International Journal for Parasitology: Parasites and Wildlife 4 (2015) 190–196

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

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

Diversity of *Cryptosporidium* in brush-tailed rock-wallabies (*Petrogale penicillata*) managed within a species recovery programme

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

ARTICLE INFO

Article history:
Received 7 December 2014
Revised 11 February 2015
Accepted 13 February 2015

Keywords:
Cryptosporidium
Molecular detection
Brush-tailed rock-wallaby
Conservation management

ABSTRACT

Host–parasite relationships are likely to be impacted by conservation management practices, potentially increasing the susceptibility of wildlife to emerging disease. *Cryptosporidium*, a parasitic protozoan genus comprising host-adapted and host-specific species, was used as an indicator of parasite movement between populations of a threatened marsupial, the brush-tailed rock-wallaby (*Petrogale penicillata*). PCR screening of faecal samples (n = 324) from seven wallaby populations across New South Wales, identified *Cryptosporidium* in 7.1% of samples. The sampled populations were characterised as captive, supplemented and wild populations. No significant difference was found in *Cryptosporidium* detection between each of the three population categories. The positive samples, detected using 18S rRNA screening, were amplified using the actin and *gp60* loci. Multi-locus sequence analysis revealed the presence of *Cryptosporidium fayeri*, a marsupial-specific species, and *C. meleagridis*, which has a broad host range, in samples from the three population categories. *Cryptosporidium meleagridis* has not been previously reported in marsupials and hence the pathogenicity of this species to brush-tailed rock-wallabies is unknown. Based on these findings, we recommend further study into *Cryptosporidium* in animals undergoing conservation management, as well as surveying wild animals in release areas, to further understand the diversity and epidemiology of this parasite in threatened wildlife.

1. Introduction

Disease emergence presents a significant risk to the conservation of endangered wildlife. The risks of disease are leading to growing concern of the cost–benefit efficiency of the supplementation strategy (Kock et al., 2010). Species recovery actions such as the supplementation of dwindling populations with captive bred animals may introduce parasites atypical to the recovery species or exacerbate prevalence of existing pathogens due to stress and immune status of captive bred individuals, which may spread these pathogens into its new environment (Moberg, 1985; Cunningham, 1996). Control of disease risks requires a sound understanding of host–parasite interactions, both in threatened species and of hosts that may contribute to disease emergence. Further, parasites specific to the target species may not survive translocation or other conservation processes, thereby unbalancing the natural host–parasite relationship (Moir et al., 2012).

Cryptosporidium, a protozoan parasite with a broad vertebrate host range and variable host specificity, represents a potential indicator of disease risks associated with conservation management. This research strategy is particularly applicable to threatened Australian marsupials where the occurrence of human derived *Cryptosporidium* species has not been conclusively determined (Hill et al., 2008; Ng et al., 2011; Dowle et al., 2013).

Of the 26 described *Cryptosporidium* species (reviewed in Ryan et al., 2014), twelve have been reported in both humans and other hosts: *C. parvum, C. hominis, C. ubiquitum, C. andersoni, C. bovis, C. cuniculus, C. muris, C. canis, C. felis, C. meleagridis, C. suis* and *C. fayeri* (Xiao et al., 2001; Gatei et al., 2002; Xiao, 2002; Leoni et al., 2006; Robinson et al., 2010; Waldron et al., 2010). Each of the *Cryptosporidium* species reported in humans have been found in the Australian environment (Ryan and Power, 2012; Abeywardena et al., 2013; Nolan et al., 2013), though human infections in Australia are predominantly *C. parvum* and *C. hominis* (Waldron et al., 2011).

Despite *Cryptosporidium* being identified in 16 marsupial species from 7 families (reviewed in O'Donoghue, 1995 and Power, 2010), identifications of *Cryptosporidium* to species level is limited to recent studies employing molecular tools (Warren et al., 2003; Hill et al., 2008; Power and Ryan, 2008; Ryan et al., 2008; Yang et al., 2011).

* Corresponding author. Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia. Tel.: +61 2 9850 9259; fax: +61 2 9850 8245.
E-mail address: elke.vermeulen@students.mq.edu.au (E.T. Vermeulen).

http://dx.doi.org/10.1016/j.ijppaw.2015.02.005

Following molecular identification, marsupials were found to be susceptible to two host-adapted *Cryptosporidium* species, *C. fayeri* and *C. macropodum* (Power and Ryan, 2008; Ryan et al., 2008). Several other host-specific genotypes have also been described in marsupials including brushtail possum genotype I from brushtail possums (*Trichosurus vulpecula*) (Hill et al., 2008) and kangaroo genotype I from western grey kangaroos (*Macropus fuliginosus)* (Yang et al., 2011).

