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PII: S0304-4017(10)00093-2
DOI: doi:10.1016/j.vetpar.2010.02.012
Reference: VETPAR 5204

To appear in: *Veterinary Parasitology*

Received date: 16-10-2009
Revised date: 3-2-2010
Accepted date: 10-2-2010

Please cite this article as: Thompson, R.C.A., Smith, A., Lymbery, A.J., Averis, S., Morris, K.D., Wayne, A.F., *Giardia* in Western Australian wildlife, *Veterinary Parasitology* (2008), doi:10.1016/j.vetpar.2010.02.012

Giardia in Western Australian wildlife

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Running title: *Giardia* in Western Australian wildlife

Abstract

Giardia has been found in numerous species of mammalian wildlife but very little information is available on the species and strains/genotypes that occur naturally in mammals in the wild. Recently, a novel genotype of *Giardia* was described in Western Australia, in the southern brown bandicoot, or quenda (*Isoodon obesulus)*. In order to determine the host range, distribution and prevalence of this novel 'quenda' genotype of *Giardia*, a comprehensive survey of this marsupial and cohabiting mammalian species was undertaken throughout the mainland and some off-shore islands of Western Australia, including urban areas. The overall prevalence of *Giardia* in 351 wildlife samples was low, with only 4.8% (17) samples testing positive. Amongst the 51 quenda samples, 11.8% (6) were positive for the 'quenda' genotype, 5.9% (3) for assemblage C/D and 2% (1) for assemblages A and E. This study has demonstrated that *Giardia* is a remarkably rare parasite in native wildlife in Western Australia, raising questions about the ecology of *Giardia* infections in wildlife.

1. Introduction

Giardia has been found in numerous species of mammalian wildlife but very little information is available on the species and strains/genotypes that occur naturally in mammals in the wild (Appelbee *et al*. 2005; Kutz *et al*. 2009). In the majority of cases, where appropriate tools for parasite characterisation have been applied, the type of *Giardia* found in free-ranging terrestrial and aquatic mammals has usually been suggestive of human origin, i.e. zoonotic genotypes/assemblages of *G. duodenalis*

(Thompson, *et al.*, 2009). This has been demonstrated in presumed pristine and/or isolated environments involving wildlife species such as beavers in North America, primates in Africa, muskoxen in the Arctic, house mice on remote islands and marine cetaceans in various parts of the world (Graczyk *et al*., 2002; Sulaiman *et al*., 2003; Kutz *et al*., 2008; Moro *et al*., 2003; Appelbee *et al.* 2005; Dixon *et al.*, 2008; Teichroeb *et al.* 2009). In all these cases, epidemiological evidence supports humans as the likely source of infection through environmental contamination, either directly or indirectly via domestic animal hosts.

G. muris, *G. simondi* and *G. microti*, which are all genetically distinct, have been described from mice, rats and microtine rodents respectively (rev. in Thompson and Monis, 2004), but there is no information on host range, prevalence of infections and geographical distribution. In all cases the hosts of these species of *Giardia* are also susceptible to zoonotic genotypes. More recently, a novel genotype of *Giardia* was described in an Australian marsupial, a bandicoot known as the quenda (*Isoodon obesulus)*, and on the basis of genetic characteristics would appear to represent a distinct species that may be endemic within Australian native fauna (Adams *et al*., 2004).

To determine the host range, distribution and prevalence of the novel 'quenda' genotype of *Giardia*, we have undertaken a comprehensive survey of *I. obsellus* and cohabiting mammalian species throughout the mainland and some off-shore islands of Western Australia, including urban areas.

2. Materials and methods

2.1 Sample collection and DNA extraction

Faecal samples were obtained from 21 native wildlife species, including 51 quenda, from nine geographic locations within Western Australia, resulting in an overall total of 351 faecal samples (see Table 1 and Figure 1). Faecal samples were divided into two equal parts, with half stored in 10% formalin solution for general endoparasite microscopy and half in 70% ethanol for PCR based investigation. Genomic DNA was extracted using the Maxwell® 16 Tissue DNA Purification Kit (Promega, Madison, USA) according to the manufacturer's instructions. Wildlife sampling was carried out under Murdoch University Animal Ethics Approvals NS1182-06 and W2172-08. All animals were live trapped using either Sheffield cage traps or Elliot traps baited with a mixture of peanut butter, rolled oats and sardines, apart from four of the 'urban' animals being cared for at the Fauna Rehabilitation Foundation.

