Small Ruminant Research 158 (2018) 48–53

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

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

Khalid Al-Habsi, Rongchang Yang, Sam Abraham, Una Ryan, David Miller, Caroline Jacobson*

School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, 6150, Australia

ARTICLE INFO

Keywords:
qPCR
Salmonella
Campylobacter jejuni
Goat
Public health
Zoonotic

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.

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 and Kambarag, 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.

* Corresponding author.
E-mail address: C.Jacobson@murdoch.edu.au (C. Jacobson).

https://doi.org/10.1016/j.smallrumres.2017.11.011
Received 3 August 2017; Received in revised form 20 November 2017; Accepted 22 November 2017
Available online 23 November 2017
0921-4488/

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, 2017), and gastrointestinal parasites in rangeland goats (Al-Habsi et al., 2017a,c). 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,′-dimethoxy-4′5,′-dichloro-6-carboxyfluorescein (JOE)-ACGTGACGACCCACGG CTTTAC-3′. A 121 bp product was amplified from the *Campylobacter* spp. *purA* using the forward primer *purA*F1 5′-CGCCCTTATCCTCAGT AGGAAA-3′, the reverse primer *purA*R1 5′-TCAGCAGGCGCTTTAA CAG-3′ and the probe 5′-6-carboxyfluorescein (FAM)-AGCTCCATTTCC CACACGCGTTGC-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 $MgCl_2$, 1 mM 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 1×10^6 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% 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