Although there are reports of *C. parvum* and *C. hominis* in marsupials, these are based only upon a molecular signature from a faecal DNA sample, and an infection has never been confirmed using other methods such as parasite isolation (Hill et al., 2008; Ng et al., 2011; Dowle et al., 2013). The molecular detection of *C. parvum* and *C. hominis* in marsupial hosts has also been associated with an inability to confirm at greater than a single locus, namely the 18S rRNA. Passage of *C. parvum* of *C. hominis* oocysts through the marsupial gut is the likely reason for identifications of these *Cryptosporidium* species in marsupials (Dowle et al., 2013). The only confirmed case of *Cryptosporidium* infection in a marsupial that was not host specific was an infection of *C. muris* in captive greater bilbies (*Macrotis lagotis*) being bred for release into natural habitat (Warren et al., 2003).

Here we use molecular methods to detect and identify *Cryptosporidium* in the brush-tailed rock-wallaby (BTRW), *Petrogale penicillata*. This species is listed as 'endangered' in New South Wales, Australia (NSW *Threatened Species Conservation Act* 1995) and 'near threatened' on the IUCN Red List of Threatened Species across eastern Australia (IUCN, 2013). There is an approved NSW Recovery Plan for the species (DECC, 2008), as well as an approved National Recovery Plan (Menkhorst and Hynes, 2010). These plans identify supplementation of small colonies with captive bred individuals as an important recovery strategy and over the last few years several translocations of individuals between captive breeding facilities and wild populations have occurred (Menkhorst and Hynes, 2010). As rock-wallaby populations have experienced variable levels of human intervention, studying their parasites provides a platform to examine the effect of conservation management on the host–parasite relationship. Hence, our aim was to detect and identify *Cryptosporidium* species infecting wild, captive bred, and supplemented brush-tailed rock-wallaby populations.

2. Methods

2.1. Sample collection and sites

Brush-tailed rock-wallabies were once abundant in south-eastern Australia but are now reduced to fragmented populations in New South Wales and Victoria (Eldridge and Close, 2005). Dispersal between populations, which are located in steep, rocky habitats, is rare (Browning et al., 2001). For this study, seven BTRW sites were sampled between March 2010 and July 2013 (Table 1). Sample collection dates were spread evenly across three seasons (Autumn, Summer and Winter), with ~10 samples collected in Spring (2010 and 2012), spread evenly across the four years. The origin of each population varied and included three categories: one site with a BTRW population kept in a captive breeding facility (captive bred), sites where free-ranging populations had been supplemented with captive bred individuals (supplemented) and two pristine sites with only free-ranging animals (wild). Fresh faecal samples were collected in vials containing silicon beads from each site opportunistically from unknown individuals during routine colony management by the Office of Environment and Heritage staff and were then stored at 4 °C until further processing. The highest number of samples was obtained from Square Top in Warrumbungle National Park since this was a major release site.

2.2. DNA extraction and PCR screening

Genomic DNA was extracted from faecal material (~150 mg) using the ISOLATE Fecal DNA kit (Bioline, London, UK) following manufacturer's instructions. The extracted DNA was stored at –20 °C until further analysis. Directly prior to each PCR, the DNA samples were treated with GeneReleaser (BioVentures, Inc., TN, USA) by combining equal volumes of DNA and GeneReleaser, and subjecting the mixture to 7 min in a 500 W microwave.

2.3. PCR screening at the 18S rRNA locus

DNA samples were initially screened for *Cryptosporidium* using nested PCR to amplify a partial fragment of the 18S rRNA. The primary reaction followed the methodology of Xiao et al. (1999) but with a lower $MgCl_2$ concentration (2 mM). The secondary reaction comprised the primers 18S IF and 18S IR and followed the method of Morgan et al. (1997). PCRs were performed using Red Hot Taq DNA polymerase (ThermoFisher Scientific, Waltham, MA, USA) as previously described (Hill et al., 2008). Both reactions were modified to increase specificity for *Cryptosporidium* by lowering the concentration of dNTPs to 50 μM.

Longer 18S rRNA fragments were generated for samples testing positive for *Cryptosporidium* using the 18S IF and 18s IR primer set. The longer fragments were amplified using the primers of Xiao et al. (1999) for both primary and secondary reactions, following conditions as previously described by Waldron et al. (2011), inclusive of dNTPs and $MgCl_2$ concentrations as described above.

2.4. PCR amplification at confirmatory loci

To confirm 18S rRNA positives, DNA samples were screened at two additional loci, actin and glycoprotein 60 (*gp60*). For the actin locus, a nested protocol (Sulaiman et al., 2002) was performed with minor modifications. To improve specificity for *Cryptosporidium,* the concentration of $MgCl_2$ was lowered to 2 mM, dNTPS to 50 μM, and the annealing temperature raised to 54 °C in the secondary reaction.

Table 1
The rate of *Cryptosporidium* detected at the different loci per screened site and site category. All sites are in New South Wales; the precise location is withheld for some sites for the safety of the animals. KV means Kangaroo Valley. Samples at the loci (18S rRNA, actin and *gp60*) were deemed as positive after DNA sequencing.