Presence of *Giardia* was tested both by the presence of cysts via microscopy following zinc sulphate concentration and by amplification of the β-giardin and 18SrRNA genes, as described below. 18S rRNA sequences were used to confirm the identity of the 'quenda' genotype, by comparison with the sequence described by Adams *et al.* (2004). Prevalences were expressed as percentage of samples testing positive by either microscopy or DNA amplification, with 95% confidence intervals calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rózsa *et al*., 2000).

2.2 Amplification of β-giardin gene by PCR

Two microlitres of DNA extract was used to amplify the *Giardia β*-giardin gene by nested PCR. In the first round, amplification was carried out using the forward primer G7 (5'-AAGCCCGACGACCTCACCCGCAGTGC-3') and the reverse primer G759 (5'GAGGCCGCCCTGGATCTTCGAGACGAC-3') (Caccio *et al.* 2002). The second round was carried out using the forward primer βF (5'-GAACGAACGAGATCGAGGTCCG-3') and the reverse primer βR (5'-CTCGACGAGCTTCGTGTT-3') described by Lalle *et* al. (2005). In the first round, each 25 µl reaction also contained 2.5 µl of 1x buffer 67 mM Tris-HCl (pH 8.8 at 25^{o}C), 16.6 mM $(NH_4)_2SO_4$, 4.5% Triton X-100 and 2 mg/ml gelatin; 200 µM dNTPs (Promega, Madison, USA); 0.75 U TTh Plus DNA polymerase (Fisher Biotec, Perth, Australia); 2 mM $MgCl_2$ and 10 pmol/µL of each primer. Cycling conditions were: initial denaturation at 95°C for 300 s followed by 35 cycles of 95ºC 30 s; 50ºC 30 s; 72ºC 60 s; final extension at 72°C 420 s. One microlitre of the product from each reaction was then used as template for the second-round PCR under the following conditions: initial denaturation at 95°C for 300 s followed by 35 cycles of 95ºC 30 s; 55ºC 30 s; 72ºC 60 s and final extension at 72°C 420 s. Amplified products were separated on a 1% agarose gel and run at 90 V for 1 hour in Tris-acetate EDTA buffer with SYBR Safe (Invitrogen, Carlsbad, USA) and visualized under UV light. Samples producing a band of approximately 500 bp were considered positive.

2.3 Amplification of 18S rRNA gene

Samples were also screened at the 18S rRNA locus by nested PCR. Two microlitres of the DNA solution were used as template. The 25 µL reactions were identical to the ones described for amplification of the *β*-giardin gene with the addition of 0.5% DMSO (dimethyl sulfoxide). Primers RH11 and RH4 as previously described (Hopkins *et al*., 1997) were used in the primary PCR and primer pair GiarF and GiarR described by Read (2002) for the secondary PCR. The amplification conditions were as follows: an initial step of 96 °C for 5 minutes, followed by 40 cycles of 96 °C for 30 s, 55 °C for 45 s and 72 °C for 45 s, with a final extension at 72 °C for 420 s. One microlitre of the PCR product from the first amplification was used as template for the secondary PCR. The conditions of the nested PCR were the same as for the primary PCR with the exception of the annealing temperature at 53 °C. Genotype E was used as PCR positive control. The samples that were positive with the β-giardin amplification and not with 18S were spiked with positive control to ensure that negative results were not due to PCR inhibition.

2.4 DNA sequencing

Positive samples were typed into assemblages by sequencing PCR products of 130bp for all 18S positive samples and 513bp (Barrow Island quenda), 487 bp (Barrow Island planigale), 487bp (Fitzgerald River Ash-grey mouse) and 494bp (Fitzgerald River bush rat) for *β*-giardin positive samples. PCR products were purified using the Wizard SV PCR and gel purification kit (Promega, Madison, USA), and sequenced using the ABI Prism BigDye™ Terminator Cycle Sequencing kit (Applied Biosystems). Sequences were assembled using the program Sequencher. Multiple alignments of the nucleotide sequences were performed using ClustalW (Higgins *et al*., 1994).

3. Results

The overall prevalence of *Giardia* in wildlife samples was low, with only 17 of 351 samples (4.8%, CI 2.9-7.6%) testing positive. Thirteen samples tested positive by microscopy and 14 by PCR (Table 1). Although there was general concordance between the two methods, *Giardia* cysts were detected by microscopy in three samples that did not return positive PCR results with either locus, while no cysts were detected in four samples that tested positive using PCR.

