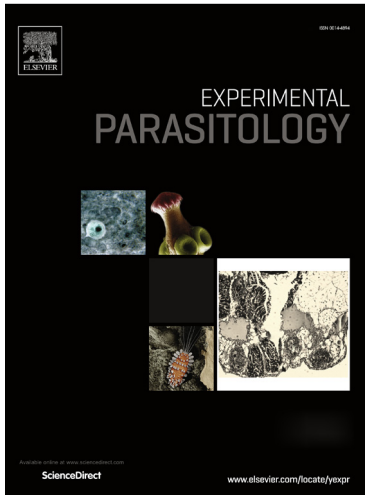

Research Brief

Cryptosporidium species in sheep and goats from Papua New Guinea

M. Koinari, A.J. Lymbery, U.M. Ryan

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Cryptosporidium species in sheep and goats from Papua New Guinea

M. Koinari[1*], A.J. Lymbery[2] and U.M. Ryan[1]

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

[2]Fish Health Unit, School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia

[*]Corresponding author at: School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia. Tel.: +61 89360 6379; Fax: +61 89310 414. E-mail address: mkoinari@gmail.com (M. Koinari).

Abstract

Species of *Cryptosporidium* are extensively recognised as pathogens of domesticated livestock and poultry, companion animals, wildlife, and are a threat to public health. Little is known of the prevalence of *Cryptosporidium* spp. in humans, domesticated animals or wildlife in Papua New Guinea (PNG). The aim of the present study was to screen goats and sheep for *Cryptosporidium* using molecular tools. A total of 504 faecal samples were collected from sheep (*n*=276) and goats (*n*=228) in village, government and institutional farms in PNG. Samples were screened by nested PCR and genotyped at the 18S rRNA and at the 60 kDa glycoprotein (*gp60*) loci. The overall prevalences were 2.2% for sheep (6/278) and 4.4% (10/228) for goats. The species/genotypes identified were *C. hominis* (subtype IdA15G1) in goats (*n*=6), *C. parvum* (subtypes IIaA15G2R1and IIaA19G4R1) in sheep (*n*=4) and in goats (*n*=2), *C. andersoni* (*n*=1) and *C. scrofarum* (*n*=1) in sheep, *C. xiao* (*n*=1) and *Cryptosporidium* rat genotype II (*n*=1) in goats. This is the first report of *Cryptosporidium* spp. identified in sheep and goats in PNG. Identification of *Cryptosporidium* in livestock warrants better care of farm animals to avoid contamination and illness in vulnerable population. The detection of zoonotic *Cryptosporidium* in livestock suggests these animals may serve as reservoirs for human infection.

Keywords: *Cryptosporidium;* sheep; goat; 18S rRNA; 60 kDa glycoprotein; zoonotic; Papua New Guinea

1 Introduction

Species of *Cryptosporidium* are globally distributed, zoonotic intestinal protozoan parasites that cause diarrheal disease in animals and are one of the main causes of serious diarrhoea in children (Kotloff et al., 2013). Clinical effects of *Cryptosporidium* infection, which include diarrhoea, weight loss and often death in lambs and goat kids, severely impact the economy of sheep and goat farming (de Graaf et al., 1999).

Globally, the prevalence of *Cryptosporidium* spp. in sheep can vary drastically from <5% to >70% (Robertson, 2009). Although fewer epidemiological studies have examined *Cryptosporidium* spp. in goats, it appears that prevalence is similarly variable, with values of <10% to >40% reported (Robertson, 2009). At least eight *Cryptosporidium* species have been identified in sheep faeces including *C. parvum*, *C. hominis*, *C. andersoni, C. suis, C. xiaoi*, *C. fayeri*, *C. ubiquitum* and *C. scrofarum,* with *C. xiaoi, C. ubiquitum* and *C. parvum* most prevalent (Ryan et al., 2005; Santin et al., 2007; Fayer and Santin, 2009; Giles et al., 2009; Yang et al., 2009; Robertson, 2009; Díaz et al., 2010a; Wang et al., 2010; Sweeny et al., 2011; Cacciò et al., 2013; Connelly et al., 2013). Three of these species; *C. parvum, C. hominis* and *C. xiaoi* have also been identified in goats (Giles et al., 2009; Robertson 2009; Diaz et al., 2010b).

