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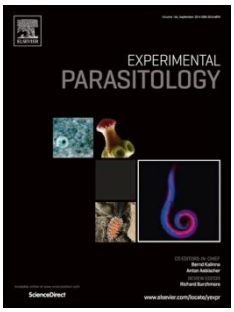

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Molecular Characterisation of *Cryptosporidium* and *Giardia* in cats (*Felis catus*) in Western Australia

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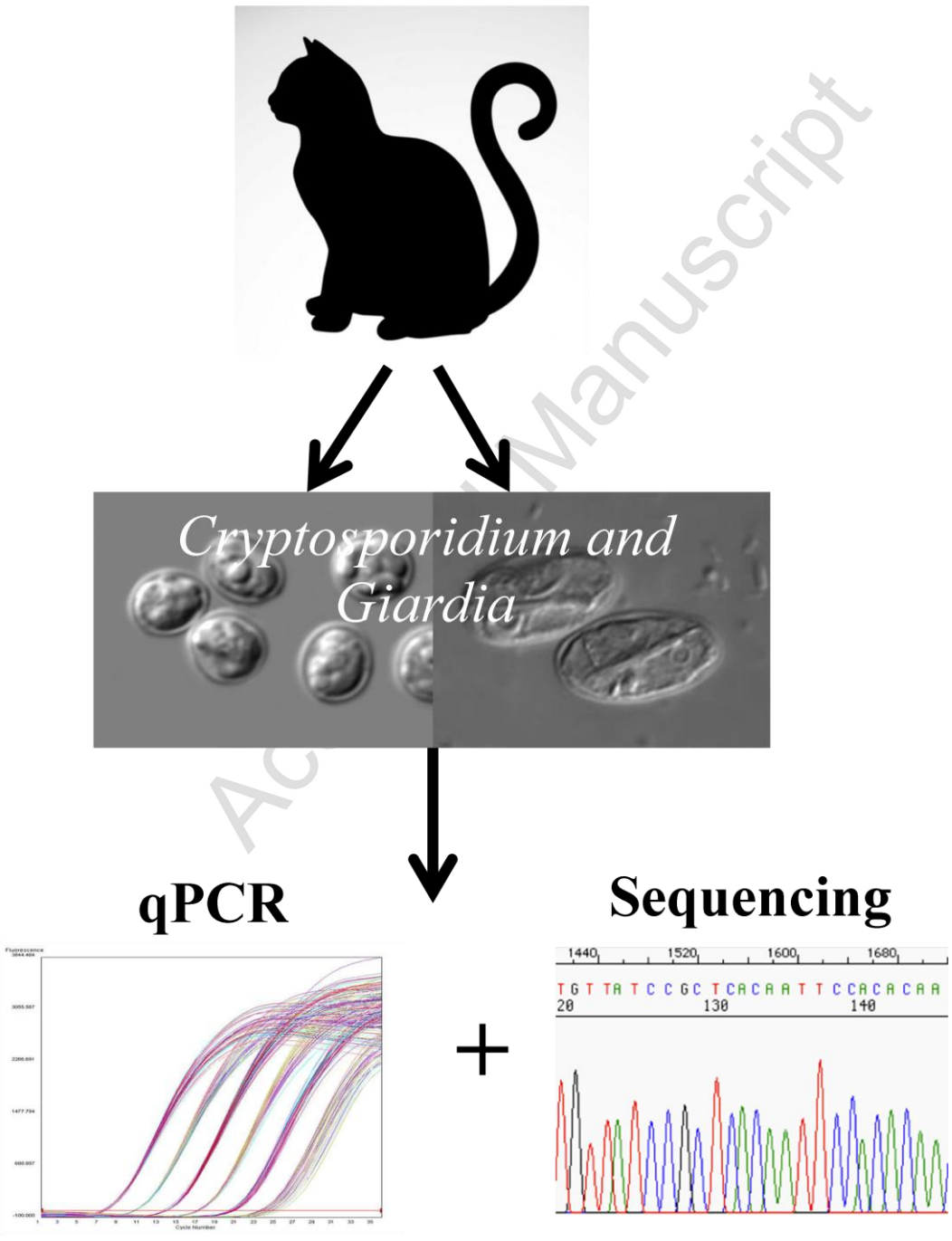
Highlights