Amongst the 51 bandicoot samples, 13 (25.5%, 15.2-39.1%) tested positive for *Giardia*. Six (11.8%, 5.2-23.4%) were positive for the 'quenda' genotype, three (5.9%, 1.6-16.4%) for assemblage C/D, two (3.9%, 0.7-13.4%) for assemblage A and one (2.0 %, 0.1-10.4%) for assemblage E (Table 1). Of the two cases of infection with assemblage A, it was possible to determine sub-genotype in one of these, as A1 in the common planigale. Infection rates were greater among quenda from the Perth metropolitan area than from other areas of the state; of 15 animals sampled from Perth, 60.0% (33.2-80.9%) were positive for *Giardia*, with prevalences of 33.3% (14.2-60.3%) for the 'quenda' genotype 20.0% (5.7-53.6%) for assemblage C and 6.7% (0.4-30.2%) for assemblage A.

4. Discussion

This study has demonstrated that *Giardia* is a remarkably rare parasite in native wildlife in Western Australia. A large number of native animals of 21 species from 9 geographical locations were screened but only 4.8% of 351 samples were found to be infected. This is

in contrast to earlier studies in Tasmania which using a combination of microscopy and an ELISA faecal antigen detection test developed for human use, reported an overall prevalence of 21% in a range of wildlife species, including bandicoots, of which 62% were infected (Bettiol *et al*. 1997). Unfortunately, molecular characterisation was not undertaken on the samples from Tasmanian wildlife.

Our aims were not only to determine the prevalence of *Giardia* in Western Australian wildlife, which appears to be very low, but also to better understand the distribution and host range of the previously described 'quenda' genotype of *Giardia*. We have confirmed that this genotype appears to be host specific; it was found in 11.8% of quenda that were sampled, but in none of the 20 other native mammal species. It is unlikely that infections were not detected since all faecal samples whether microscopically positive or negative, were screened by PCR. Further, when *Giardia* was detected by microscopy, infections were moderate to heavy based on a qualitative microscopy score. Since *Giardia* is an enteric parasite that is spread through environmental contamination with subsequent ingestion of infective cysts, the present results raise questions about how the quenda genotype is maintained in nature. It is possible that this could be achieved through direct contact from one infected animal to another via cysts adherent to the fur. However, the ground foraging habit of quenda within the topsoil and leaf litter also increase their susceptibility to ingesting contaminated substrate. They are not a communal species and while territories may overlap they are typically solitary except when breeding. Therefore, close and sustained direct contact is otherwise unlikely.

184 This study has also demonstrated that other species of native mammals apart from the
185 quenda, at least in Western Australia, do not harbour distinct strains of *Giardia*. *G.*
186 *duodenalis* was the only other species detected in this study, in 5 bandicoots, 1 planigale,
187 2 bush rats and 1 ash grey mouse, which were infected with assemblages C/D, A, F/C and
188 E (Table 1). All these infected animals were located in environments where domestic
189 hosts, including humans, encroach onto wildlife habitats or vice versa. Even Fitzgerald
190 River, which is one of the most pristine environments sampled, is surrounded on three
191 sides by agriculture and, although the area sampled is officially closed to the public, is
192 visited by campers and presumably incursions by domestic or feral cats and dogs are
193 common. Consequently, humans or domestic animals could have been the source of
194 environmental contamination from which wildlife contracted infection. This also raises
195 the question of whether the finding of *G. duodenalis* assemblages in wildlife in the
196 present study represent 'real' infections or are indicative of environmentally acquired
197 cysts that have not encysted and are passing through the intestine. This question can also
198 be applied to reports of the occurrence of domestic assemblages of *Giardia* in other
199 terrestrial and aquatic mammalian wildlife (Graczyk *et al*., 2002; Moro *et al*., 2003;
200 Sulaiman *et al*., 2003; Appelbee *et al.* 2005; Kutz *et al*., 2008). However, in beavers,
201 epidemiological evidence indicates that at least in certain cases, zoonotic assemblages of
202 *Giardia* can be amplified in wildlife.
203
204 Of 51 quenda examined in the present study, the majority infected with *Giardia* were
205 found on the outskirts, or in, the urban area of the city of Perth. Although accidental
206 infection of urban quenda with 'domestic' strains of *G. duodenalis* is understandable in

urban areas, it is interesting that the presumed host specific 'quenda' genotype is also more common in urban areas than in the wild. The absence of the novel *quenda* genotype in the broad range of native animals considered here from both urban and non-urban environments, the most comprehensive survey of Western Australian wildlife we are aware of, both confirms its presence and supports the suggestion of Adams *et al*. (2004) that it is bandicoot specific.

The results support the need for similar studies in other mammals such as the beaver in 'pristine' environments to determine whether they also harbour their own 'host adapted' forms of *Giardia*.

