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Identification of zoonotic Cryptosporidium and Giardia genotypes infecting animals in Sydney’s Water Catchments.

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Abstract

To identify the animal sources for Cryptosporidium and Giardia contamination, we genotyped Cryptosporidium and Giardia spp. in wildlife from Sydney’s water catchments using sequence analysis at the 18S rRNA locus for Cryptosporidium and 18S rRNA and glutamate dehydrogenase (gdh) for Giardia. A total of 564 fecal samples from sixteen different host species were analysed. Cryptosporidium was identified in 8.5% (48/564) samples from eight host species and Giardia was identified in 13.8% (78/564) from seven host species. Eight species/genotypes of Cryptosporidium were identified. Five G. duodenalis assemblages were detected including the zoonotic assemblages A and B.

Keywords: Cryptosporidium; Giardia; catchment; zoonotic genotypes.
1. Introduction

*Cryptosporidium* and *Giardia* are the most common parasitic cause of diarrhea in humans and animals worldwide (Xiao and Fayer, 2008). These organisms infect the gastrointestinal epithelium to produce a diarrhea that is self limited in immunocompetent persons but chronic and potentially life threatening in immunocompromised patients and malnourished children (Xiao and Feng, 2008).

Currently 22 different species of *Cryptosporidium* and over 40 genotypes are recognised, with new genotypes continually being identified (cf. Xiao and Fayer, 2008; Xiao, 2010). Of these, *C. hominis* and *C. parvum* are responsible for most human infections.

There are 6 recognised species of *Giardia* with *Giardia duodenalis* being the species that infects humans. There are several cryptic species within *G. duodenalis* including assemblage A and B that have been reported to occur in humans and animals; assemblage C and D in dogs, assemblage E in cattle, sheep and pigs, assemblage F in cats and assemblage G in rats (Caccio and Ryan, 2008).

While the most common mode of transmission of *Cryptosporidium* and *Giardia* in humans is via person-to-person contact, outbreaks have occurred due to the contamination of drinking water sources. The Sydney Catchment Authority (SCA) uses a risk management approach to protect the quality of Sydney’s drinking water (Cox et al., 2003). As part of this strategy, risk assessment of the drinking water supply in relation to *Cryptosporidium* and *Giardia* is based firstly on identifying sources of *Cryptosporidium/Giardia* and the types of *Cryptosporidium/Giardia* that may be contributed from each source. Management priorities can then focus on identified sites, especially those that input oocysts that
may be infectious to humans. The aim of the present study was to use molecular tools to better characterise species/genotypes of Cryptosporidium and Giardia in Sydney’s catchments and to indicate likely potential sources of water contamination.

2. Materials and methods

2.1 Sampling.

Sydney’s drinking water catchment areas supply water to nearly 4 million people living in Sydney, Wollongong and the lower Blue Mountains. The outer catchment contains high numbers of both stock and native animals, while the native animals dominate the inner catchment lands. Feral animals are also found across the catchment. A total of 564 fecal samples from a range of different animals (Table 1) from the inner and outer catchment areas were collected opportunistically over a period of 3 years by SCA staff, researchers engaged on collaborative SCA projects and members of the Game Council of NSW. Samplers were experienced and requested to note the animal of origin of the fecal sample. Samplers were also requested to avoid collecting multiple samples from the one area to avoid duplicating samples from the same animal.

2.2 DNA isolation

Genomic DNA was extracted from 200mg of each faecal sample using a QIAamp DNA Mini Stool Kit (Qiagen, Hilden, Germany) or from 250mg of each
faecal sample using a Power Soil DNA Kit (MolBio, Carlsbad, California). A negative
control (no faecal sample) was used in each extraction group.

