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**Original research**

# Molecular characterisation of *Salmonella enterica* serovar Typhimurium and *Campylobacter jejuni* faecal carriage by captured rangeland goats

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**Highlights**

- Zoonotic *S.* Typhimurium and *C. jejuni* faecal carriage by goats identified using qPCR
- High frequency for *S.* Typhimurium faecal carriage immediately after capture and transport
- Low frequency of faecal carriage detection one to three months after arrival at the feedlot
- Repeated *S.* Typhimurium faecal carriage detection not common
- Meat hygiene and effluent management implications for goats following capture and transport

**Abstract**

Western Australian rangeland goats were surveyed for faecal carriage of *Salmonella enterica* and *Campylobacter* spp. Faecal samples were collected from 125 goats on four occasions. The first sample was collected immediately upon arrival at a commercial goat depot (feedlot). Subsequent samples were collected at one month intervals thereafter. Frequency of detection and faecal carriage intensity were determined using qPCR targeting the *S. enterica* outer membrane protein (*ompF*) and *Campylobacter* spp. purine biosynthesis gene (*purA*). *Salmonella enterica* were identified in 40/500 of faecal samples, with *S. enterica* faecal carriage detected in 30% (38/125) goats over the duration of the study. *Campylobacter* spp. were identified in 12/500 of samples, with *Campylobacter* spp. detected in 10% (12/125) goats over duration of the study. Frequency of detection was highest at the first sample collection for both *S. enterica* (26%) and *Campylobacter* spp. (8%). Repeat detection of *Salmonella* was observed for only a single goat (0.8%). *Salmonella* qPCR positive samples were characterised at *ompF* and *invA* genes as *S. enterica*. Further

characterisation at STM2755 and STM4497 genes confirmed the isolates were *S. enterica* serovar Typhimurium. Characterization at the 16S rRNA and hippuricase (*hipO*) genes revealed all *Campylobacter* spp. positive samples were *C. jejuni.* This study demonstrates that qPCR can be used for rapid identification of faecal carriage in goat faecal samples and showed evidence of carriage of zoonotic *S*. Typhimurium and *C. jejuni* by captured rangeland goats. The findings have implications for management of goats at abattoirs and in confined feeding facilities.

**Keywords:** qPCR; *Salmonella*; *Campylobacter jejuni*; goat; public health; zoonotic

## 1. Introduction

Undomesticated rangeland goats are naturalised throughout Australian rangelands. These goats are opportunistically captured and utilised for meat production (Meat and Livestock Australia, 2015). Following capture, rangeland goats may be managed in goat depots (feedlots) for variable periods prior to slaughter. Diarrhoea and ill-thrift (weight loss or poor growth rates) are cited as important issues for captured rangeland goats under intensive management conditions in goat depots (Meat and Livestock Australia, 2016). The causes of diarrhoea and ill-thrift are not well described. It has been suggested that stress associated with capture, transport and domestication, as well as high stocking densities in feedlots, may contribute to increased shedding and transmission of disease agents with veterinary and public health importance, such as *Salmonella* (Meat and Livestock Australia, 2016).

*Salmonella* and *Campylobacter* occur naturally in the gut as commensals, and infections are often asymptomatic (Kusiluka et al., 1996; Duffy et al., 2009; Markey et al., 2013). However, *Salmonella (S.) enterica* and *Campylobacter* spp. (*C. jejuni* and *C. coli*) have

been associated with diarrhoea, weight loss, lethargy and inappetance in goats, with sustained periods of stress identified as a risk factor for manifestation of disease in both goats and sheep (Bulgin and Anderson, 1981; McOrist and Miller, 1981; Richards et al., 1989; Sharma et al., 2001; Markey et al., 2013). Outbreaks of acute diarrhoea due to *S. enterica* (*S.* Adelaide*, S.* Typhimurium*, S.* Muenchen and *S.* Singapore) with 38% mortality rate have been reported in Australian rangeland goats (McOrist and Miller, 1981), but the epidemiology of *Salmonella* and *Campylobacter* infections and specific risk factors for faecal carriage in rangeland goats are not well described.