- Prevalence of *Cryptosporidium* and *Giardia* in cats determined by PCR
- Oo/cyst shedding determined by qPCR
- First report of *Cryptosporidium ryanae* and rat genotype III in cats

Graphical Abstract



ABSTRACT

Little is known of the prevalence of *Cryptosporidium* and *Giardia* in domestic cats in Western Australia and their potential role as zoonotic reservoirs for human infection. In the present study, a total of 345 faecal samples from four different sources were screened for the presence of *Cryptosporidium* and *Giardia* by PCR and genotyped by sequence analysis. Oocyst numbers and cyst numbers for *Cryptosporidium* and *Giardia* respectively were also determined using quantitative PCR assays. *Cryptosporidium* and *Giardia* were detected in 9.9% (95% CI 6.7-13.0) and 10.1% (95% CI 7.0-13.3) of cats in Western Australia respectively. Sequence analysis at the 18S rRNA locus identified five *Cryptosporidium* species/genotypes; *C. felis* (n=8), *C. muris* (n=1), *C. ryanae* (n=1), *Cryptosporidium* rat genotype III (n=5) and a novel genotype most closely related to *Cryptosporidium* rat genotype III in one isolate. This is the first report of *C. ryanae* and *Cryptosporidium* rat genotype III in cats. For *Giardia,* assemblage F the most commonly identified species, while only 1 assemblage sequence was detected. Since most human cases of cryptosporidiosis are caused by *C. parvum* and *C. hominis* and human cases of giardiasis are caused by *G. duodenalis* assemblage A and B, the domestic cats in the present study are likely to be of low zoonotic risk to pet owners in Perth. Risk analyses identified that elderly cats (more than six years) were more prone to *Cryptosporidium* and *Giardia* infections than kittens (less than six months) (P =0.009). Clinical symptoms were not associated with the prevalence of *Cryptosporidium* and *Giardia* infections in cats.

Keywords: Cryptosporidium; Giardia; cats*; C. felis*; *C. ryanae*; rat genotype III, Assemblage A, Assemblage F.

1. Introduction

The domestic cat or house cat (*Felis silvestris* subspecies catus), through geographical expansion, is currently the most widespread feline species worldwide (Driscoll et al., 2007; Johnson et al., 2011). *Cryptosporidium* and *Giardia* are common enteric protozoan parasites (Cacciò et al., 2002; Lalle, 2005; FitzGerald et al., 2011) that cause clinical and subclinical infections in cats of all ages (Santis-Kerr et al., 2006; FitzGerald et al., 2011). They also infect other companion animals including dogs (Palmer et al., 2008a; Yoshiuchi et al., 2010), and may result in significant gastroenteritis in a vast number of mammalian hosts worldwide (Cacciò et al., 2002; Xiao, 2010). The infective stages of these parasites (the oocysts/cysts) are found in the gastrointestinal tract and faeces of infected hosts. The oocysts/cysts are extremely robust, able to remain in the environment for long periods of time, and are not readily inactivated by chlorine-based disinfectants (Yoshiuchi et al., 2010; Surl et al., 2011). Transmission occurs primarily through the faecal-oral route and these protozoan parasites have been responsible for numerous documented waterborne outbreaks worldwide (Baldursson and Karanis, 2011).