Sheep and dairy goats were introduced to Papua New Guinea (PNG) in the early 19th century by colonial administrators and missionaries (Quartermain, 2004). There are two predominant breeds of sheep (PNG Priangan sheep and the Highlands Halfbred) and one breed of goat (PNG goat genotype) in PNG (Quartermain, 2004). Currently, sheep and goats are raised in government stations for breeding and distribution to smallholder farms and in research institutional farms. Little is known about *Cryptosporidium* in sheep and goats in PNG and therefore the aim of the present study was to determine the prevalence and genotypes of *Cryptosporidium* in these two hosts in PNG.

2 Materials and Methods

2.1 Sample collection

Faecal samples from a total of 228 goats and 276 sheep were collected from February 2011 to April 2011 from government, institutional and smallholder farms in a variety of agro-economic zones in PNG.

Farm management: The flocks from the government (Menifo) and institutional (Labu, Baisu and Tambul) farms grazed pasture in fenced areas (20-60 ha) at daytime. At night time, the flocks were kept in houses with wooden, slatted floors in institutional farms and on the ground in the government farm. At the time of sample collection, the combined numbers of sheep and goats in Menifo, Labu, Baisu, and Tambul were 55, 125, 70 and 143, respectively. The subsistence farmers kept few animals, usually less than 20, which grazed free range or were tethered and housed at night on slatted floors or on the ground underneath the farmer's house. Most animals grazed on native grasses and shrubs. Smallholder farmers also fed their animals with starchy vegetables (mostly sweet potatoes). The animals drank from troughs (sourced from water supply or rainwater tanks), rainwater run-off water or ponds.

Herd health programs: The floors of the resting houses were not swept. The animals were penned on dirty floor, ground or on bare concrete floors. The farmers at the institutions and government farms sheared their sheep, whereas, the smallholder farmers did not and explained that they did not have the resources for it. Most farm managers reported that the most common signs of illness in their animals were diarrhoea and coughing, followed by itching and hair loss. The three large institutional flocks were drenched with benzimidazole (Panacur) nominally at bimonthly intervals. At the time of sampling, animals had been drenched two months previously in Labu, four months previously in Baisu and Tambul and six months previously in Menifo. Most smallholder farmers did not know about causes of diseases in their sheep and goats or the use of anthelmintic drugs for parasite control. For instance, a smallholder farmer reported the death of his entire flock (n=25) and noticed nematode worms in the gut of a dead sheep.

All animals sampled were adults. Faecal samples were obtained from the rectum of randomly selected animals and examined visually for consistency, mucus and macroscopic parasites. All sample collection methods used were approved by the Murdoch University Animal Ethics Committee (approval number R2368/10). The faecal samples were preserved in 70% ethanol and transported to Murdoch University, Australia, for further analysis.

2.2 DNA isolation and genotyping of Cryptosporidium sp.

Total DNA was extracted from 250 mg of faeces using a PowerSoil® DNA Isolation Kit (MO BIO laboratories, Carlsbad, California, USA). All samples were screened for the presence of *Cryptosporidium* spp. at the 18S rRNA locus using a nested PCR as previously

described (Morgan et al., 1997). *Cryptosporidium parvum* and *C. hominis*-positive isolates were subtyped at the 60 kDa glycoprotein locus (*gp60*) as described by Sulaiman et al. (2005). All positive isolates were sequenced as previously described (Koinari et al., 2013).