Apart from potential for impacts on goat health and production, there are important implications for faecal carriage of *S. enterica* and *Campylobacter* spp. along the entire goat meat supply chain. *Salmonella enterica* and some *Campylobacter* spp. have zoonotic potential, therefore faecal carriage is associated with public health risks via contamination of carcases and water sources (Davies et al., 2004; Garcia et al., 2010). There are also important economic consequences for processors, with many meat export markets having zero tolerance for contamination of meat products.

Molecular methods including quantitative PCR (qPCR) for detection of *Salmonella* and *Campylobacter* in faeces and food can offer some advantages over culture, including speed, and greater specificity, sensitivity and reproducibility (Maciel et al., 2011; Singh et al., 2011; Zhang et al., 2011; Whiley et al., 2016). Molecular tools based on qPCR have been used to characterise faecal carriage of *S. enterica* and *Campylobacter* spp. for sheep (Yang et al., 2014; Yang et al., 2017), and gastrointestinal parasites in rangeland goats (Al-Habsi et al., 2017a; Al-Habsi et al., 2017c). The aim of the present study was to determine faecal carriage of *S. enterica* and *Campylobacter* spp. in captured rangeland goats in Western Australia using molecular tools.

## 2. Materials and Methods

### *2.1 Animals and faecal sample collection*

Rangeland goats (*n*=125) were captured from a sheep and cattle rangeland grazing property, North Wooramel station, located 78 km east of Denham and 113 km south east of Carnarvon in the Gascoyne region of Western Australia. Goats were transported by road to a commercial goat depot (feedlot) near Geraldton, Western Australia. On arrival at the feedlot (S1), goats weighed 30.7 ± 0.3 kg (mean ± standard error) with an estimated age of 9–12 months based on dentition. Only male goats were included in the study.

Faecal samples were collected from each goat on four occasions (S1-S4). The first sample collection (S1) occurred immediately after arrival at the feedlot. Subsequent sampling (S2-S4) occurred monthly thereafter. Faecal samples were collected directly from the rectum and stored on ice or in a refrigerator (4.0°C) until DNA extraction.

Goats were housed in four group pens (30-33 goats per pen) for the duration of the study. Grain-based pellets, hay and water were supplied *ad libitum*. Straw-bedding was provided with bare dirt covering the majority of available pen space. No pasture was available for the duration of the study. Goats were consigned for slaughter after the conclusion of the experiment when they reached acceptable slaughter weight.

All procedures were approved and monitored by the Murdoch University Animal Ethics Committee (approval number R2617/13).

### *2.2 DNA isolation*

Genomic DNA was extracted from 200 mg 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, quantification and sequencing*

All samples were screened for the presence of *S. enterica* and *Campylobacter* spp. using a quantitative PCR (qPCR) at the outer membrane protein (*ompF*) and purine biosynthesis gene (*purA*) respectively as described by Yang et al. (2014). Briefly, a 96 base pair (bp) product was amplified from the *S. enterica ompF* using the forward primer *ompF*1 5'-TCGCCGGTCGTTGTCCAT-3', the reverse primer *omp*R1 5'-AACCGCAAACGCAGCAGAA-3' and the probe 5'-2'7,'-dimeth-oxy-4'5,'-dichloro-6-carboxyfluorescein (JOE)-ACGTGACGACCCACGGCTTTAC-3'. A 121 bp product was amplified from the *Campylobacter* spp. *purA* using the forward primer *purA*F1 5'-CGCCCTTATCCTCAGTAGGAAA-3', the reverse primer *purA*R1 5'-TCAGCAGGCGCTTTAACAG-3' and the probe 5'-6-carboxyfluorescein (FAM)-AGCTCCATTTCCCACACGCGTTGC-3'.