Based on biological and molecular characterisation there are 26 *Cryptosporidium* species (Ryan et al., 2014) with over 40 genotypes (Xiao, 2010) recognised at present. Eight species are responsible for most human cryptosporidiosis cases; *C. hominis, C. parvum, C. meleagridis, C. cuniculus, C. ubiquitum, C. viatorum, C. felis* and *C. canis*, (Ryan et al., 2014) with *C. hominis* and *C. parvum* responsible for the majority of human infections.

Giardia duodenalis is the species that infects mammals and consists of different genetic groups (assemblages) with different host specificities; assemblage A in humans and other mammals, assemblage B primarily in humans and primates, assemblages C and D in dogs,

assemblage E in hoofed livestock, assemblage F in cats, assemblage G in rodents and assemblage H reported from a seal and a gull (Ballweber et al., 2010; Ryan and Cacciò, 2013). Therefore, infected animals can be important reservoirs of giardiasis for humans as cross infections have been observed, and assemblage F has also been detected in humans (Gelanew et al., 2007; Sprong et al., 2009).

Although the prevalences of *Cryptosporidium* and *Giardia* infection in domestic cats have been well documented worldwide (cf. Lucio-Foster et al., 2010; Sabshin et al., 2012; Hoopes et al., 2013; Sotiriadou et al., 2013; Scorza et al., 2014), few studies have been conducted on their prevalence in the Western Australia (WA) cat population. Palmer et al. (2008a) reported a prevalence of 2.4% and 2.0% in *Cryptosporidium* and *Giardia* respectively in cats in Australia. Of the *Giardia* positives, all but one were identified as assemblage F, with one assemblage D identified. The lack of zoonotic *Giardia* assemblages identified in that study was hypothesised to be due to the low *Giardia* prevalence in the human population (Palmer et al., 2008a). Molecular epidemiological studies of *Cryptosporidium* in cats in Australia have shown that they seem to be largely infected with *C. felis* (Morgan et al., 1998; Sargent et al., 1998; McGlade et al., 2003; Palmer et al., 2008a; 2008b).

The aim of the present study was to determine the prevalence of *Cryptosporidium* and *Giardia* species in domestic cats in the Perth metropolitan area using molecular tools, to quantify the levels of *Cryptosporidium* and *Giardia* oo/cyst shedding in these cats using quantitative PCR (qPCR) and to identify the risk factors associated with *Cryptosporidium* and *Giardia* in cats.

2. Materials and methods

2.1. Questionnaires

Questionnaires were designed based on similar studies in the literature (McGlade et al., 2003; Palmer et al., 2008a) with modifications to include information on demographic data (age, sex, breed, weight and sterilisation status), management data (diet, use of anti-parasitic treatments, indoor or outdoor cats) as well as any currently known medical history or clinical symptoms (blood in stools, anorexia, weight loss and vomiting).

2.2 Sample Collection

Sampling groups were selected based on results of previous studies where factors such as age, diet, and environment were considered to influence the level of parasitism in cats (Coman, 1972; Pavlov and Howell, 1977; Wilson-Hanson and Prescott, 1982; Sargent et al., 1998; Hills et al., 2000; Palmer et al., 2008a). In addition, veterinary establishments were selected based on fifteen different postal codes to collect faecal samples from a wide geographical area around Perth metropolitan region.

2.2.1. Sample Groups

Feline faecal samples (n = 345) were collected from four main sources in the Perth metropolitan area during August 2010 to September 2011. Faecal samples from the cat refuge centre were collected from 179 kittens and cats in the refuge facility, with ages ranging from five weeks to 12 years old. The medical histories of these animals were unknown.

Samples were also collected from three pet shops from kittens (n = 29) aged four weeks to six months of age. These pet shops were selected based on their location and

willingness to be involved in the survey. At one breeding establishment, samples were collected from adult cats and young kittens (n = 10). The ages ranged from five weeks to eight years old from two breeds of cats (eight Abyssinians and two mixed breeds).