2.3 PCR amplification

All samples were screened at the 18S rRNA locus for both parasites and
positives were genotyped by sequencing. A two-step nested PCR protocol was used to
amplify the 18S rDNA gene of Cryptosporidium as previously described (Ryan et al.,
2003). All 18S positives were amplified at the actin gene as previously described
(Sulaiman et al., 2002). Attempts were also made to amplify C. parvum/C. hominis
positives at the heat shock protein (hsp70), acetyl Co A and gp60 loci as previously
described respectively (Morgan et al., 2001; Foo et al., 2007 and Strong et al., 2000).
Amplification of a fragment of the Giardia 18S rRNA gene was performed as
described by Hopkins et al., (1997) and Read et al., (2002). A subset of 18S positive
samples (n = 45) were also analysed at the Giardia glutamate dehydrogenase (gdh)
locus. A 530 bp fragment of the gdh gene was obtained using primers designed as part
of the present study: Gdh1 (5\textquotesingle-TTCCGTRTYCAGTACAACTC-3\textquotesingle) and Gdh2 (5\textquotesingle-
ACCTCGTTCTGRGTCGGCAGCA-3\textquotesingle) for the primary amplification, and Gdh3 (5\textquotesingle-
TGACYGAGCTYACAGAGGCACGT-3\textquotesingle) and Gdh4 (5\textquotesingle-
GTGGCGCARGGCATGATGCA-3\textquotesingle) for the nested amplification. The conditions for
the primary and secondary amplification of the gdh gene fragment were identical and
consisted of 35 cycles (94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min) in a T-
personal thermocycler (Whatman-Biometra, Goettingen, Germany), with an initial hot
start at 94 °C for 2 min and a final extension at 72 °C for 7 min. Reactions were
visualized using 1% agarose gels stained with SYBR Safe (Invitrogen). Primer
specificity was confirmed by BLAST searches in GenBank and amplification testing
on human, bacterial and Cryptosporidium DNA.

PCR contamination controls were used including negative controls and
separation of preparation and amplification areas. The amplified DNA fragments from
the secondary PCR product were separated by gel electrophoresis and purified using
the freeze-squeeze method (Ng et al., 2006). A spike analysis (addition of 0.5 IL of
positive control DNA into each sample) was conducted on randomly selected negative
samples from each group of DNA extractions to determine if negative results were
due to PCR inhibition.

2.4 Sequence and phylogenetic analysis

Purified PCR products were sequenced using an ABI Prism™ Dye Terminator
Cycle Sequencing kit (Applied Biosystems, Foster City, California) according to the
manufacturer’s instructions with the exception that the annealing temperature was
raised to 58 °C. The results of the sequencing reactions were analysed and edited
using Chromas lite version 2.0 (http://www.technelysium.com.au), compared to
existing Cryptosporidium 18S rDNA sequences in GenBank using BLAST searches
and aligned with reference genotypes from GenBank using Clustal W
(http://www.clustalw.genome.jp).

Phylogenetic trees were constructed for Cryptosporidium at the 18S locus and
Giardia at the gdh locus with additional isolates from GenBank. Distance estimation
was conducted using TREECON (Van de Peer and De Wachter, 1994), based on
evolutionary distances calculated with the Tamura-Nei model and grouped using
Neighbour-Joining. Parsimony analyses were conducted using MEGA version 3.1
(MEGA3.1: Molecular Evolutionary Genetics Analysis software, Arizona State University, Tempe, Arizona, USA). Bootstrap analyses were conducted using 1,000 replicates to assess the reliability of inferred tree topologies. Maximum Likelihood (ML) analyses were conducted using the program PhyML (Dereeper et al., 2008) and the reliability of the inferred trees was assessed by the approximate likelihood ratio test (aLRT) (Anisimova and Gascuel, 2006).