An internal amplification control (IAC) consisting of a fragment of a coding region from Jembrana disease virus (JDV) cloned into a pGEM-T vector (Promega) and IAC primers were used as described previously (Yang et al., 2013). Each 15 µL PCR mixture contained 1× PCR buffer (10 mM Tris–HCl, 50 mM KCl), 4 mM $MgCl_2$, 1mM deoxynucleotide triphosphates, 1.0 U KAPA DNA polymerase (MolBio), 0.2 µM each forward and reverse primer, 0.2 µM each forward and reverse IAC primers, 50 nM probe, 50 nM IAC probe, 10 copies IAC template and 1 µL sample DNA. The PCR cycling conditions consisted of 95 °C for 3 min, then 45 cycles of 95 °C for 20 s and 60 °C for 45 s.

*2.4 Specificity and sensitivity of qPCR*

The analytical specificities of the multiplex qPCR assays were assessed by testing DNA from *S. enterica* (*S.* Typhimurium, *S.* Wandsbek II 21:$_{z10:z6}$, *S.* Bredeney, *S.* Muenchen, *S.* Adelaide, *S.* Waycross, *S.* Infantis), *C. jejuni*, *C. coli*, *Chlamydia pecorum*, *Chlamydia abortus*, *Yersinia enterocolitica*, *Streptococcus bovis* (ATCC33317), *Enterococcus durans* (ATCC

11576), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Serratia marcescens* (ATCC 14756 pigmented), *Citrobacter freundii* (NCTC 9750), *Enterobacter cloacae* (ATCC 13047), *Coxiella burnetii*, *Giardia duodenalis* assemblages A and E from sheep, *Cryptosporidium* spp. (*n* = 5), *Isospora* sp., *Tenebrio* sp., *Cyclospora* sp., *Toxoplasma gondii*, *Trichostrongylus colubriformis*, *Teladorsagia circumcincta*, *Haemonchus contortus* and *Eimeria* sp., as well as human, sheep and cattle genomic DNA (Yang et al., 2014).

To determine the sensitivity of the assay, 10-fold serial dilutions of plasmids containing the cloned PCR products amplified from each of the two bacteria (*S. enterica* and *Campylobacter* spp.) were prepared from $1\times10^6$ 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 % relative standard deviation were calculated. Template copy numbers were converted to numbers of organisms present on the basis that *ompF* (*S. enterica*) and *purA* (*Campylobacter* spp.) are single copy genes (Pearson et al., 2007; Tatavarthy and Cannons, 2010; GenBank CP000814) and bacterial genomes are haploid. Therefore the detected plasmid numbers were equivalent to the numbers of *S. enterica* and *Campylobacter* spp.

*2.5 Inhibition and efficiency analysis of qPCR*

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. If inhibition was evident, then the sample was diluted and re-amplified (Yang et al., 2014). Amplification efficiency (*E*, a measure of inhibition), was estimated by using the slope of the standard curve and the formula $E = -1 + 10^{(-1/\text{slope})}$ (Nybo, 2011). To estimate amplification efficiency on faecal samples, serial dilutions of five 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 vs. the log 10 of the dilution and a linear regression was performed using the Rotor-Gene 6.0 software.

*2.6 Molecular characterisation of* S. enterica *and* Campylobacter *spp.*

Initially qPCR-positive *Salmonella* samples were subjected to one-step PCR for the *S. enterica* gene *ompF* (578 bp amplicon) and *invA* (521 bp amplicon) gene as described by Tatavarthy and Cannons (2010) and Swamy et al. (1996) respectively. Positive *S. enterica* isolates were further investigated using serovar specific STM2755 (406 bp amplicon) and STM4497 (523 bp amplicon) primers and PCR conditions described by Shanmugasundaram et al. (2009). The primers' specificity were assessed by testing DNA from an isolate of *S.* Typhimurium (Abraham et al., 2016) as a positive control and four isolates of *S.* Anatum, *S.*Dublin, *S.* Enteritidis and *S.* Hadar as negative controls.