Site	Population category	No. of samples	18S rRNA (298 bp)	18S rRNA (825 bp)	Actin	*gp60*
KV Mountain	Wild	55	7	7	2	3
KV River	Supplemented	43	2	1	0	1
KV Creek	Supplemented	10	4	3	0	0
Nattai	Wild	30	3	3	1	1
Square Top	Supplemented	123	5	4	0	0
Waterfall Springs	Captive bred[a]	39	2	2	1	2
Jenolan Caves	Supplemented	24	0	0	0	0

[a] Wallabies in a captive breeding facility.

All amplifications were performed using Red Hot Taq DNA Polymerase.

Amplification of the *gp60* locus was achieved using a nested protocol with primary amplification achieved using the primers outF and outR (Power et al., 2009) and secondary reactions using ATGF and StopR (Waldron et al., 2009). Red Hot Taq was used for both amplifications. All PCR reactions performed included a negative control (H_2O) and a positive control of DNA extracted from purified oocysts of *C. parvum*.

2.5. Sequencing of positive samples

All amplicons generated for 18S rRNA, actin and *gp60* were sequenced to enable *Cryptosporidium* species identification. Amplicons from each of the four PCRs which contained a band of the expected size when resolved by electrophoresis (2% agarose in TBE with SYBR Green staining) were purified using the QIAQuick PCR purification kit (Qiagen, Venlo, the Netherlands). Purified amplicons were sequenced in both directions (Macrogen, Seoul, Korea) using appropriate primers for amplifications, with the exception of the short fragment of the 18S rRNA, which were only sequenced with the primer 18S IF (Morgan et al., 1997).

2.6. Sequencing and phylogenetic analyses

The sense and antisense sequence fragments for each locus were aligned with Geneious (version 6.1.7, Biomatters LtD, New Zealand) and manually examined for quality and read errors. Consensus sequences for each positive sample were extracted and searched against GenBank using BlastN function in Geneious. To enable species identification within a phylogenetic framework, samples positive for 18S rRNA (~825 bp) were trimmed to the same length and aligned with *Cryptosporidium* reference sequences from GenBank using Clustal W (Larkin et al., 2007). A phylogenetic tree was constructed based on this alignment using neighbour-joining. Sequences generated in this study have been submitted to GenBank under accession numbers KP730299-KP730329.

2.7. Statistical analysis

To test differences of *Cryptosporidium* detection rate between sites and site categories, samples were tested at the 18S rRNA (~298 bp) locus for presence or absence and checked for significant differences with a chi-square test in Minitab (version 17.1.0, Minitab Inc.).

3. Results

3.1. Cryptosporidium screening

DNA was extracted from 324 samples and screened for *Cryptosporidium* using 18S rRNA PCR. Of the 324 screened faecal samples, 43 contained the expected amplicon. DNA sequencing and Blast searches identified 23 samples as being *Cryptosporidium,* giving a total positive identification rate of 7.1% in BTRW. *Cryptosporidium* positive samples were obtained from three site types (captive bred, supplemented and wild). Positives were found to be present across most study sites except for Jenolan Caves. There was no significant difference in *Cryptosporidium* detection between captive-bred, wild and supplemented as categories ($\chi^2 = 3.811$, DF = 2, $p = 0.149$). However, there was a significant difference between the sites ($\chi^2 = 23.6$, DF = 6, $p < 0.001$). Kangaroo Valley Creek had the highest rate of positive samples (40%), but this site had the lowest amount of samples tested (n = 10; Table 1).

3.2. Species identification at the 18S rRNA locus

From the initial positive samples (n = 23), 20 samples yielded sequence data for the larger 18S rRNA fragment (825 bp), which was used to generate a phylogeny (Fig. 1). Four samples from supplemented sites and three samples from wild sites clustered with the *C. parvum* and *C. hominis*. Three samples from supplemented sites, one from a captive bred site and one from a wild site grouped with the marsupial-specific species *C. fayeri* and *C. macropodum*. A further four samples from a wild site and one from a captive-bred site grouped with *C. meleagridis*. Two samples from a wild site were grouped with *C. ubiquitum*.

3.3. Species confirmation using actin and gp60

Sequence analysis at the actin and *gp60* loci resulted in amplicons and sequence data from only eight samples across all loci (Table 2). At the actin locus, although 15 samples generated a band of the expected size (~1066 bp), only four were identified using BlastN searches as *Cryptosporidium*, with two samples being *C. fayeri* and two being *C. meleagridis*. For *gp60*, seven samples generated an amplicon with three samples assigned to *C. fayeri* and four to *C. meleagridis* (Table 2). However, the four samples from Kangaroo Valley Mountain identifying as *C. meleagridis* may represent the same individual sampled twice over two time points. For those samples identified at the 18S rRNA as *C. hominis* and *C. parvum*, neither actin nor *gp60* could be amplified. An exception was sample 973 identified as *C. hominis* at the 18S rRNA and *C. fayeri* by *gp60* sequencing.