3. Results

3.1 Cryptosporidium

Cryptosporidium was detected in 8.5% (48/564) (6.2-10.8 % Confidence Interval (CI)) of samples based on the 18S rRNA locus (Table 1). The 48 positive isolates were detected in eight different host species (Table 1). The prevalence of Cryptosporidium in cattle was 4.3% (3/70). Two of the positives were identified as C. bovis, and the third positive was C. hominis (isolate SCA352). Only 1 deer (isolate SCA333) was positive out of 137 isolates screened (0.7%) and the target 18S rRNA sequence was identical to Cryptosporidium environmental sequence isolate 8059 (GenBank accession no. AY737603) from water previously identified in New York storm water in the US and grouped as W9 (Jiang et al., 2005). The prevalence of Cryptosporidium in dingos/wild dogs was 22.7% (10/44). Nine of the positives were identified as C. canis (1 SNP difference) and one isolate (SCA318) was C. hominis and exhibited 1 single nucleotide polymorphism (SNP) from C. hominis. Two of nineteen foxes (10.5%) were positive for Cryptosporidium. One isolate (SCA285) was identified as C. canis and one isolate (SCA291) has one SNP from C. macropodum.
The prevalence of Cryptosporidium in kangaroos was 16.9% (27/160). Of the 27 positives identified, two were *C. macro podium*, six isolates were *C. parvum* (isolates SCA3, SCA4, SCA 5, SCA8, SCA14, SCA16) and 18 were *C. hominis* (SCA1, SCA2, SCA6, SCA7, SCA10-SCA13, SCA15, SCA17-SCA19, SCA22-SCA24, SCA31, SCA39, SCA40). The 18S positive isolates for *C. parvum* and *C. hominis* were only able to be confirmed at one of the five loci tested, as the four other loci were not able to be amplified in the samples. Of the six *C. parvum* positives, all were identical to reference *C. parvum* sequences, with the exception of SCA5, which had 1 SNP to GenBank sequence EF375893 from an alpaca (Twomey et al., 2008). Of the 18 *C. hominis* positives, 6 were 100% identical to reference *C. hominis* sequences, 10 had 8 T’s in the hypervariable region compared to the more usual 11 T’s, isolate SCA6, in addition to having 8 Ts, also had 1 SNP compared to a human isolate (GenBank accession no DQ388386-Wielinga et al., 2008) and isolate SCA40 had 1 SNP. The three positives from pigs (3/27-11.1% prevalence) were all identified as pig genotype II and the one positive from sheep was *C. xiaoi*. One (isolate SCA350) of seventeen from wallabies (5.9% prevalence) was identified as *C. parvum* with 4 SNPs.

PCR and sequence analysis at the actin locus was only successful for five of the forty-eight 18S positives isolates despite numerous attempts; SCA271, SCA90, SCA310 from dogs and SCA285 from a fox, all of which were confirmed as *C. canis* and SCA241 from a cow which was confirmed as *C. bovis*.

Distance-based, parsimony and ML analysis (data not shown) of Cryptosporidium 18S rRNA sequences and a range of Cryptosporidium species and genotypes obtained from GenBank produced similar results and confirmed the results of the GenBank blast searches.
3.2 Giardia

Giardia was detected in 13.8% (78/564) (11.0-16.7 % CI) of samples at the 18S rRNA locus (Table 1). 18S rRNA sequences were successfully obtained for all 78 positives. The prevalence of Giardia in cattle was 14.3% (10/70). Nine were assemblage E and one was a mixed B and E infection. In deer, the prevalence of Giardia was 21.2% (29/137). Eighteen were assemblage A, one was assemblage B, one was a mixed A and B infection, seven were assemblage E and 2 were a mixed assemblage A and E. In dingos/wild dogs, the prevalence was 29.5% (13/44). Three were assemblage A, five were assemblage C, two were assemblage E, three were a mixed A and E infection. In foxes, the prevalence was 31.6% (6/19). One was assemblage D, one was E, one was a mixed A and D and three were a mixed A and E infection. One assemblage C infection was identified in a goat and one assemblage A infection was identified in a horse. In kangaroos, the prevalence was 11.3% (18/160). Eight were assemblage A, two were assemblage B, four were assemblage C and four were assemblage D.