Samples that were qPCR-positive for *Campylobacter* spp. were subjected to PCR for the *Campylobacter* spp. 16S rRNA gene (287 bp amplicon) using primers and PCR conditions described by Lubeck et al. (2003). Positive *Campylobacter* spp. isolates were further confirmed by species specific PCR at the hippuricase (*hipO*) (344 bp amplicon) gene previously described by Persson and Olsen (2005). PCR products were separated by gel electrophoresis and purified using an in-house filter tip method (Yang et al., 2013). Purified PCR products were sequenced using an ABI Prism Dye Terminator Cycle Sequencing kit (Applied Biosystems) using an annealing temperature of 58 °C. Nucleotide sequences were analysed 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). The target genes and primers used for the amplification and sequencing of *S. enterica* and *Campylobacter* spp. isolates and their length are given (Table 1).

*2.7 Statistical analyses*

Goats were classified as positive (DNA detected) or negative (no DNA detected) for *S. enterica* and *Campylobacter* spp. Frequency of faecal carriage detection was determined by proportion of positive goats for each sample occasion. Statistical analyses were performed using IBM SPSS Statistics for Mac (version 24). Two-tailed Chi-square tests (Pearson Chi-square or Fisher's exact test) were used to compare frequency of faecal carriage detection between sampling occasions and different pens at the goat depot. Prevalence confidence intervals (95%) were calculated using Jeffrey's interval method (Brown et al., 2001).

**3. Results**

*3.1 Specificity, sensitivity and efficiency for qPCR*

Specificity and sensitivity of the multiplex bacterial qPCR are shown in Table 2 (adapted from Yang et al., 2014). Evaluation demonstrated no cross-reactions with other genera; the qPCR only amplified the relevant bacterial species.

*3.2 Faecal carriage and intensity for* S. enterica *and* Campylobacter *spp.*

A total of 40/500 faecal samples were qPCR-positive for *S. enterica* and 12/500 were PCR-positive for *Campylobacter* spp. over the four sampling occasions. Frequency of detection faecal carriage are shown in Table 3. Faecal carriage for both *S. enterica* and *Campylobacter* spp. were highest at S1 (immediately after capture and transport) and decreased ($P<0.05$) at subsequent samplings (Table 3). Neither *S. enterica* or *Campylobacter* spp. were identified at S4 (Table 3). Concurrent (mixed) *S. enterica* and *Campylobacter* spp. faecal carriage was identified in 5/125 (4%) of the goats at S1 only. Faecal carriage was detected in 1/125 (0.8%) goat on three sampling occasions (S1-S3). Faecal carriage was not identified on two or four sampling occasions in any goats. Frequency of faecal carriage

detection was higher (*P*=0.033) for pen 3 (4/31) compared to pen 2 (0/33) or pen 4 (0/31) at S2, but otherwise there was no difference in faecal carriage frequency between pens (*P*>0.05).

*3.3* Salmonella enterica *and* Campylobacter *spp. molecular typing*

All *Salmonella*-positive sequences (*n*=40) were confirmed as *S. enterica* at the *ompF* and *invA* loci (98–99% homology to GenBank isolates CP014979 and CP014971 respectively). Further amplification and sequencing using serovar specific primers (STM2755 and STM4497) confirmed all *S. enterica*-positive isolates as *S.* Typhimurium (100% homology to GenBank isolate CP013720).

All *Campylobacter*-positive samples (*n*=12) were identified *C. jejuni* based on amplicon sequencing of both the 16S rRNA and *hipO* loci (99% homology to GenBank isolates CP001876 and KJ659824 respectively).

**4. Discussion**

This study identified faecal carriage of two important caprine pathogens with zoonotic potential by captured rangeland goats, namely *S.* Typhimurium and *C. jejuni*. The temporal pattern of faecal carriage observed has important implications for the goat meat industry, with the highest frequency of detection for both *S.* Typhimurium and *C. jejuni* identified on arrival at the feedlot, immediately following capture and road transport. This is an important observation because the first sampling occasion reflected level of faecal carriage likely to be observed at abattoirs for goats consigned directly to slaughter following capture and transport by road from rangeland properties to processing facilities, and this is common practice. The high frequency of *S.* Typhimurium faecal carriage observed at the first sampling occasion (26%) indicates further investigation of the factors that impact shedding

by goats in lairage, including the impact of pre-slaughter management practices, are warranted because high frequency of faecal carriage has important implications for meat hygiene and abattoir effluent management.