3 Results

Cryptosporidium was detected in 2.2% (6/276; 95% CI 2.8 - 6.2) of sheep and 4.4% (10/228; 95% CI 2.8 - 6.2) of goats at the 18S rRNA locus. Three species of *Cryptosporidium* were detected in sheep, namely *C. parvum* (*n*=4), *C. andersoni* (*n*=1) and *C. scrofarum* (*n*=1). Four species/genotypes were detected in goats; *C. hominis* (*n*=6), *C. parvum* (*n*=2), *C. xiaoi* (*n*=1) and rat genotype II (*n*=1) (Table 1). Rat genotype II, *C. xiaoi*, *C. scrofarum* and *C. andersoni* isolates were detected in animals from smallholder farms. The *C. hominis* isolates were from smallholder (*n*=4) and institutional (*n*=2) farms, while *C. parvum* was identified in animals from all three types of farms; government (*n*=1), institutional (*n*=3) and smallholder (*n*=1). Analysis of the *gp60* gene identified the presence of two *C. parvum* subtypes; IIaA15G2R1 (*n=3*) and IIaA19G4R1 (*n*=2) in sheep and goats and a *C. hominis* subtype (IdA15G1) (*n*=1) in a goat (Table 1). The partial 18S and *gp60* nucleotide sequences were deposited in the GenBank database under the accession numbers KJ584567-KJ584584.

4 Discussion

This is first study to identify and molecularly characterise *Cryptosporidium* in sheep and goats in PNG and analysis revealed a high diversity of *Cryptosporidium* parasites within these animal populations. The results of the present study complement recent findings of *C. parvum* in fish from freshwater aquaculture, wild freshwater and wild saltwater, and *C. hominis* in a wild marine fish in PNG (Koinari et al. 2013). The only other previous study of *Cryptosporidium* in PNG identified *Cryptosporidium* antibodies in 24% of young children from Goroka (Groves et al., 1994).

Although point prevalences were low for *Cryptosporidium* in the present study, the true prevalence may be underestimated as only single faecal samples were screened at one time point and intermittent shedding and seasonal variation are common (O'Handley et al.,

1999). In addition, only adult animals were screened and prevalences are known to be much higher in younger animals (Santin et al., 2007). Most importantly, the identification of *Cryptosporidium* in livestock warrants better care of farm animals to avoid contamination and illness in vulnerable populations, as *Cryptosporidium* spp. are known for causing diarrhoea and mortality in young animals in both natural and artificial infections (de Graaf et al., 1999; Quilez et al., 2008; Giles et al., 2009).

The three species (*C. parvum, C. andersoni* and *C. scrofarum)* identified in sheep from the present study have also been reported in sheep in previous studies (Ryan et al., 2005; Santin et al., 2007; Quilez et al., 2008; Giles et al., 2009). In addition, *C. andersoni* is frequently reported in cattle and occasionally in humans, while *C. scrofarum* is commonly identified in pigs (Xiao, 2010). *Cryptosporidium ubiquitum* is a common species found in sheep in other countries (Ryan et al., 2005; Santin et al., 2007; Wang et al., 2010; Yang et al., 2009); however, it was not identified in the present study.

Three species, *C. hominis, C. parvum* and *C. xiaoi,* detected in goats in the present study have also been reported in goats in other studies (Goma et al., 2007; Geurden et al., 2008; Quilez et al., 2008; Giles et al., 2009; Diaz et al., 2010b). For example, molecular analyses confirmed infections with *C. hominis* and *C. parvum* in diarrheic goat kids in the UK (Giles et al., 2009) and *C. parvum* in goats in Spain (Quilez et al., 2008). *Cryptosporidium xiaoi* is commonly reported in sheep (Fayer and Santin, 2009) and occasionally in goats (Diaz et al., 2010b). This is the first report of rat genotype II in goats. Rat genotype II has been reported in house rats in China (Lv et al., 2009), and in the Philippines (Ng-Hublin et al., 2013), brown rats in the Philippines (Ng-Hublin et al., 2013) and in wild black rats in Northern Australia (Paparini et al., 2012). The goat in which rat genotype II was identified was from a smallholder farm in Bena-Bena, PNG. Smallholders usually keep their goats in night houses, which are built very close to their own homes in order to avoid theft. The goat could have acquired this genotype from the house rats; however, further studies are required to confirm this and to determine if the goat was actually infected or just passing oocysts from ingestion of rat faeces. Identification of species such as *C. andersoni*, *C. scrofarum* and *C. xiaoi* in smallholder flocks probably reflects the management system. Typically, these small ruminants are tethered and/or allowed to graze freely on shrubs and grasses along road sides, near homes and gardens, where they share the feeding grounds with other livestock, especially cattle and pigs.

