolated from the faeces of 5% of sheep, 3% of fleeces and 0.6% of pre-chill carcases at two Australian abattoirs,[39] but higher than that observed in a study that detected *E. coli* O157 in 2.5% in sheep faeces and 2.9% on hides at a Saudi Arabian abattoir.[40] Other studies have reported prevalences of 0% in sheep from North Wales,[41] 3.9% in sheep from Iran[7] and 18% in sheep from Turkey.[6]

The overall prevalence of *S. enterica* (3.6%, point prevalence 0–6.4%) was comparable with a longitudinal study that found the *S. enterica*point prevalence ranged from 0% to 24% in lambs on farms across Australia,[26] but lower than that observed in studies that identified *Salmonella* in 20% of faecal samples, 13% of fleeces and 1.3% of pre-chilled carcases at two Australian abattoirs,[39] and 23.2% of faecal samples and 67.6% of hides at an abattoir in Saudi Arabia.[40]

The differences in bacterial prevalence observed in the literature may be related to a number of factors, including diagnostic method used, contamination levels of the pastures, hygienic practices and slaughtering processes in the slaughterhouses, as well as the stress levels, age, sex or sampling season of the sheep included in the investigations.

Shedding of bacterial pathogens has implications beyond the farm gate. It can result in contamination of meat carcases with potentially zoonotic pathogens, affecting public health. The median concentrations of both *Campylobacter* spp. and *S. enterica* in sheep faeces were lower than those previously observed in a study by Yang et al.[26] of Australian lambs on farms across Australia (3.9×10^5 and 9.4×10^4organisms/g faeces, respectively). An important difference in the present study is that sheep were selected at random and age was not known, whereas the study by Yang et al. was a longitudinal study of lambs with faecal samples collected on three occasions between weaning (≈ 12 weeks old) and the period pre-slaughter (≈ 29 weeks of age).[26]

The factors that determine shedding (both prevalence of animals shedding and concentration in faeces) in sheep are not well described, but age is likely to have a significant effect, with younger animals more susceptible to infection. Common stressors in sheep meat enterprises (e.g. transport, curfew–lairage time where feed and water are withheld, mixing of groups of animals, seasonal factors and management practices such as shearing) may affect the shedding of bacterial pathogens in faeces. However, the effect of these factors on shedding has not been well described. Nonetheless, there is evidence that protracted fasting may result in the proliferation of undesirable enteric bacteria (such as *Salmonella* spp. and *E. coli* spp.).[42] The time spent off-feed prior to sampling was not known for the lines sampled in the present study. The prevalence and median concentration of organisms in faeces would be expected to increase where sheep are consigned to slaughter directly from saleyards and therefore subjected to protracted fasting times (>24 h) and additional stressors related to mixing of animals and transport between sale yards and abattoirs.

Apart from potential contamination of carcases and meat products, the presence of *Campylobacter* spp. in abattoir effluent (inlet or outlet) on all four sampling occasions, and both *S. enterica* and *E. coli* O157 on three of the four sampling occasions, has implications for the

management of effluent waste-water from abattoirs, particularly where this water may come into contact with humans.

Conclusions

In the present study, *Campylobacter* spp., *S. enterica* and *E. coli* O157/O145 were identified in sheep faeces at two saleyards in Western Australia. The overall prevalence was generally low, but point prevalence of all three pathogens ranged considerably in healthy sheep, suggesting that further work to determine the risk factors for shedding of bacterial organisms in meat sheep in the pre-slaughter period (on-farm, sale yards and lairage at abattoirs) could reduce the risk of contamination of meat products. It is important that abattoir effluent be adequately treated, as effluent was also shown to contain zoonotic bacterial pathogens.