Acknowledgements

This work was funded by the Australian Research Council and the Western Australian Department of Environment and Conservation under ARC-Linkage Project ID: LP0775356.

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Table 1

Giardia in native mammalian wildlife from Western Australia

Location	Species	Number tested	Microscopy positive	PCR positive & genotype
Urban (Perth)	Quenda (*Isoodon obesulus*)	14	9	4 x *quenda* 1 x *quenda* 1 x A 3 x C
Lake Magenta	Brush-tailed possum (*Trichosurus vulpecula*)	7		
Lake Magenta	Red tailed phascogale (*Phascogale calura*)	1		
Lake Magenta	Heath mouse (*Pseudomys shortridgei*)	4		
Lake Magenta	Western mouse (*Pseudomys occidentalis*)	2		
Upper Warren	Chuditch (*Dasyurus geoffroii*)	21		
Upper Warren	Brush-tailed possum (*Trichosurus vulpecula*)	101		
Upper Warren	Quenda (*Isoodon obesulus*)	9	2	1 x *quenda*
Upper Warren	Woylie (Bettongia penicillata)	83		
Upper Warren	Numbat (Myrmecobius fasciatus)	1		
Fitzgerald River	Western chestnut mouse (*Pseudomys nanus*)	3		
Fitzgerald River	Chuditch (*Dasyurus geoffroii*)	1		
Fitzgerald River	Dibbler *(Parantechinus apicalis*)	1		
Fitzgerald River	Ash-grey mouse (*Pseudomys albocinereus*)	2		1 x E
Fitzgerald River	Bush rat (*Rattus fuscipes*)	12		1 x F+C
Lorna Glen	Spinifex hopping mouse (*Notomys alexis*)	1		
Lorna Glen	Bilby (*Macrotis lagotis*)	3		
Lorna Glen	Brush-tailed possum (*Trichosurus vulpecula*)	4		
Faure Island	Western barred bandicoot (*Perameles bougainville*)	10		
Faure Island	Banded-hare wallaby (*Lagostrophus fasciatus*)	6		
Barrow Is	Brush-tailed possum (*Trichosurus vulpecula*)	17		
Barrow Is	Boodie (*Bettongia lesueur*)	3		
Barrow Is	Common planigale (*Planigale maculata*)	5		1 x A1
Barrow Is	Western chestnut mouse (*Pseudomys nanus*)	3		
Barrow Is	Quenda (*Isoodon obesulus*)	28		1 x E
Barrow Is	Spectacled-hare wallaby (*Lagorchestes conspicullatus*)	2		
Barrow Is	Water rat (*Hydromys chrysogaster*)	1		
Barrow Is	*Pseudantechinus* sp.	2		
Julimar	Quenda (*Isoodon obesulus*)	4	2	

GenBank accession numbers of novel *β*-giardin sequences: GU574799 *Isoodon obesulus*; GU574800 *Planigale maculata*; GU574801 *Pseudomys albocinereus*; GU574802 *Rattus fuscipes*

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Lake Magenta	Red tailed phascogale (*Phascogale calura*)	1		
Lake Magenta	Heath mouse (*Pseudomys shortridgei*)	4		
Lake Magenta	Western mouse (*Pseudomys occidentalis*)	2		
Upper Warren	Chuditch (*Dasyurus geoffroii*)	21		
Upper Warren	Brush-tailed possum (*Trichosurus vulpecula*)	101		
Upper Warren	Quenda (*Isoodon obesulus*)	9	2	1 x *quenda*
Upper Warren	Woylie (Bettongia penicillata)	83		
Upper Warren	Numbat (Myrmecobius fasciatus)	1		
Fitzgerald River	Western chestnut mouse (*Pseudomys nanus*)	3		
Fitzgerald River	Chuditch (*Dasyurus geoffroii*)	1		
Fitzgerald River	Dibbler *(Parantechinus apicalis*)	1		
Fitzgerald River	Ash-grey mouse (*Pseudomys albocinereus*)	2		1 x E
Fitzgerald River	Bush rat (*Rattus fuscipes*)	12		1 x F+C
Lorna Glen	Spinifex hopping mouse (*Notomys alexis*)	1		
Lorna Glen	Bilby (*Macrotis lagotis*)	3		
Lorna Glen	Brush-tailed possum (*Trichosurus vulpecula*)	4		
Faure Island	Western barred bandicoot (*Perameles bougainville*)	10		
Faure Island	Banded-hare wallaby (*Lagostrophus fasciatus*)	6		
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