4. Discussion

The level of detection of *Cryptosporidium* in BTRW (7.1%) is consistent with observations of *Cryptosporidium* in other marsupials which range between 6.7% and 12.2% (Power et al., 2004; Hill et al., 2008; Yang et al., 2011; Dowle et al., 2013). Here, *Cryptosporidium* detection in BTRW is based on sequence identifications using a 298 bp fragment of the 18S rRNA. PCR is commonly employed for detection of *Cryptosporidium* in faecal samples as this approach has greater sensitivity than microscopy, both in detection and identification of species (Fall et al., 2003; Power et al., 2003; Ryan et al., 2008; Dowle et al., 2013). In our study, a larger fragment (~825 bp) failed to amplify three samples confirmed as *Cryptosporidium* using the smaller fragment, indicating that selection of optimal amplification methods should be considered when undertaking molecular detection of this parasite.

Despite no significant difference in the detection of *Cryptosporidium* between captive bred and free ranging animals, the identity of *Cryptosporidium* species in BTRW determined by sequencing raises concern for the health status of captive and wild BTRW. *Cryptosporidium fayeri* has previously been identified in six marsupial hosts including the related yellow-footed rock-wallaby *P. xanthopus* (Morgan et al., 1997; Power et al., 2003, 2009; Ryan et al., 2008; Power, 2010; Yang et al., 2011; Nolan et al., 2013). *Cryptosporidium fayeri* does not appear to cause disease in marsupials (Ryan et al., 2008). *Cryptosporidium meleagridis* has been identified in a range of vertebrates, including avian and mammalian hosts, as well as humans (Akiyoshi et al., 2003; Xiao et al., 2004). While *C. meleagridis* is the most common infection of *Cryptosporidium* in humans after *C. parvum* and *C. hominis* (Elwin et al., 2012), human infections are rare in Australia (Waldron et al., 2011). Consequently, it is unlikely that the captive bred animals were infected from human sources, but by other host species, such as free ranging birds, inhabiting the captive breeding site. The wild site, where most of the *C. meleagridis* isolates were found, is secluded from humans and thus transmission between humans and BTRW is unlikely. This is supported by the *gp60* analysis.