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Conflict of interest statement

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Table 1. Bacterial shedding (organisms per gram of faeces) and prevalence (%) from sheep at saleyards in Western Australia over a 4-month period

Time and sample numbers	*Escherichia coli* O157/O145			*Campylobacter*			*Salmonella enterica*		
	Prevalence % (95% CI)	Shedding (organisms/g faeces)		Prevalence % (95% CI)	Shedding (organisms/g faeces)		Prevalence % (95% CI)	Shedding (organisms/g faeces)	
		Range	Median		Range	Median		Range	Median
Saleyard A									
April (n=60)	5 (0–10.5)	4.2×10^3–2.1×10^4	1.6×10^4	3.3 (0–7.9)	1.8×10^3–2.7×10^3	2.3×10^3	1.7 (0–4.9)	–	2.3×103*
May (n=60)	3.3 (0–7.9)	260–3.3×10^3	1.7×10^3	6.7 (0.4–13.0)	430–2.0×10^6	5.4×10^5	0	0	0
June (n=58)	3.4 (0–8.1)	2.5×10^3–6.0×10^3	4.2×10^3	12.1 (3.7–20.5)	430–6.1×10^5	3.2×10^4	3.4 (0–8.1)	2.9×10^3–6.0×10^3	4.5×10^3
July (n=60)	0	0	0	11.7 (3.5–19.8)	355–3.2×10^4	4.4×10^3	6.7 (0.4–13.0)	296–1.3×10^4	4.0×10^3
Subtotal (n=238)	2.9 (0.8–5.1)	260–2.1×10^4	2.8×10^3	8.4 (4.9–11.9)	355–2.0×10^6	6.7×10^3	2.9 (0.8–5.1)	296–1.3×10^4	3.8×10^3
Saleyard B									
April (n=58)	25.9 (14.6–37.1)	660–3.5×10^4	5.4×10^3	1.7 (0–5.1)	–	2.6×103*	5.2 (0–10.9)	2.3×10^3–2.0×10^4	5.6×10^3
May (n=58)	8.6 (1.4–15.8)	910–1.3×10^5	2.2×10^3	5.2 (0–10.9)	1.2×10^3–4.8×10^4	2.2×10^4	5.2 (0–10.9)	2.2×10^3–3.1×10^3	2.7×10^3
June (n=60)	15.0 (6.0–24.0)	146–2.0×10^4	2.0×10^3	5.0 (0–10.5)	2.2×10^3–5.3×10^3	3.5×10^3	1.7 (0–4.9)	–	7.9×103*
July (n=60)	6.7 (0.4–13.0)	$1.1x10^3$–2.8×10^3	1.6×01^3		0	0	5.0 (0–10.5)	2.5×10^3–2.2×10^5	4.2×10^3
Subtotal (n=236)	14.0 (9.6–18.4)	146–1.3×10^4	2.6×10^3	3.0 (0.8–5.1)	1.2×10^3–4.8×10^4	3.7×10^3	4.2 (1.7–6.8)	2.2×10^3–2.2×10^5	5.5×10^3
Overall (n=474)	8.4 (5.9–10.9)	146–2.1×10^4	2.7×10^3	5.7 (3.6–7.8)	355–2.0×10^6	4.7×10^3	3.6 (1.9–5.3)	296–2.2×10^5	4.5×10^3

*1 positive only. CI, confidence interval.

Table 2. Bacterial load (organisms per gram of faeces) and prevalence (%) in effluent at a Western Australian abattoir over four sampling periods