Samples were also collected from kittens and cats belonging to various veterinary hospital staff as well private cat owners (n = 127). Both of these groups were categorised as "privately owned cats" The ages of the cats ranged from 8 weeks to 18 years old. All samples were collected under Murdoch University animal ethics permit R2364/10.

Each faecal specimen was scored according to their consistency, which was recorded by participants at the time of collection and confirmed by one person who was trained in this area. The faecal consistency scores were subsequently correlated with parasite status. Demographic information was also recorded

2.3 DNA Isolation

Total DNA was extracted from 200 mg of each faecal sample using a Power Soil DNA purification Kit (MolBio, Carlsbad, California) with some modifications as described by Yang et al., (2013). Briefly, the faeces for DNA extraction were subjected to four cycles of freeze/thaw (liquid nitrogen followed by boiling water) to ensure efficient lysis of oocysts before being processed using the manufacturer's protocol. A blank control (no faecal sample) was used in each extraction group.

2.3. Cryptosporidium and Giardia PCR analysis

All samples were screened at the 18S rRNA locus for *Cryptosporidium* using a two-step nested PCR described by Ryan et al., (2003). A spike analysis (addition of ~10 ng of *C.*

hominis DNA positive control into each sample) was conducted on randomly selected *Cryptosporidium* negative samples (n = 42) to determine if negative results were due to PCR inhibition.

Amplification of a fragment of the *Giardia* 18S rRNA gene was performed as described by Hopkins et al., (1997) and Read et al., (2002). Fragments of the glutamate dehydrogenase (*gdh)* and the *β-giardin* genes were amplified as previously described (Read et al., 2004; Sulaiman et al., 2004). Approx 10 ng of *Giardia duodenalis* assemblage A from a human was used as a positive control and a negative control (no DNA) was included in all reactions.

Quantitation of *Cryptosporidium* and *Giardia* oo/cysts in faecal samples was achieved using qPCR assays at the actin and *gdh* loci as previously described (Yang et al., 2014a; Yang et al., 2014b). Both the actin and *gdh* qPCR assays used in the present study have previously been extensively validated for specificity and sensitivity (Yang et al., 2014a; Yang et al., 2014b). For standard curve generation, partial fragments of the actin and *gdh* gene were amplified as previously described (Yang et al., 2014a; Yang et al., 2014b) and individually cloned into pGEM-T vectors (Promega, USA). Plasmid DNA was isolated by alkali\SDS lysis followed by column purification using QIAprep Spin Columns (Qiagen) in accordance with the manufacturer's protocol. Plasmid mini-preparations were sequenced using T7 sequencing primer (Stratagene, La Jolla, CA, USA) and clones with the correct sequence then used. The plasmid copy numbers were calculated based on the plasmid size (base pairs) and DNA concentration. 10-fold series dilutions of plasmid were conducted from 10,000 copies down to 1 copy of the plasmid template for sensitivity testing and these were then spiked into faecal samples and the DNA extracted and amplified as described above and mean detection limits, RSQ (R squared) values and % Relative Standard Deviation (RDS) were calculated. For *Giardia*, copy numbers detected were converted to cyst numbers on the basis that the *gdh* gene in *Giardia* is a single copy gene (Yee and Denis, 1992) and the fact that there are 4

haploid nuclei per cyst. Therefore, every 4 copies of *gdh* detected by qPCR were equivalent to 1 cyst. For *Cryptosporidium*, target copy numbers detected were converted to numbers of oocysts based on the fact that the actin gene in *Cryptosporidium* is a single copy gene (Kim et al., 1992) and there are 4 haploid sporozoites per oocyst. Therefore, every 4 copies of actin detected by qPCR were equivalent to 1 oocyst.