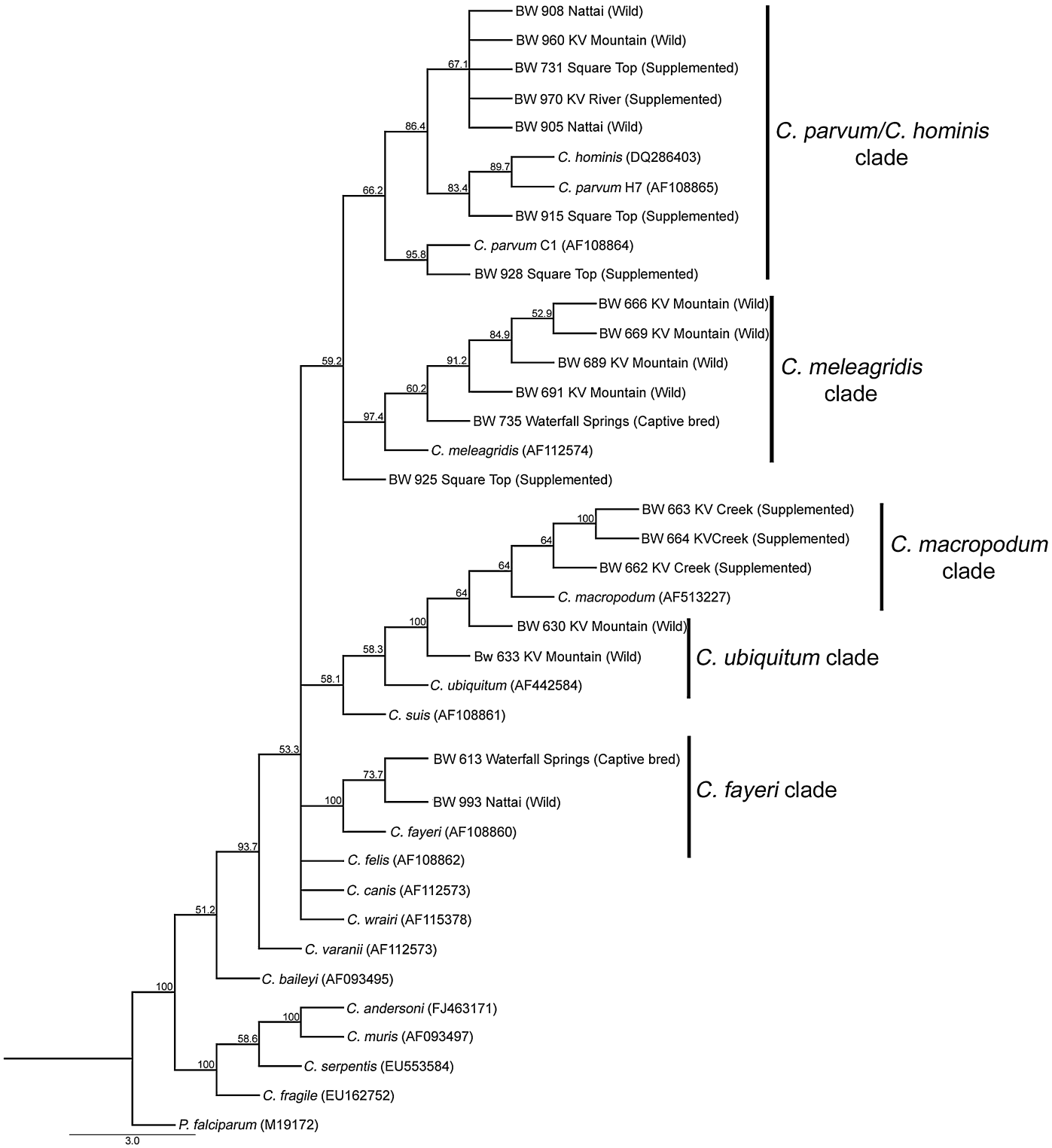

Fig. 1. Samples were identified within a phylogenetic framework with the tree constructed using neighbour-joining with bootstrap test (1,000 replicates, displayed at nodes) using the 18S rRNA locus (878 bp). KV denotes Kangaroo Valley.

The *C. meleagridis gp60* sequences from BTRW isolates displayed greater genetic similarity to *gp60* sequences from avian hosts (Stensvold et al., 2014), yet they were distinct from described sequences, indicating a new *gp60 C. meleagridis* subtype. This finding is the first report where a zoonotic species of *Cryptosporidium* was confirmed across multiple loci in a wild marsupial host. As such, much is unknown about the diversity and pathogenicity of *C. meleagridis* in wild marsupials and thus further study is required to understand the extent to which this species has penetrated marsupial hosts and likely transmission routes.

Table 2
Species identification across three loci (18S rRNA, actin and *gp60*) for the samples positive at the 18S rRNA locus for *C. fayeri* and *C. meleagridis*. Samples identified at the 18S rRNA locus as other *Cryptosporidium* species (Fig. 1) were omitted from this table since they could not be amplified at other loci. NP indicates no product was amplified. KV means Kangaroo Valley.

Label BW #	Location	Site type	18S rRNA (298 bp)	% Similarity	18S rRNA (825 bp)	% Similarity	Actin	% Similarity	*gp60*	% Similarity
613	Waterfall Springs	Captive bred	*C. fayeri*	99.6%	*C. fayeri*	99.5%	*C. fayeri*	99.9%	*C. fayeri* (subtype A10)	99.7%
666	KV Mountain	Wild	*C. meleagridis*	99.2%	*C. meleagridis*	99.9%	NP	NP	NP	NP
669	KV Mountain	Wild	*C. meleagridis*	99.8%	*C. meleagridis*	99.6%	*C. meleagridis*	99.9%	*C. meleagridis* (subtype IIIgA)	91.4%
689	KV Mountain	Wild	*C. meleagridis*	99.6%	*C. meleagridis*	99.9%	*C. meleagridis*	99.7%	*C. meleagridis* (subtype IIIgA)	91.2%
691	KV Mountain	Wild	*C. meleagridis*	99.2%	*C. meleagridis*	99.7%	NP	NP	*C. meleagridis* (subtype IIIgA)	91.6%
735	Waterfall Springs	Captive bred	*C. meleagridis*	99.1%	*C. meleagridis*	99.8%	NP	NP	*C. meleagridis* (subtype IIIbA)	88.8%
973	KV River	Supplemented	*C. hominis*	100.0%	NP	NP	NP	NP	*C. fayeri* (subtype A10)	99.9%
993	Nattai	Wild	*C. fayeri*	99.6%	*C. fayeri*	99.4%	*C. fayeri*	99.4%	*C. fayeri* (subtype A7)	99.8%

Cryptosporidium parvum and *C. hominis* were also identified in BTRW samples; however, these identifications were only possible at a single locus, the 18S rRNA. Only one of these samples could be amplified at one of the two confirmatory loci where it was typed as *C. fayeri*. Both *C. parvum* and *C. hominis* have been reported in a range of marsupials but similar to this study, other studies also failed to confirm identifications at loci other than the 18S rRNA (Hill et al., 2008; Ng et al., 2011; Dowle et al., 2013). Some isolates were inferred to be *C. ubiquitum* and *C. macropodum* through a GenBank match at the 18S rRNA locus but failed to amplify at subsequent loci (Fig. 1). While *C. macropodum* is specific to marsupials, particularly macropods (Power and Ryan, 2008), *C. ubiquitum* is typical to cattle but is commonly identified in humans as well (Fayer et al., 2010). So far, no report has been made of *C. ubiquitum* in marsupials (Ryan and Power, 2012).

