Veterinary Parasitology: Regional Studies and Reports 7 (2017) 32–35

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

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

Short Communication

Zoonotic *Cryptosporidium* and *Giardia* shedding by captured rangeland goats

Khalid Al-Habsi, Rongchang Yang, Andrew Williams, David Miller, Una Ryan *, Caroline Jacobson

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

ARTICLE INFO

Article history:
Received 13 September 2016
Accepted 28 November 2016
Available online 5 December 2016

Keywords:
Cryptosporidium
Giardia
Rangeland goats
Zoonotic

ABSTRACT

Faecal shedding of *Cryptosporidium* and *Giardia* by captured rangeland goats was investigated using a longitudinal study with four faecal samples collected from 125 male goats once monthly for four months, commencing immediately after capture and transport to a commercial goat depot (feedlot). Goats were composite breed and aged approximately 9–12 months on arrival. Faecal samples were screened for *Cryptosporidium* and *Giardia* presence and concentration using quantitative PCR and sequencing at the 18S ribosomal RNA locus (*Cryptosporidium*), and glutamate dehydrogenase and β-giardin loci (*Giardia*). Longitudinal prevalence for *Cryptosporidium* was 27.2% (point prevalence range 3–14%) with 3 species identified: *C. xiaoi* (longitudinal prevalence 13.6%), *C. ubiquitum* (6.4%) and *C. parvum* (3.2%). Sub-typing at the gp60 locus identified *C. ubiquitum* XIIa, *C. parvum* IIaA17G2R1 and *C. parvum* IIaA17G4R1. This is the first report of the zoonotic *C. parvum* subtype IIaA17G4R1 in goats. The pattern of genotypes shed in faeces changed over the duration of study with *C. ubiquitum* identified only at the first and second samplings, and *C. parvum* identified only at the fourth sampling. Longitudinal prevalence for *Giardia duodenalis* was 29.6% (point prevalence range 4–12%) with all positives sub-typed as assemblage E. Only 2/125 goats were identified to be shedding *Cryptosporidium* or *Giardia* on more than one occasion. This is the first report of *Cryptosporidium* and *Giardia* genotypes in captured rangeland goats. Faecal shedding of zoonotic *Cryptosporidium* spp. and potentially zoonotic *G. duodenalis* has implications for food safety and effluent management. *Keywords*: *Cryptosporidium; Giardia*; Rangeland goats; zoonotic.

1. Introduction

Strong growth in the Australian goat meat industry has been largely supported by goats derived from rangeland (extensive) production systems. Rangeland goats are a composite breed naturalised throughout Australian rangelands, typically unmanaged (undomesticated) and opportunistically captured and utilised for meat production. Diarrhoea and ill-thrift are cited as important issues for rangeland goats following capture, particularly under intensive management conditions in feedlots prior to slaughter (MLA, 2016). Causes of diarrhoea and ill thrift in captured rangeland goats are not well described, although it is suggested that stress associated with capture, transport and domestication of wild goats, and high stocking densities in feedlots increase shedding and transmission of disease agents with veterinary and public health importance, e.g. *Eimeria* and *Salmonella* (MLA 2016).

Reviews of available evidence have concluded that *Cryptosporidium* spp. and *Giardia duodenalis* may cause diarrhoea, weight loss and mortalities in goat kids, although evidence of disease in goats post-weaning age is less clear (de Graaf et al., 1999; O'Handley and Olsen 2006; Geurden et al., 2010; Santin, 2013). Six *Cryptosporidium* species and genotypes have been reported in goats; *C. parvum, C. hominis, C. xiaoi, C. ubiquitum, C. andersoni* and rat genotype II (Robertson, 2009; Koinari et al., 2014; Ryan et al., 2014; Peng et al., 2016). *Giardia duodenalis* assemblages A, B and E have been identified in goats (Robertson, 2009; Zhang et al., 2012; Peng et al., 2016). Importantly, some *Cryptosporidium* and *G. duodenalis* genotypes reported in goats have public health significance, having zoonotic potential and capacity for contamination of water supplies (Robertson, 2009; Ryan et al., 2014).