At the more informative gdh locus, a total of 45 of the 78 18S positives were successfully amplified and sequenced but only 40 were included in phylogenetic analysis as full-length sequences could not be obtained for those five. There was ~86% agreement between the 18S and gdh loci across the 45 gdh sequences successfully sequenced. Four isolates (SCA187, SCA387, SCA 459, SCA486) were typed as assemblage E at the 18S locus but were assemblage A at the gdh locus. One isolate (SCA475) was assemblage A at the 18S locus but assemblage E at the gdh locus and one isolate (SCA291) was a mixed assemblage A and E at the 18S locus but
assemblage B at the \textit{gdh} locus. Mixed infections have been identified in 11 isolates at
the 18S locus.

Phylogenetic analyses of the partial nucleotide sequence of the \textit{gdh} locus
using Distance, Parsimony and ML analyses produced similar results (Fig.1 NJ tree
shown). At the \textit{gdh} locus, isolates SCA293, SCA63 and SCA87 and from two Eastern
grey kangaroos and a dog respectively grouped with assemblage C but exhibited
considerable variation from the reference assemblage C isolate U60982 (isolate Ad-
136 - dog-derived sample grown in suckling mice) with up to 30 SNPs (Table 2).
However, all these substitutions were silent as the sequences were identical at the
protein level. This is the first report of assemblage C in cattle and marsupials.
Similarly isolates SCA66, SCA140 from kangaroos and isolate SCA278 from a fox
grouped with assemblage D but exhibited considerable variation from the reference
assemblage D isolate (U60986-isolate Ad-148- dog-derived sample grown in suckling
mice) with up to 25 SNPs (Table 3). As with the assemblage C isolates, these
substitutions were all silent. This is the first report of assemblage D in kangaroos and
foxes.

Isolate SCA152 from a kangaroo was 100\% identical to BIV reference isolate
AD-45 (AY178739) from a human. Isolates SCA107 and SCA338 from a kangaroo
and a deer respectively had between 2 and 7 SNPs from BAH12c14 (EF685683) a
reference BIII isolate. Nineteen isolates were typed as assemblage AI variants at the
\textit{gdh} locus; fourteen isolates had 3 SNPs from Portland, a reference AI isolate
(EF685701) and five isolates exhibited 12 SNPs from Portland.
4. Discussion

In the present study, the overall prevalence of Cryptosporidium and Giardia in a range of animals in the SCA catchment was 8.5% and 13.8% respectively. The highest Cryptosporidium prevalence was recorded in dogs (23.8%). The highest Giardia prevalence was recorded in foxes (31.6%), followed by dogs (28.6%). A previous study which examined the prevalence of Cryptosporidium and Giardia in four watersheds within the SCA catchment, reported a prevalence of 23.9% (33/138) for Cryptosporidium from ten different host species and 30% (40/133) for Giardia from nine hosts using immunomagnetic purification following by microscopy (Cox et al., 2005). The lower prevalence reported in the present study may have been due to the fact that (oo)cysts were not purified and concentrated from faecal samples, rather DNA was extracted directly from faecal samples. The present study was also carried out during a prolonged drought in the catchment. This may have led to there being fewer animals, particularly young animals, in the catchment during this period thus reducing the potential for transmission. Previous studies worldwide that have examined the prevalence of Cryptosporidium in wildlife in catchments have reported overall prevalences of 7.0% to 23.5% (Feng et al., 2007; Ziegler et al., 2007; Jellison et al., 2009).

In the present study, zoonotic Cryptosporidium species were identified in four hosts based on 18S rRNA sequences: cattle, dogs, kangaroos and a wallaby. Cryptosporidium hominis was identified in one cow, one dog and 18 kangaroo isolates. Cryptosporidium parvum/parvum-like was identified in six kangaroos and 1 wallaby. Cryptosporidium hominis has previously been reported in a goat and sheep in the UK (Giles et al., 2009). Cryptosporidium hominis has not been reported
previously in cattle or dogs but *C. parvum* has been reported in dogs in Italy (Giangaspero et al., 2006).