Failure to amplify *C. parvum* and *C. hominis* isolates from marsupials at other loci has been attributed to low numbers of oocysts and the multi copy nature of the 18S rRNA locus compared to single copy confirmatory loci (Hill et al., 2008; Power et al., 2009; Ng et al., 2011). Indeed, oocyst counts in possums and bandicoots confirm low oocyst numbers (Hill et al., 2008; Dowle et al., 2013). The question remains if the presence of these human infective species is merely passage of oocysts through the marsupial gut or a true infection. Whole genome amplification could be employed to boost amplification of low oocyst numbers. This method has previously proved successful on clinical samples of *C. parvum* and *C. hominis* across three loci (Bouzid et al., 2010). Identification at the 18S rRNA locus alone has been found to underestimate mixed infections as this technique preferentially amplifies a predominant genotype (Reed et al., 2002). Mixed infections of *Cryptosporidium* were considered rare but they have only been studied so far in humans, mainly AIDS patients (Cama et al., 2006) and children (Xiao et al., 2001), and in calves (Tanriverdi et al., 2003). The role of mixed infections in *Cryptosporidium* pathology is still unclear. No study has so far described mixed infections in marsupials (Ryan and Power, 2012). The difficulty to amplify at discriminatory loci for genotypes such as *C. parvum* in marsupials highlights the need to identify *Cryptosporidium* using a multi-locus approach.

Another difficulty encountered in this study was the potential for pseudo-replication. When working with an endangered species one encounters issues with sample collection and numbers available for stringent analyses. For instance, the Kangaroo Valley Mountain population is estimated to comprise less than 10 individuals. As we identified *C. meleagridis* in four samples from Kangaroo Valley Mountain collected over two sampling periods, it is possible that the same individual has been sampled multiple times. A possible solution to reduce bias relative to sampling would be to apply microsatellite (MSAT) analysis to identify individuals. This method has been widely applied to many species ranging from large carnivores to small marsupials using faecal DNA, to monitor threatened populations, analyse their genetic diversity and wide-scale demographics of large populations (Spencer et al., 1995; Dool et al., 2013; Wultsch et al., 2014).

The findings in this study suggested that there was no direct effect of captive breeding and translocation on *Cryptosporidium* in brush-tailed rock-wallabies. In Australia, translocation policies are developed by the representative State bodies, and veterinary screening is not mandated but is increasingly employed to monitor the health of captive bred animals before release (Short, 2009). Health screening and its relation to the success of a recovery program is further complicated by a diverse number of potential pathogens and a lack of baseline data on risks that selected pathogens may pose to wildlife species. If unusual parasite species atypical to the host group are found, such as *C. meleagridis* in BTRW, consideration as to whether the animal should be used for

translocation or isolated from the population would form part of the management response. The pathology of *Cryptosporidium* in wild marsupials is also currently unknown (reviewed in Ryan and Power, 2012), making such a risk assessment difficult for BTRW. The identification of *Cryptosporidium* species with varying host specificity found in both captive bred and wild brush-tailed rock-wallabies indicates that further research is required into the diversity and pathology of this parasite in Australian wildlife.

Acknowledgements

Funding for this research was provided by the Australian Research Council, Office of Environment and Heritage and the Australian Museum through the ARC Linkage program, project ID LP110200569. The animal ethics approval numbers for the collections are AEC 050207/02 and AEC 080728/01. The authors would like to thank Todd Soderquist, Celia Thomson, Melinda Norton and Juliet Dingle for collecting the BTRW faecal samples.

Conflict of interest

The authors declared that there is no conflict of interest.

References

Abeywardena, H., Jex, A.R., von Samson-Himmelstjerna, G., Haydon, S.R., Stevens, M.A., Gasser, R.B., 2013. First molecular characterisation of *Cryptosporidium* and *Giardia* from *Bubalus bubalis* (water buffalo) in Victoria, Australia. Infect. Genet. Evol. 20, 96–102.

Akiyoshi, D.E., Dilo, J., Pearson, C., Chapman, S., Tumwine, J., Tzipori, S., 2003. Characterization of *Cryptosporidium meleagridis* of human origin passaged through different host species. Infect. Immun. 71, 1828–1832.

Bouzid, M., Heavens, D., Elwin, K., Chalmers, R.M., Hadfield, S.J., Hunter, P.R., et al., 2010. Whole genome amplification (WGA) for archiving and genotyping of clinical isolates of *Cryptosporidium* species. Parasitology 137, 27–36.

Browning, T.L., Taggart, D.A., Rummery, C., Close, R.L., Eldridge, M.D.B., 2001. Multifaceted genetic analysis of the "Critically Endangered" brush-tailed rock-wallaby *Petrogale penicillata* in Victoria, Australia: implications for management. Conserv. Genet. 2, 145–156.

Cama, V., Gilman, R.H., Vivar, A., Ticona, E., Ortega, Y., Bern, C., et al., 2006. Mixed *Cryptosporidium* infections and HIV. Emerg. Infect. Dis. 12 (6), 1025–1028.

Cunningham, A.A., 1996. Disease risks of wildlife translocations. Conserv. Biol. 10, 349–353.