The other key observation was the decline in faecal carriage detection one month subsequent to capture and transport. Persistent faecal carriage detection over more than one sample occasion (monthly intervals) was not common (0.8% goats) for rangeland goats housed in the goat depot. The decline in faecal carriage frequency suggests investigations based on faecal samples from goats that are habituated to their surroundings are likely to underestimate faecal carriage potential following a period of stress. Comparison of frequency of faecal carriage of *S.* Typhimurium and *C. jejuni* between studies is problematic because factors apart from geographic location, including diagnostic techniques, husbandry and management practices, herd size and season will impact detection.

Risk factors for *S.* Typhimurium and *C. jejuni* faecal carriage were not tested in this observational study, but the higher frequency of faecal carriage identified at the first sample collection was likely attributable to stress associated with trapping and transport of the wild goats, including mixing of unfamiliar animals, increased stock density, food deprivation, or contaminated feed/water during the trapping period. Transportation stress in goats has been associated with increased plasma cortisol levels (Kannan et al., 2003; Al-Kindi et al., 2005), and this has been shown to play a major role in the recrudescence of *Salmonella* resulting in increased shedding (Verbrugghe et al., 2011). Transportation stress in goats (domestic and feral) has been associated with lowered immunity (Kannan et al. 2000; Kannan et al., 2002), elevated plasma glucose concentration (Sanhouri et al., 1992; New et al., 1996; Rajion et al., 2001), and clinical salmonellosis (McOrist and Miller 1981).

Concurrent faecal carriage with both *S.* Typhimurium and *Camplyobacter* spp. was not commonly observed (4% goats) in this study. However, co-infection with protozoan

parasites including *Entamoeba, Cryptosporidium, Giardia* and *Eimeria* have been previously reported in the goats included in the present study (Al-Habsi et al., 2017a, Al-Habsi et al., 2017b; Al-Habsi et al., 2017c). Consistent with observations for *S.* Typhimurium and *C. jejuni*, the first sampling occasion had highest point prevalence for *Cryptosporidium* and *Giardia (*Al-Habsi et al., 2017a) and *Eimeria* (Al-Habsi et al., 2017c)*,* and supports the suggestion that stress related to capture and transport increases faecal pathogen shedding by rangeland goats.

Both *S.* Typhimurium and *C. jejuni* observed in this study have zoonotic potential. *Salmonella enterica* serovar Typhimurium is a typical broad-host-range pathogen, and is among the serotypes most frequently associated with disease in humans (Coburn et al., 2007), including Australia where *S.* Typhimurium was reported as the most frequently reported serovar in all states and territories, and associated with 43.9% of the 127,195 cases of human salmonellosis reported between 2000 and 2013 (Ford et al., 2016). Likewise, outbreaks of *C. jejuni* have been widely reported in Australia (Hundy and Raupach, 2003; Parry et al., 2012; Moffatt et al., 2016), with campylobacteriosis also a major cause of foodborne outbreaks (Unicomb et al., 2009). Reports of *Salmonella* recovery from 28.9% goat carcasses in Australia (Duffy et al., 2009) and 51.2% goat carcasses in Saudi Arabia (Bosilevac et al., 2015) demonstrate carcass contamination during processing. Salmonellosis has been associated with consumption of raw or improperly cooked goat meat in Japan (Kadaka et al., 2000). Similarly, *Campylobacter* recovery from goat carcases has been widely reported in Ethiopian, Ghanaian and Grecian abattoirs (Woldemariam et al., 2009; Lazou et al., 2014; Karikari et al., 2017), raw retail goat meats respectively in Ethiopia and Iran (Dadi and Asrat, 2008; Rahimi et al., 2010), and goat meat has been reported to act as major source of human and environmental contamination by *Campylobacter* spp. in Congo (a Mpalang et al., 2014).

