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# Investigation into potential transmission sources of *Giardia duodenalis* in a threatened marsupial (*Petrogale penicillata*)

Elke T. Vermeulen [a,*], Deborah L. Ashworth [b], Mark D.B. Eldridge [a,c], Michelle L. Power [a]

[a] Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia
[b] Office of Environment and Heritage, PO Box 1967, Hurstville, NSW 2220, Australia
[c] Australian Museum Research Institute, Australian Museum, 6 College Street, Sydney, NSW 2010, Australia

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ABSTRACT

Assemblages of the protozoan parasite *Giardia duodenalis* common in humans and domestic species are increasingly identified in wildlife species, raising concern about the spill-over of pathogens from humans and domestic animals into wildlife. Here, the identity and prevalence of *G. duodenalis* in populations of a threatened marsupial, the brush-tailed rock-wallaby (*Petrogale penicillata*), was investigated. Identification of *G. duodenalis* isolates, across three loci (18S rRNA, β-giardin and *gdh*), from rock-wallaby fecal samples ($n = 318$) identified an overall detection rate of 6.3%. No significant difference in *G. duodenalis* detection was found among captive, wild and supplemented populations. Isolates were assigned to the zoonotic assemblages A and B at 18S rRNA, with sub-assemblages AI and BIV identified at the β-giardin and *gdh* loci, respectively. Assemblages AI and BIV have previously been identified in human clinical cases, but also in domestic animals and wildlife. The identification of these assemblages in brush-tailed rock-wallabies suggests there are transmission routes of *G. duodenalis* from humans or other animals to Australian wildlife, both in captivity and in the wild.



## 1. Introduction

*Giardia duodenalis* (*syn. Giardia intestinalis* and *Giardia lamblia*) is a protozoan parasite that infects a broad range of mammalian hosts, including humans (Cacciò and Ryan, 2008). *Giardia* taxonomy relies heavily on molecular data which has shown *G. duodenalis* to be a species complex that is divided into assemblages (A–H) and sub-assemblages (e.g., AI–AIV) that vary in host specificity (Feng and Xiao, 2011) and pathogenicity (Read et al., 2002). The zoonotic assemblages A and B typically infect humans but have been found in a range of other mammals, including wildlife, while the other assemblages (C–H) are usually host-specific (reviewed in Feng and Xiao, 2011).

Spill-over of zoonotic assemblages of *G. duodenalis* into wildlife populations have been associated with agriculture and recreational activities (reviewed in Thompson, 2013). Recent molecular studies of *G. duodenalis* in terrestrial and marine wildlife have identified zoonotic assemblages (A and B) more commonly than host-specific assemblages (C–H) (Ash et al., 2010; Johnston et al., 2010; Thompson et al., 2010; Delport et al., 2014). The proximity of wild host populations to areas inhabited by humans was associated with observed increases of the prevalence of zoonotic assemblages of *G. duodenalis* (Delport et al., 2014).

The spill-over of zoonotic *G. duodenalis* assemblages has also occurred in Australia, where wild animals have mostly been reported to be infected with zoonotic assemblages rather than host-specific ones (McCarthy et al., 2008; Thompson et al., 2008, 2010; Ng et al., 2011). To date *G. duodenalis* has been reported in 27 marsupial species across 8 families (Marino et al., 1992; Bettiol et al., 1997; Buckley et al., 1997; Millstein and Goldsmith, 1997; Thompson et al., 2008, 2010; Ng et al., 2011), but identifications to assemblage and sub-assemblage are restricted to recent molecular studies (Adams et al., 2004; McCarthy et al., 2008; Thompson et al., 2008, 2010; Ng et al., 2011). The most frequent identifications of *Giardia* in marsupials are of the assemblages commonly found in humans (A and B) (McCarthy et al., 2008; Thompson et al., 2008, 2010; Ng et al., 2011) or assemblages specific to domestic or feral animals, for example C, D (canines) and E (pigs) (Thompson et al., 2010; Ng et al., 2011). A possibly unique marsupial assemblage, the 'quenda genotype', was identified in a bandicoot (*Isoodon obesulus*), but appears to be only found in this host (Adams et al., 2004; Thompson et al., 2010). These identifications suggest there is a transmission route of *G. duodenalis* from

* Corresponding author.
*E-mail addresses:* elke.vermeulen@students.mq.edu.au (E.T. Vermeulen), deborah.ashworth@environment.nsw.gov.au (D.L. Ashworth), mark.eldridge@austmus.gov.au (M.D.B. Eldridge), michelle.power@mq.edu.au (M.L. Power).

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humans and/or domesticated animals and marsupial species, as well as other wildlife, with which the marsupials can be in contact.

Management practices such as captive breeding may involve close contact between wildlife and humans, as well as other domestic and commensal animals, and thus allow for a potential transmission route for parasites (Warren et al., 2003). To investigate transmission routes of *G. duodenalis* in Australian wildlife, we used molecular tools to detect and characterize *G. duodenalis* isolates in a threatened marsupial species undergoing conservation management. Moreover, we investigated whether the prevalence and identity of *G. duodenalis* varied with increased exposure to humans and other animals. The brush-tailed rock-wallaby (BTRW, *Petrogale penicillata*) was chosen for this study, as it is listed as 'near threatened' (IUCN, 2013) and a state-wide Recovery Plan has been operating in New South Wales since 2005 (DECC, 2008). As part of this Recovery Program, BTRW have been bred in captivity and translocated to various locations (DEC, 2005; DECC, 2008; Menkhorst and Hynes, 2010), resulting in BTRW populations with varying levels of exposure to humans and other animals.