Cryptosporidium hominis and *C. parvum* are the most common causes of cryptosporidiosis in humans worldwide (Xiao, 2010). In the present study, *C. hominis* (subtype IdA15G1) was found in goats and *C. parvum* (subtypes IIaA15G2R1 and IIaA19G4R1) was found in both sheep and goats. Both the *C. parvum* IIa subtypes and *C. hominis* Id subtype identified in the present study were previously identified in fish in PNG (Koinari et al., 2013). The *C. parvum* subtype IIaA15G2R1, has been reported in sheep and goats in previous studies in Belgium, Spain, Brazil, China and Australia (Diaz et al., 2010a; Geurden et al., 2008; Paz et al., 2014; Yang et al., 2014; Ye et al., 2014). *C. parvum* subtype IIaA15G2R1 is a common subtype in cattle and humans (Feng et al., 2013; Xiao, 2010) in the Americas, Europe, Northern Africa and Asia (Alyousefi et al., 2013; Amer et al., 2010; Brook et al., 2009; Diaz et al., 2010a; Geurden et al., 2009; Helmy et al., 2013; Iqbal et al., 2012; Meireles et al., 2011; Quilez et al., 2008; Rahmouni et al., 2014; Rieux et al., 2013; Santin et al., 2008; Soba and Logar, 2008). It has also been found in yak in China (Mi et al., 2013) and in buffalo in Egypt (Helmy et al., 2013). The *C. parvum* subtype IIaA19G4R1 was identified in both a goat and a sheep in the present study. Previously, *C. parvum* subtype IIaA19G4R1 was identified in cattle in Northern Ireland (Thompson et al., 2007) and Australia (Ng et al., 2008) and freshwater fish (tilapia and silver barb) from PNG (Koinari et al., 2013).

These findings suggest that sheep and goats may be important reservoirs of *C. hominis* and zoonotic *C. parvum* subtypes in PNG. The detection of *C. hominis* in goats presumably reflects the very close association between humans and goats. Further research is necessary to characterize the prevalence of various *Cryptosporidium* species and genotypes in young lambs, goats and cattle and other hosts such as humans to more fully understand the transmission dynamics of *Cryptosporidium* in PNG.

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336 **Table 1.** Species and subtypes of *Cryptosporidium* identified in sheep and goats in the
337 present study.
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343 **Table 1. Species and subtypes of *Cryptosporidium* identified in sheep and goats in the**

344 **present study.**

Sample	Host	Species identified at the 18S locus	Type of farm	*gp60* subtype
GE51	Goat	*C. hominis*	Smallholder	_
GE53	Goat	*C. hominis*	Smallholder	_
GE66	Goat	*C. hominis*	Smallholder	_
GE78	Goat	*C. hominis*	Smallholder	IdA15G1
GM14	Goat	*C. hominis*	Research Institution	_
GW10	Goat	*C. hominis*	Research Institution	_
GM35	Goat	*C. parvum*	Research Institution	IIaA19G4R1
GW19	Goat	*C. parvum*	Research Institution	IIaA15G2R1
SW29	Sheep	*C. parvum*	Research Institution	_
SE03	Sheep	*C. parvum*	Government	IIaA15G2R1
SE83	Sheep	*C. parvum*	Smallholder	IIaA15G2R1
SW106	Sheep	*C. parvum*	Research Institution	IIaA19G4R1
SE67	Sheep	*C. scrofarum*	Smallholder	_
SE79	Sheep	*C. andersoni*	Smallholder	_
GE102	Goat	*C. xiaoi*	Smallholder	_
GE01	Goat	Rat genotype II	Smallholder	_

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350 Highlights

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352 • Detection of *Cryptosporidium* spp. in adult sheep and goats from Papua New Guinea
353 using molecular tools.
354 • In sheep, *C. parvum, C. andersoni* and *C. scrofarum* were identified.
355 • In goats, *C. hominis, C. parvum, C. xiaoi* and rat genotype II were identified.
356 • Subtypes detected were *C. hominis* IdA15G1 and *C. parvum* IIaA15G2R1 and
357 IIaA19G4R1.
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Graphical abstract