This study is the first molecular description of *S. enterica* and *Camplobacter* spp. reported for Australian rangeland goats. Molecular methods including qPCR offer some advantages for enumeration and detection of *Salmonella* and *Campylobacter* in faeces and food, including greater specificity, sensitivity and reproducibility than conventional diagnostic methods (Maciel et al., 2011; Singh et al., 2011; Zhang et al., 2011; Whiley et al., 2016). The present study demonstrated rapid detection of *S. enterica* and *Campylobacter* spp. in faecal samples of captured rangeland goats through qPCR, and offered high specificity to detect *S. enterica* in faecal samples of rangeland goats when specific primer pairs that target a putative hexulose-6-phosphate synthase (STM2755) and cytoplasmic proteins (STM4497) are utilised. These genes are present in serovar Typhimurium but not in any other *S. enterica* (Chan et al., 2003; Shanmugasundaram et al., 2009; Park et al., 2009; Park and Ricke, 2015; Ogunremi et al., 2017). The qPCR method offers some potential advantages as a diagnostic test for in that it can be used for rapid detection (screening) of pathogens in faecal samples, and can be run as multiplex PCR for a range of other pathogens (for example, *Cryptosporidium, Giardia, Eimeria* and helminths) during outbreak investigations and routine surveillance. The high minimum detection limit of the qPCR assay (~1250 organisms/g faeces) meant that the rate of detection of faecal carriage may have under-estimated the proportion of samples that would have been positive by culture using methods that include selective enrichment. Unfortunately it was not within the scope of the study to validate the agreement for detection and quantitation of the qPCR with culture methods. Inhibitors in faecal samples can hinder PCR amplification (Wilson, 1997), but for this assay inhibition determined by IAC amplification was only ~2% samples (Yang et al., 2014). It is likely the sensitivity of the assay could be increased with inclusion of a pre-enrichment step. Further validation of the qPCR for quantification of bacterial shedding intensity with culture methods is warranted.

There were some weaknesses of this study that should be addressed in future investigations. The observational study design and small number of facilities included in the study meant that risk factors for faecal carriage were not determined. Only male goats were included in the study. Male goats are preferentially managed in Australian goat depots for several reasons. Following capture, producers may choose to retain female goats to maintain goat populations, and consign males to goat depots or directly to slaughter. Live export markets have preference for entire male goats, therefore goat depots preferentially source males because these can be marketed into export or domestic meat markets, or for live export. Finally, wild male goats are not castrated when captured, therefore males and females need to be housed and managed separately in the depot. Including both males and females in the study would confound the effect of pen on pathogen shedding, growth and diarrhoea.

Another weakness of this study is that pathogen faecal carriage by wild goats prior to capture, between capture and transport, and at slaughter were not determined. Developing methods for collection of fresh faecal samples from wild goats prior to capture is challenging, but as animals need to be trapped and transported in order to enter goat depots or slaughter facilities, post-capture sampling is appropriate to characterise animal health and public health risks associated with pathogen faecal carriage. Follow up studies could include faecal sample collection for animals in trap yards (soon after capture) if suitable handling facilities to restrain wild goats are available. The findings of this study prompted a follow-up experiments of rangeland goats at slaughter that were sourced from a wider geographical area.

As described above, the high minimum detection limit of the qPCR assay meant that the rate of detection of faecal carriage may have under-estimated the proportion of samples that would have been positive using methods (including culture) that include

selective enrichment. A further limitation was lack of isolation (by culture) limited the ability for evaluating the genotypic characteristic of the isolate and downstream public health impact including antimicrobial resistance (AMR). Notwithstanding these limitations, the observations demonstrate clearly that faecal carriage of *S.* Typhimurium and *C. jejuni* in rangeland goats in the period immediately following capture and transport are sufficiently high to warrant further investigation into the risk factors for shedding, and how these risks can be managed to reduce impacts on abattoir and meat hygiene, and the health and productivity of goats in confined feeding facilities.

**Conclusion**

This study demonstrates that rangeland goats may act as reservoirs for *S.* Typhimurium and *C. jejuni,* and utilisation of qPCR has identified high frequency of detection of faecal carriage of *S.* Typhimurium in goats immediately following capture and transport. The qPCR method for detection may have underestimated the rate of faecal carriage that would be detected culture using methods that include selective enrichment. Further studies are warranted to determine the animal production and public health impacts of *S.* Typhimurium and *C. jejuni* in rangeland goats, and the impact of pre-slaughter management on faecal carriage.