The epidemiology of *Cryptosporidium* and *Giardia* in rangeland goats in Australia is not described, and may have implications for management of goats pre-slaughter. The present study therefore aimed to investigate the faecal shedding of *Cryptosporidium* and *Giardia* species by captured rangeland goats using molecular tools.

2. Materials and methods

2.1. Animals and sample collection

Sampling occurred once monthly for four months (S1 to S4) from 125 male rangeland goats (composite breed) following capture and

* Corresponding author.
E-mail address: Una.Ryan@murdoch.edu.au (U. Ryan).

http://dx.doi.org/10.1016/j.vprsr.2016.11.006
2405-9390/

beginning immediately after arrival at a commercial goat depot (feedlot) near Geraldton, Western Australia in February 2014. On arrival (S1), goats weighed 30.7 ± 0.3 kg (mean ± SEM) with estimated age 9–12 months based on dentition. Goats were housed in four group pens (approximately 30 goats per pen). Grain-based pellets, hay and water were supplied ad libitum. Goats were consigned for slaughter after conclusion of the experiment when they reached acceptable slaughter weight.

Faecal samples were collected directly from the rectum and stored on ice or a refrigerator (4.0 °C) until DNA extraction was performed. Sample collection methods were approved by Murdoch University Animal Ethics Committee (approval number R2617/13).

2.2. DNA isolation

Four freeze–thaw cycles were employed followed by genomic DNA extraction from 200 mg of each faecal sample using a Power Soil DNA Kit (MolBio, Carlsbad, California), which includes a mechanical bead disruption step using glass beads to increase the efficiency of DNA extraction. A negative control (no faecal sample) was used in each extraction group.

2.3. PCR screening, amplification and sequencing

Faecal samples were screened for the presence of *Cryptosporidium* and *Giardia* spp. using quantitative PCR (qPCR) as previously described (Yang et al., 2014a; Yang et al., 2014b). Analytical specificity and sensitivity testing of the qPCR assays was previously described (Yang et al., 2014a; Yang et al., 2014b), with no cross-reactions with other genera and detection of all *Cryptosporidium* and *Giardia* isolates tested. The assays detected 2 *Cryptosporidium* oocysts and 1 *Giardia* cyst per μl of faecal DNA extract. Mean RSQ and % RDS were 0.99 and 1.5%. for *Cryptosporidium*, and 0.98 and 5.5% for *Giardia* respectively. The number of oocyst equivalents per gram of faeces was calculated on the premise that one oocyst contains 40 fg of genomic DNA (Guy et al., 2003).

Cryptosporidium qPCR positives were amplified at the 18S rRNA locus using a nested PCR as previously described (Morgan et al., 1997). Subtyping at the *gp60* locus was conducted for *C. parvum* (Ng et al., 2008) and *C. ubiquitum* (Li et al., 2014). *Giardia* positive isolates were amplified at the glutamate dehydrogenase (*gdh*) and β-giardin (*bg*) loci (Read et al., 2004; Lalle et al., 2005). Triose-phosphate isomerase (*tpi*) assemblage *E*-specific primers (Geurden et al., 2008) were used to confirm the assemblages typed at the *gdh* and *bg* loci. Purified PCR products were sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, California). 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).

2.4. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics Version 21 for Mac. Goats were classified as positive (parasite DNA detected) or negative (no parasite DNA detected) for *Cryptosporidium* and *Giardia*. Point prevalence was determined by proportion of positive goats for each sample occasion. Two-tailed Chi-square tests were used to compare point prevalence between sampling occasions. Longitudinal prevalence was calculated as the proportion of goats with parasite DNA detected on at least one occasion. Prevalence 95% confidence intervals were calculated using Jeffrey's interval method (Brown et al., 2001). Differences in shedding intensity for *Cryptosporidium* and *Giardia* between time points were assessed using a univariate general linear model with timepoint included as a fixed factor and least squares difference post hoc test. Levene's test was used to determine for equality of variance ($P > 0.05$). *P*-values of 0.05 were used to declare statistical significance.