## 2. Methods

### 2.1. Sample collection and DNA preparation

Brush-tailed rock-wallabies (BTRW) were once abundant across south-eastern Australia but since European settlement have been reduced to fragmented populations (Eldridge and Close, 2005). Dispersal between these remnant populations is now rare (Browning et al., 2001). For this study, seven sites in New South Wales were selected and assigned to one of three categories according to its management history: a site which housed captive bred animals (captive bred), a site which comprised free-ranging animals with no direct human contact (wild) and sites where free-ranging populations had been supplemented with captive bred animals (supplemented) (Table 1). For each site fecal samples were opportunistically collected between March 2010 and July 2013 during routine colony management by Office of Environment and Heritage staff who are experienced at recognizing BTRW scats. Fecal samples were subsequently stored at 4 °C until DNA extraction. Genomic DNA was extracted from feces (~150 mg) using the ISOLATE Fecal DNA kit (Bioline, London, UK) and following manufacturer's instructions. The extracted DNA was subsequently stored under −20 °C.

### 2.2. Parasite detection and identification

DNA samples were screened at the 18S rRNA locus using a previously described nested PCR protocol (Hopkins et al., 1997; Read et al., 2002). DNA extracted from trophozoites of *G. duodenalis* assemblage B was used as a positive control for all PCRs. Samples deemed positive at the 18S rRNA locus through gel electrophoresis (2% agarose) in TBE with SYBR safe staining (Promega, Australia) were amplified at two additional loci (β-giardin and *gdh*) to identify *Giardia* assemblages. Amplification of the β-giardin and *gdh* loci was achieved using nested and semi-nested PCRs respectively (Cacciò et al., 2002; Read et al., 2004; Lalle et al., 2005). All PCRs included a negative control (PCR-grade water) and a positive control (DNA extracted from cultured *G. duodenalis* trophozoites source). Samples displaying a single band of the expected size for each locus in gel electrophoresis were then purified using the QIAQuick PCR purification kit (Qiagen, Venlo, Netherlands) and sequenced in the forward and reverse direction (Macrogen, Seoul, Korea).

The sense and anti-sense sequences were aligned manually and checked for reading errors. To identify the assemblage and sub-assemblage of the *G. duodenalis* isolates, a consensus sequence was compared to previously published *G. duodenalis* sequences in GenBank through BlastN using Geneious (version 6.1.7, Biomatters Ltd., New Zealand). Sequences generated for 18S rRNA were submitted to the European Nucleotide Archive under accession numbers LN811452–LN811463 and sequences at the β-giardin and *gdh* loci were submitted to Genbank under accession numbers KP756604–KP756614.

Statistical analysis was performed in Minitab version 17.1.0 (LEAD Technologies, Inc., PA, USA). Prevalence of *G. duodenalis* detection and identification compared between sites and site categories was tested with a Chi Square test.

## 3. Results

### 3.1. Detection of G. duodenalis

In this study, 318 wallaby fecal samples were screened for *G. duodenalis* using a small fragment of the 18S rRNA. A positive amplicon of the correct size was detected in 70 DNA samples (22%) (Table 1) representing all site categories (wild, supplemented and captive bred). Based on initial prevalence estimates from PCR detection at the small 18S rRNA fragment, there was no significant difference in prevalence of *G. duodenalis* between site categories ($\chi^2 = 3.912$, DF = 2, $p = 0.141$) or between sites ($\chi^2 = 9.609$, DF = 6, $p = 0.142$).

### 3.2. Identification of G. duodenalis assemblages

Sequencing of 12 of the 70 18S rRNA positives confirmed samples to be *G. duodenalis* assemblages A or B (Table 2). The 12 samples identified at the 18S rRNA locus were obtained from wallaby feces from three supplemented sites (Kangaroo Valley Creek and River, and Square Top) and a captive-bred site (Waterfall Springs). To gather further genetic data on the isolates the 70 samples positives detected at the 18S rRNA locus were amplified at the β-giardin and the *gdh* loci. For β-giardin, 5 amplicons were positive with sequence analysis identifying 4 amplicons as assemblage AI. One sequenced sample was obtained from a wild site (Nattai) and three from a supplemented site (Square Top). At the *gdh* locus, 34 samples were positive and only 7 were successfully sequenced, with each sample identified as assemblage BIV. Four of these samples were from a wild site (Kangaroo Valley Mountain) and three were from supplemented sites (Kangaroo Valley River and Square Top). Thus, a total of 20 *G. duodenalis* isolates were identified across the three loci (Table 2).

Only the isolates identified through sequencing across the three loci were considered for statistical analysis. There was no significant difference in identification frequency between categories ($\chi^2 = 0.202$, DF = 2, $p = 0.904$). When sites with animals that had

**Table 1**
*Giardia duodenalis* screening and detection across three loci (18S rRNA, β-giardin and *gdh*) in seven sampled brush-tailed rock-wallaby populations. kV denotes 'Kangaroo Valley'.

| Site | Category | Total screened | 18S rRNA | β-Giardin | *gdh* |
|---|---|---|---|---|---|
| kV Mountain | Wild | 55 | 17 | 0 | 10 |
| kV River | Supplemented | 51 | 13 | 0 | 5 |
| kV Creek | Supplemented | 10 | 2 | 0 | 1 |
| Nattai | Wild | 30 | 4 | 1 | 1 |
| Square Top | Supplemented | 109 | 24 | 3 | 15 |
| Waterfall Springs | Captive bred | 39 | 3 | 0 | 1 |
| Jenolan Caves | Supplemented | 24 | 7 | 0 | 1 |



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