This is also the first report of *C. hominis* and *C. parvum* in kangaroos and wallabies. Of the twenty-seven *Cryptosporidium* positives in kangaroos in this study, twenty-five (>92%) were zoonotic. Earlier studies of kangaroos in the Sydney catchment area identified only *C. fayeri* and *C. macropodum* (Power et al., 2004). A recent study by Hill et al., (2008) examined the prevalence of *Cryptosporidium* in the common brush-tail possum (*Trichosurus vulpecula*) adapted to urban settings compared to possums inhabiting remote woodlands. The authors reported that while both populations were shedding a genetically distinct “possum” genotype, the urban-adapted population was also shedding an additional five isolates that were *C. parvum/hominis*-like (Hill et al., 2008). Whether the kangaroos in the present study were actually infected or were simply passively transmitting the oocysts is unknown. We were unable to amplify these isolates at other loci and therefore the definitive identification of *C. hominis* and *C. parvum* in kangaroos and wallabies requires confirmation by further studies. The lack of amplification at other loci is unlikely to be due to PCR inhibition spike analysis indicated that this was not an issue but may be due to low levels of *Cryptosporidium* present in the samples and the fact that the 18S locus is multi-copy whereas the other loci are single copy.

At the combined 18S and *gdh* loci zoonotic *G. duodenalis* Assemblages A and B were identified in cattle (A and B), deer (A, and B), dogs (A), a dingo (A), foxes (A), a horse (A) and kangaroos (A and B). Assemblage A is commonly reported in cattle and assemblage B has been reported in cattle in Canada and Portugal (Coklin et al., 2007; Mendonca et al., 2007). Assemblage A and B have been reported in dogs and foxes in a number of studies (cf. Caccio and Ryan, 2008). Assemblage A and B
have been reported in horses (Traub et al., 2005). Previous studies have also reported
assemblages A and B in marsupials (Thompson et al., 2008). In that study, *Giardia*
sp. (A and B) were identified in 13.4% (28/209) of samples from captive marsupials
and 13.7% (29/212) of samples from wild marsupials (Thompson et al., 2008).

A higher proportion of assemblage A was detected (40/78) compared to
assemblage B (5/78) in the animals screened in the present study at the 18S locus. AII
is generally found in animals, whereas genotype AII has mainly been identified in
humans. However, AII (and many other A genotypes) have also been detected in
animals (cf. Caccio and Ryan, 2008). Similarly assemblage B was thought to be
largely restricted to humans, however more recently, assemblage B has been reported
in a variety of hosts (cf. Caccio and Ryan, 2008). In the present study, no assemblage
A was identified in the cattle samples at the 18S locus although it has been reported in
previous studies of Australian cattle (McCarthy et al., 2008). At the *gdh* locus, 3 cattle
isolates, which were typed as assemblage E at the 18S locus, were typed as AII
variants. The assignment of *Giardia* isolates to different assemblages using different
markers has been found in both human and animal isolates and has been found using
different combinations of gene markers (Caccio and Ryan, 2008). There are various
explanations for ‘assemblage swapping’ such as mixed infections and/or meiotic
recombination (Caccio and Ryan, 2008). Mixed infections were identified in 11
isolates in the present study (14.1%). Mixed infections have been reported in previous
studies ranging from 2.0% to 21.0%, with higher prevalences in less economically
developed countries (Caccio and Ryan, 2008). Mixed infections are known to occur
both at the inter- and at the intra-assemblage levels (for example, A plus B, or AII plus
AII).
In previous studies, the identification of *Giardia duodenalis* assemblages C, D, and E in unusual hosts have generally been made with 18S rRNA-based tools, and generally could not be confirmed with the use of other genotyping tools (Traub et al., 2004; Feng and Xiao, 2011). In the present study, Assemblage E was detected in a dog and fox and Assemblage C and D were identified in kangaroos at the 18S locus and confirmed at *gdh* locus.