DECC, 2008. NSW, D.o.E.a.C.C. (Ed.), Recovery Plan for the Brush-Tailed Rock-Wallaby (*Petrogale penicillata*).

Dool, S.E., Puechmaille, S.J., Dietz, C., Juste, J., Ibáñez, C., Hulva, P., et al., 2013. Phylogeography and postglacial recolonization of Europe by *Rhinolophus hipposideros*: evidence from multiple genetic markers. Mol. Ecol. 22, 4055–4070.

Dowle, M., Hill, N.J., Power, M.L., 2013. *Cryptosporidium* from a free-ranging marsupial host: bandicoots in urban Australia. Vet. Parasitol. 198, 197–200.

Eldridge, M.D.B., Close, R.L., 2005. Brush-tailed rock-wallaby *Petrogale penicillata*. In: Strahan, R. (Ed.), The Mammals of Australia. Reed Books, Sydney, pp. 383–385.

Elwin, K., Hadfield, S.J., Robinson, G., Chalmers, R.M., 2012. The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000–2008. Epidemiol. Infect. 140, 673–683.

Fall, A., Thompson, R.C.A., Hobbs, R.P., Morgan-Ryan, U.M., 2003. Morphology is not a reliable tool for delineating species within *Cryptosporidium*. J. Parasitol. 89 (2), 399–402.

Fayer, R., Santín, M., Macarisin, D., 2010. *Cryptosporidium ubiquitum* n. sp. in animals and humans. Vet. Parasitol. 172 (1–2), 23–32.

Gatei, W., Ashford, R.W., Beeching, N.J., Kang'ethe Kamwati, S., Greensill, J., Anthony Hart, C., 2002. *Cryptosporidium muris* infection in an HIV-infected adult, Kenya. Emerg. Infect. Dis. 8, 204–206.

Hill, N.J., Deane, E.M., Power, M.L., 2008. Prevalence and genetic characterization of *Cryptosporidium* isolates from common brushtail possums (*Trichosurus vulpecula*) adapted to urban settings. Appl. Environ. Microbiol. 74, 5549–5555.

IUCN, 2013. IUCN Red List of Threatened Species. Version 2013.2.

Kock, R.A., Woodford, M.H., Rossiter, P.B., 2010. Disease risks associated with the translocation of wildlife. Rev. Sci. Tech. 29, 329–350.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., et al., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Leoni, F., Amar, C., Nichols, G., Pedraza-Díaz, S., McLauchlin, J., 2006. Genetic analysis of *Cryptosporidium* from 2414 humans with diarrhoea in England between 1985 and 2000. J. Med. Microbiol. 55, 703–707.

Menkhorst, P., Hynes, W.H., 2010. Department of Sustainability and Environment, V. (Ed.), National Recovery Plan for the Brush-Tailed Rock-Wallaby *Petrogale penicillata*. East Melbourne.

Moberg, G.P., 1985. Animal Stress. American Physiological Society, Bethesda, Maryland, USA.

Moir, M.L., Vesk, P.A., Brennan, K.E.C., Poulin, R., Hughes, L., Keith, D.A., et al., 2012. Considering extinction of dependent species during translocation, ex situ conservation, and assisted migration of threatened hosts. Conserv. Biol. 26, 199–207.

Morgan, U.M., Constantine, C.C., Forbes, D.A., Thompson, R.C.A., 1997. Differentiation between human and animal isolates of *Cryptosporidium parvum* using rDNA sequencing and direct PCR analysis. J. Parasitol. 83, 825–830.

Ng, J., Yang, R., Whiffin, V., Cox, P., Ryan, U., 2011. Identification of zoonotic *Cryptosporidium* and *Giardia* genotypes infecting animals in Sydney's water catchments. Exp. Parasitol. 128, 138–144.

Nolan, M.J., Jex, A.R., Koehler, A.V., Haydon, S.R., Stevens, M.A., Gasser, R.B., 2013. Molecular-based investigation of *Cryptosporidium* and *Giardia* from animals in water catchments in southeastern Australia. Water Res. 47, 1726–1740.

O'Donoghue, P.J., 1995. *Cryptosporidium* and cryptosporidiosis in man and animals. Int. J. Parasitol. 25, 139–195.

Power, M.L., 2010. Biology *of Cryptosporidium* from marsupial hosts. Exp. Parasitol. 124, 40–44.

Power, M.L., Ryan, U.M., 2008. *A new species of* Cryptosporidium *(*Apicomplexa: Cryptosporidiidae*) from eastern grey Kangaroos (*Macropus giganteus*).* J. Parasitol. 94, 1114–1117.

Power, M.L., Shanker, S.R., Sangster, N.C., Veal, D.A., 2003. Evaluation of a combined immunomagnetic separation/flow cytometry technique for epidemiological investigations of *Cryptosporidium* in domestic and Australian native animals. Vet. Parasitol. 112, 21–31.