**Conflict of Interest**

None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper. Meat and Livestock Australia approved the manuscript for publication, but were not involved in the collection, analysis or interpretation of data.

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**Table 1**: Target genes and primers used for amplification and sequencing of *S. enterica* and *Campylobacter* isolates in rangeland goats.

| Target/ Primer | Nucleotide sequence (5'–3') | PCR | Product size (bp) | Reference |
|---|---|---|---|---|
| ***S. enterica*** | | | | |
| ompF/ ompF1 | TCGCCGGTCGTTGTCCAT | qPCR | 96 | Yang et al., 2014 |
| ompF/ ompR1 | AACCGCAAACGCAGCAGAA | | | |
| ompF/ ompF1 | CCTGGCAGCGGTGATCC | one-step PCR | 578 | Tatavarthy & Cannons, 2010 |
| ompF/ ompF-seqR | TGGTGTAACCTACGCCATC | | | |
| invA/ invA-F | TTGTTACGGCTATTTTGACCA | one-step PCR | 521 | Swamy et al., 1996 |
| invA/ invA-R | CTGACTGCTACCTTGCTGATG | | | |
| STM2755/ STM2755-F | AGCTTGCCCTGGACGAGTT | one-step PCR | 406 | Shanmugasundaram et al., 2009 |
| STM2755/ STM2755-R | TGGTCGGTGCCTGTGTGTA | | | |
| STM4497/ STM4497-F | GGAATCAATGCCCGCCAATG | one-step PCR | 523 | Shanmugasundaram et al., 2009 |
| STM4497/ STM4497-R | CGTGCTTGAATACCGCCTGTC | | | |
| ***Campylobacter* spp.** | | | | |
| purA/ purAF1 | CGCCCTTATCCTCAGTAGGAAA | qPCR | 121 | Yang et al., 2014 |
| purA/ purAR1 | TCAGCAGGCGCTTTAACAG | | | |
| 16S rRNA/ OT1559 | CTGCTTAACACAAGTTGAGTAGG | one-step PCR | 287 | Lubeck et al., 2003 |
| 16S rRNA*/ 18-1* | TTCCTTAGGTACCGTCAGAA | | | |
| hipO/ hipO-F | GACTTCGTGCAGATATGGATGCTT | one-step PCR | 344 | Persson & Olsen, 2005 |
| hipO/ hipO-R | GCTATAACTATCCGAAGAAGCCATCA | | | |

**Table 2**: Specificity and sensitivity analysis for qPCR (adapted from Yang et al., 2014)

| | *S. enterica* | *C. jejuni* |
|---|---|---|
| **Specificity** | | |
| Cross-reactions with other genera | nil | nil |
| **Sensitivity** | | |
| Mean minimum detection (organisms/g faeces) | ~1250 | ~1250 |
| R-squared value | 0.99 | 0.99 |
| % relative standard deviation | 4.5 | 3.5 |
| Frequency PCR inhibition (%) | ~2 | ~2 |
| Mean efficiency (%) | 97.4 | 103.8 |

**Table 3**: Frequency of detection and faecal carriage intensity for *S.* Typhimurium and *C. jejuni* in faecal samples collected from 125 rangeland goats on four occasions (S1-S4)

| | Frequency of faecal carriage detection (%) | |
|---|---|---|
| Sampling occasion | *S.* Typhimurium | *C. jejuni* |
| February (S1) | 25.6 [a] | 8 [a] |
| March (S2) | 4.8 [b] | 0.8 [b] |
| April (S3) | 1.6 [b] | 0.8 [b] |
| May (S4) | 0 [b] | 0 [b] |
| Longitudinal frequency of detection[#] (95% CI) | 30.4 (22.8, 38.8) | 9.6 (5.4, 15.7) |

[abc]Faecal carriage frequency (%) detection for each bacteria at different sampling occasions (in columns) with different superscripts are significantly different ($P<0.05$).

[#]Goats with faecal carriage detected on at least one sampling occasion.

CI: confidence interval