Molecular tools can provide information on the host specificity and the human-infective potential of *Cryptosporidium* and *Giardia* (oo)cysts in wildlife in drinking water catchments. Likewise, surveys of isolates from animals can expand our understanding of the types of these parasites that different animals may carry. In the present study both recognised zoonotic and non-zoonotic *Cryptosporidium* and *Giardia* species/genotypes were identified. The most significant finding was the high prevalence of zoonotic *Cryptosporidium* and *Giardia* species/genotypes in marsupials, as in Australia, marsupials are one of the dominant mammalian groups within watersheds (Power et al., 2005). This contrasts with the results of previous studies. While most of the faecal samples collected for this study were scats and not taken from the source animal directly, opening the possibility of misidentification of the host, and the *Cryptosporidium* oocysts were typed at only one locus, the results suggest that further studies are required to clarify the potential role that marsupials play in contamination of the catchment with human-infectious (oo)cysts. Catchment protection programs should be informed by an understanding of and, where necessary, controlling pathogen inputs from wildlife in addition to anthropogenic and agricultural sources. The potential for pathogen transmission and amplification between wildlife, feral animals and livestock should be understood. Attention should be directed to studying the transport of pathogens from wildlife to water and to
appropriate levels of monitoring pathogens in watersheds, even those deemed protected or pristine.

Acknowledgements

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Traub, R.J., Monis, P.T., Robertson, I., Irwin, P., Mencke, N., Thompson, R.C.A. 2004. Epidemiological and molecular evidence supports the zoonotic transmission of Giardia among humans and dogs living in the same community. Parasitology 128, 253-262

alpaca (*Lama pacos*) holdings in the South-West of England. Veterinary Journal 175, 419-422.


Figure 1. Phylogenetic relationships of *Giardia* isolates inferred by Neighbor Joining analysis of Kimura’s distances calculated from pair-wise comparisons of glutamate dehydrogenase (*gdh*) sequences. Percentage bootstrap support (>70%) from 1,000 replicate samples is indicated at the left of the supported node.
Research Highlights

- Identification of zoonotic Cryptosporidium species (*C. parvum* and or *C. hominis*) in kangaroos, a wallaby, a cow and a dog.

- First report of *C. hominis* in kangaroos.

- Identification of zoonotic Giardia in kangaroos.

- Novel Giardia assemblage A1 variants identified in cattle and deer.

- First report of Giardia assemblage D in kangaroos and foxes.

- First report of Giardia assemblage C in cattle and marsupials.

- Novel Giardia assemblage C variants identified in kangaroos and a deer.

- Novel Giardia assemblage D variants identified in kangaroos and a dog.
Table 1. Prevalence of Cryptosporidium and Giardia species in various animal hosts

at the 18S rRNA locus. Numbers in parenthesis indicate the numbers of each

genotype identified.