Power, M.L., Slade, M.B., Sangster, N.C., Veal, D.A., 2004. Genetic characterisation of *Cryptosporidium* from a wild population of eastern grey kangaroos *Macropus giganteus* inhabiting a water catchment. Infect. Genet. Evol. 4, 59–67.

Power, M.L., Cheung-Kwok-Sang, C., Slade, M., Williamson, S., 2009. Cryptosporidium fayeri: diversity within the *gp60* locus of isolates from different marsupial hosts. Exp. Parasitol. 121, 219–223.

Reed, C., Sturbaum, G.D., Hoover, P.J., Sterling, C.R., 2002. *Cryptosporidium parvum* mixed genotypes detected by PCR-restriction fragment length polymorphism analysis. Appl. Environ. Microbiol. 68 (1), 427–429.

Robinson, G., Wright, S., Elwin, K., Hadfield, S.J., Katzer, F., Bartley, P.M., et al., 2010. Re-description of *Cryptosporidium cuniculus* Inman and Takeuchi, 1979 (Apicomplexa: Cryptosporidiidae): morphology, biology and phylogeny. Int. J. Parasitol. 40, 1539–1548.

Ryan, U.M., Power, M.L., 2012. *Cryptosporidium* species in Australian wildlife and domestic animals. Parasitology 139, 1673–1688.

Ryan, U.M., Power, M.L., Xiao, L., 2008. Cryptosporidium fayeri *n. sp. (*Apicomplexa: Cryptosporidiidae*) from the red kangaroo (*Macropus rufus*).* J. Eukaryot. Microbiol. 55, 22–26.

Ryan, U.M., Fayer, R., Xiao, L., 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology 141 (13), 1667–1685.

Short, J., 2009. The characteristics and success of vertebrate translocations within Australia. In: The Australian Animal Welfare Strategy. Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT.

Spencer, P.B., Odorico, D.M., Jones, S.J., Marsh, H.D., Miller, D.J., 1995. Highly variable microsatellites in isolated colonies of the rock-wallaby (*Petrogale assimilis*). Mol. Ecol. 4, 523–525.

Stensvold, C.R., Beser, J., Axen, C., Lebbad, M., 2014. High applicability of a novel method for gp60-based subtyping of *Cryptosporidium meleagridis*. J. Clin. Microbiol. 52, 2311–2319.

Sulaiman, I.M., Lal, A.A., Xiao, L., 2002. Molecular phylogeny and evolutionary relationships of *Cryptosporidium* parasites at the actin locus. J. Parasitol. 88, 388–394.

Tanriverdi, S., Arslan, M.O., Akiyoshi, D.E., Tzipori, S., Widmer, G., 2003. Identification of genotypically mixed *Cryptosporidium parvum* populations in humans and calves. Mol. Biochem. Parasitol. 130 (1), 13–22.

Waldron, L.S., Ferrari, B.C., Power, M.L., 2009. Glycoprotein 60 diversity in C. hominis and C. parvum causing human cryptosporidiosis in NSW, Australia. Exp. Parasitol. 122, 124–127.

Waldron, L.S., Cnheung-Kwok-Sang, C., Power, M.L., 2010. Wildlife-associated *Cryptosporidium fayeri* in human, Australia. Emerg. Infect. Dis. 16, 2006–2007.

Waldron, L.S., Dimeski, B., Beggs, P.J., Ferrari, B.C., Power, M.L., 2011. Molecular epidemiology, spatiotemporal analysis, and ecology of sporadic human cryptosporidiosis in Australia. Appl. Environ. Microbiol. 77, 7757–7765.

Warren, K.S., Swan, R.A., Morgan-Ryan, U.M., Friend, J.A., Elliot, A., 2003. Cryptosporidium muris *infection in bilbies (*Macrotis lagotis*).* Aust. Vet. J. 81, 739–741.

Wultsch, C., Waits, L.P., Kelly, M.J., 2014. Noninvasive individual and species identification of jaguars (*Panthera onca*), pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*) in Belize, Central America using cross-species microsatellites and faecal DNA. Mol. Ecol. Resour. 14, 1171–1182.

Xiao, L., 2002. Identification of the *Cryptosporidium* pig genotype in a human patient. J. Infect. Dis. 185, 1846–1848.

Xiao, L., Bern, C., Limor, J., Sulaiman, I., Roberts, J., Lal, A.A., et al., 2001. Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru. J. Infect. Dis. 183, 492.

Xiao, L., Fayer, R., Ryan, U., Upton, S.J., 2004. *Cryptosporidium* taxonomy: recent advances and implications for public health. Clin. Microbiol. Rev. 17, 72–97.

Xiao, L.H., Escalante, L., Yang, C.F., Sulaiman, I., Escalante, A.A., Montali, R.J., et al., 1999. Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. Appl. Environ. Microbiol. 65, 1578–1583.

Yang, R., Fenwick, S., Potter, A., Ng, J., Ryan, U., 2011. Identification of novel *Cryptosporidium* genotypes in kangaroos from Western Australia. Vet. Parasitol. 179, 22–27.