3. Results

3.1. Cryptosporidium and giardia prevalence

A total of 36/500 faecal samples were qPCR-positive for *Cryptosporidium* and 38/500 were PCR-positive for *Giardia*. Point and longitudinal prevalences are shown in Table 1. Point prevalence fell between the first and second sampling for both *Cryptosporidium* and *Giardia*. By S4, point prevalence for both *Cryptosporidium* and *Giardia* were not different to S1. Two goats were *Cryptosporidium*-positive on two occasions (S1 and S3; S3 and S4) and one goat was *Giardia*-positive on two occasions (S1 and S4). No goats were *Cryptosporidium*- or *Giardia*-positive on more than two occasions. Concurrent *Cryptosporidium* and *Giardia* infections were identified at each sampling occasion (Table 1).

3.2. Cryptosporidium species and subtypes

Overall 29/36 *Cryptosporidium*-positive samples were successfully sequenced at the 18S locus and three *Cryptosporidium* species were detected; *C. xiaoi* ($n = 17$), *C. ubiquitum* ($n = 8$) and *C. parvum* ($n = 4$). Sub-typing at the gp60 locus identified all eight *C. ubiquitum* positives as XIIa, while *C. parvum* positives were subtyped as IIaA17G2R1 ($n = 1$) and IIaA17G4R1 ($n = 3$). Point prevalence and longitudinal prevalence for species identified by sequencing are shown in Table 1. *Cryptosporidium xiaoi* was identified at all four sampling occasions. *Cryptosporidium ubiquitum* was not identified after S2. *Cryptosporidium parvum* was identified at S4 only. No mixed genotype *Cryptosporidium* infections were identified in any goats at a single sampling occasion. For the two goats that were *Cryptosporidium*-positive on two occasions, one goat was positive for *C. ubiquitum* (S3) and *C. parvum* IIaA17G4R1 (S4), and the other goat was positive for *C. ubiquitum* (S1) with the second isolate (S4) not successfully sequenced. Representative sequences were submitted to GenBank under the accession numbers: KX813706, KX813707, KX813708 and KX813709.

3.3. Giardia assemblages

Overall 26/38 *Giardia* qPCR positives were successfully typed at the *gdh* and *bg* loci and all were identified as *Giardia duodenalis* assemblage E. This was confirmed using assemblage *E*-specific *tpi* primers. No positive samples from S4 were successfully sequenced. Representative sequences were submitted to GenBank under the accession numbers: KX813710 and KX813711.

3.4. Cryptosporidium and giardia faecal shedding intensity

Faecal shedding intensity (concentration) in positive samples are shown in Table 1. There was no effect of sampling occasion on shedding intensity in positive goats for either *Cryptosporidium* ($P = 0.374$) or *Giardia* ($P = 0.400$).

4. Discussion

This is the first report of *Cryptosporidium* and *Giardia* genotypes from Australian rangeland goats. The key observations were the low point prevalence of zoonotic genotypes, and a change in pattern of zoonotic *Cryptosporidium* genotypes shed in faeces changed over time with *C. ubiquitum* most prevalent immediately following capture, transport, and arrival at the feedlot, and *C. parvum* only evident after 3 months in the feedlot. This is the first report of the zoonotic *C. parvum* IIaA17G4R1 genotype in goats. *Giardia duodenalis* assemblage E was also identified, and is potentially zoonotic. Faecal shedding of zoonotic *Cryptosporidium* and potentially zoonotic *G. duodenalis* has impacts for public health, including management of effluent to manage risk of contamination of water supplies (Robertson, 2009).