<table>
<thead>
<tr>
<th>Animal Host</th>
<th>n</th>
<th>Crypto +ve</th>
<th>Prevalence %</th>
<th>Species/genotype identified</th>
<th>Giardia +ve</th>
<th>Prevalence %</th>
<th>Species/genotype identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carp</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Cattle</td>
<td>70</td>
<td>3</td>
<td>4.3 (0-9.0)</td>
<td><em>C. bovis</em> (2) <em>C. hominis</em> (1)</td>
<td>10</td>
<td>14.3 (6.1- 22.5)</td>
<td>E (9) B+E (1)</td>
</tr>
<tr>
<td>Deer</td>
<td>13</td>
<td>7</td>
<td>0.7 (0-2.2)</td>
<td>W9 (1)</td>
<td>29</td>
<td>21.2 (14.2-28)</td>
<td>A (18); B (1); A+B (1) E (7); A+E (2)</td>
</tr>
<tr>
<td>Dingos/wild dogs</td>
<td>44</td>
<td>10</td>
<td>22.7 (10.3-35.1)</td>
<td><em>C. canis</em> (9) <em>C. hominis-like</em> (1)</td>
<td>13</td>
<td>29.5 (16.1-43.0)</td>
<td>A (3), C (5), E (2), A+E (3)</td>
</tr>
<tr>
<td>Foxes</td>
<td>19</td>
<td>2</td>
<td>10.5 (0-24.3)</td>
<td><em>C. canis</em> (1) <em>C. macropodum-like</em> (1)</td>
<td>6</td>
<td>31.6 (10.7-52.5)</td>
<td>D (1); A+D (1) E (1); A+E (3)</td>
</tr>
<tr>
<td>Fruit Bat</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
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<tr>
<td>Goat</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>1</td>
<td>25 (0-67.4)</td>
<td>C</td>
</tr>
<tr>
<td>Horse</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>1</td>
<td>9.1 (0-26.1)</td>
<td>A (1)</td>
</tr>
<tr>
<td>Kangaroo</td>
<td>16</td>
<td>0</td>
<td>16.9 (11.1-22.7)</td>
<td><em>C. macropodum</em> (2) <em>C. parvum</em> (6) <em>C. hominis</em> (18) <em>C. parvum-like</em> (1)</td>
<td>18</td>
<td>11.3 (6.4-16.1)</td>
<td>A (8) B (2) C (4) D (4)</td>
</tr>
<tr>
<td>Pigs</td>
<td>27</td>
<td>3</td>
<td>11.1 (0-23 CI)</td>
<td>Pig genotype II (3)</td>
<td>0</td>
<td>0</td>
<td>-</td>
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<tr>
<td>Rabbit</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>-</td>
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<td>Shag</td>
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<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Sheep</td>
<td>2</td>
<td>1</td>
<td>50 (0-119.3)</td>
<td><em>C. xiaoi</em> (1)</td>
<td>0</td>
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<tr>
<td>Wallaby</td>
<td>17</td>
<td>1</td>
<td>5.9 (0-17.2)</td>
<td><em>C. parvum</em> (1)</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Wombat</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>4</td>
<td>8.5 (6.2-10.8)</td>
<td>-</td>
<td>78</td>
<td>13.8 (11-16.7)</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2. Polymorphisms in *Giardia duodenalis* assemblage C isolates compared with C reference isolate Ad148 (U60986) at the glutamate dehydrogenase (*gdh*) locus. Polymorphic sites are numbered with reference to the full-length gene.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Nucleotide position</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCA66</td>
<td>492 498 522 587 570 579 603 615 627 633 636 705 711 720 723 732 753 762 783 831 843 861 867 873 879 897 903 912 936 915 918 945 963</td>
</tr>
<tr>
<td>SCA2728</td>
<td>C T C G C T C A T C C C T C A G G C C C C A T C G T T</td>
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<tr>
<td>SCA140</td>
<td>T C C G T C C A C C C T C C A A C T T T A C T A C C</td>
</tr>
<tr>
<td>U60986</td>
<td>C T T T A T C T G C T T C T T C T G A A A T T C T T G T C G T T</td>
</tr>
</tbody>
</table>

Table 3. Polymorphisms in *Giardia duodenalis* assemblage D isolates compared with D reference isolate Ad136 (U60982) at the glutamate dehydrogenase (*gdh*) locus. Polymorphic sites are numbered with reference to the full-length gene.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Nucleotide position</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCA87</td>
<td>498 507 510 570 579 586 600 624 630 636 747 762 768 831 835 843 846 861 864 867 873 891 897 903 912 915 918 945 963</td>
</tr>
<tr>
<td>SCA63</td>
<td>A C C C G T C G C C C C C C C A G G C G T T</td>
</tr>
<tr>
<td>SCA293</td>
<td>C C C C T A G C T T A T C G C C C C T C T T G A T A C C</td>
</tr>
<tr>
<td>U60982</td>
<td>-D T C T C T G T C T T C G C C A T T C T C T T T G A C G T T</td>
</tr>
</tbody>
</table>
Prevalence of *Cryptosporidium* and *Giardia* in animal hosts in Sydney water catchments

C. *hominis* and C. *parvum*