Sample occasion, location and sample numbers	***Escherichia coli* O157/O145**			***Campylobacter***			***Salmonella enterica***		
	Prevalence % (95% CI)	**Load (organisms/g effluent)**		**Prevalence % (95% CI)**	**Load (organisms/g effluent)**		**Prevalence % (95% CI)**	**Load (organisms/g effluent)**	
April		Range	Median		Range	Median		Range	Median
Inlet (n=12)	8.3 (0–24)	-	1.2×103*	8.3 (0–24)	-	1.8×104*	0	0	2.3×10^3
Outlet (n=12)	8.3 (0–24)	-	2.6×103 *	8.3 (0–24)	-	6.7×103 *	8.3 (0–24)	5.4×10^3–5.4×10^3	0
May									
Inlet (n=12)	8.3 (0–24)	-	5.0×103 *	8.3 (0–24)	-	2.9×103 *	16.7 (0–37.8)	3.5×10^3–4.0x10^3	4.5×10^3
Outlet (n=12)	8.3 (0–24)	-	3.6×104 *	0			8.3 (0–24)	-	8.4×103 *
June									
Inlet (n=12)	0	0	0	33.3 (6.7–60.0)	1.5×10^3–7.8×10^5	4.1×10^4	0	0	3.8×10^3
Outlet (n=12)	0	0	0	8.3 (0–24.0)	-	3.2×104 *	0	0	5.6×10^3
July									
Inlet (n=12)	0	0	0	58.3 (30.4–86.2)	1.8×10^3–4.5×10^5	4.7×10^3	0	0	2.7×10^3
Outlet (n=12)	8.3 (0–24)	-	2.4×104 *	25 (0.5–49.5)	6.9×10^3–6.6×10^4	4.0×10^4	16.7 (0–37.8)	1.0×10^3–1.3×10^3	7.9×10^3
Total (n=96)	5.2 (0.8–9.7)	293–3.6×10^4	7.8×10^3	18.8 (10.9–26.6)	1.5×10^3–7.8×10^5	3.2×10^4	6.3 (1.4–11.1)	1.0×10^3–8.4×10^3	4.2×10^3

*1 positive only. CI, confidence interval.

Prevalence and pathogen load of *Campylobacter* spp., *Salmonella enterica* and *E. coli* O157/O145 serogroup in sheep faeces collected at sale yards and in abattoir effluent in Western Australia

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Table S1. *E. coli* strains used for qPCR specificity testing

***E. coli* strain**	**Serogroup[a]**	**qPCR result**
S3	O138	Negative
S4	O109	Negative
S6	O141	Negative
S9	O141	Negative
S10	O141	Negative
S12	O8	Negative
S15	O8	Negative
S16	O8	Negative
S21	O20	Negative
S22	O20	Negative
S25	O8	Negative
S26	O8	Negative
S27	O8	Negative
S30	O141	Negative
S32	O141	Negative
S33	O141	Negative
EC3990	O145	Negative
EC4136a	O145	Positive
EC4457a	O26	Negative
ETEC 24	O157:K88	Positive
ETEC 95	0157:K88	Positive
03/13/4/134	O139	Negative
01/13/2/25	O89	Negative
03/13/4/91	O149	Negative
03/13/4/59	O89	Negative
ATCC 25922	O6	Negative
O157[b]	O157	Positive

[a]The serogroup of non-ATCC *E. coli* strains were previously identified.[27-30]

[b]Positive control: *E. coli* O157 Latex Test Kit (Thermo Scientific, Cat. Number: DR0620)

Table S2. Additional bacteria used for specificity testing

Species	Strain/serovar
Bacillus subtilis	ATCC 6633
C. jejuni, C. coli	
Chlamydia pecorum, Chlamydia abortus	
Citrobacter freundii	NCTC 9750
Coxiella burnetii	
Cryptosporidium spp. (n=5)	
Cyclospora sp.	
E. coli	ATCC 25922
Eimeria sp.	
Enterobacter cloacae	ATCC 13047
Enterococcus durans	ATCC 11576
Giardia duodenalis	assemblages A and E from sheep
Haemonchus contortus	
Human sheep and cattle genomic DNA	
Isospora sp	
S. enterica serovar	Typhimurium, Wandsbek II 21:z10:z6, Bredeney, Muenchen, Adelaide,Waycross, Infantis
Serratia marcescens	ATCC 14756 pigmented
Streptococcus bovis	ATCC 33317
Teladorsagia circumcincta	
Tenebrio sp	
Toxoplasma gondii	
Trichostrongylus colubriformis	
Yersinia enterocolitica	