2.4. PCR product purification and sequencing

The amplified DNA fragments from the secondary PCR were separated by gel electrophoresis and purified using an in house filter tip method and used for sequencing without any further purification as previously described (Yang et al., 2013). Briefly, positive bands were cut from the gel and the gel fragment transferred to a 100µl filter tip (with the tip cut off) (Axygen, FisherBiotech, WA), and then placed in a 1.5 ml Eppendorf tube and spun at full speed in a microfuge for 15 seconds. The filter tip was then discarded and the eluent was retained and used for sequencing without any further purification using an ABI Prism Terminator Cycle Sequencing kit (Applied Biosystems, USA), according to the manufacturer's instructions with the exception that the annealing temperature was at 58ºC.

2.5. Statistical analysis

Statistical analysis was performed using SPSS 20.0 (Statistical Package for the Social Sciences) for Windows (SPSS Inc. Chicago, USA). Odds ratio risk analyses with Pearson's chi-squared ($\chi 2$) test for independence or Fisher's exact two-sided test for significance were conducted to determine if there was any significance between the prevalence of *Cryptosporidium* and *Giardia* and risk factors such as faecal consistency scores, , age, sex

and diet. Overall prevalences were calculated for cats that were classified as positive by using the exact binomial method (Thrusfield, 2007). One sample t-test was conducted to determine sample distribution and Wilcoxon signed ranks test was conducted to determine if age played a part in parasite infections.

3. Results

3.1 The prevalence of Cryptosporidium and Giardia by PCR

The overall prevalence of *Cryptosporidium* by PCR at the 18S rRNA locus from the four sources of cats in WA was 9.9% (34/345), with the highest prevalence in cats from the refuge centre (13.4%), followed by privately owned cats (7.1%) and pet shop kittens (3.4%) (Table 1). No positives were detected from the cats from the breeder. A total of 35 *Giardia* positives were detected by combined screening of three loci (18S rRNA, *β-Giardin* and *gdh*), an overall prevalence of 10.1%. Of these three loci, twenty-five positives were detected at the *gdh* locus; seven and six positives were detected at the 18S rRNA and *β-Giardin* loci, respectively The highest prevalence of *Giardia* was detected in the cats from the breeder (60.0%), followed by the refuge centre cats (10.6%), and privately owned cats (7.9%). No *Giardia* positives were detected from pet shop cats. Co-infections of *Cryptosporidium* and *Giardia* were detected in four samples (Cat 20, Cat 44, Cat 161 and Cat 321) by PCR. None of the co-infected cats had diarrhea or other known clinical signs.

3.2. Genotyping Cryptosporidium and Giardia positive samples

Sixteen of the 34 *Cryptosporidium* samples were sequenced and five species/genotypes were identified; *C. felis* in eight isolates (Cat 20, 61, 110, 115, 118, 143, 321 and 322), *C. muris* in one isolate (Cat 97), *C. ryanae* in one isolate (Cat 44), *Cryptosporidium* sp. rat genotype III in five isolates (Cat 81, 85, 86, 89 and 94) and a novel genotype most closely related to *Cryptosporidium* sp. rat genotype III (98.8% similarity) in one isolate (Cat 100).

Seven *Giardia* PCR positives that were detected at the 18S rRNA locus were sequenced. Sequencing analysis revealed that six of the isolates (Cat 3, 17, 132, 161, 162 and 321) were *G. duodenalis* assemblage F and one isolate (Cat 164) was *G. duodenalis* assemblage A. At the *β-giardin* locus, four of the six positives were successfully sequenced. Sequence analysis revealed that all four isolates (Cat 132, 138, 161 and 162) were assemblage F. At the *gdh* locus, 10 of the 25 positive *Giardia* isolates were chosen for sequencing. Sequence analysis revealed that the 9 isolates (Cat 3, 132, 138, 161 and 162, 277, 279, 320 and 321) were assemblage F and Cat 164 was identified as Assemblage A, sub-assemblage A1 by aligning with reference AI sub-assemblage KJ027437. 18S sequences of representative *Cryptosporidium* isolates have been submitted to GenBank: *C. felis* (Cat 20, Cat 118, Cat 321 - KP216703, KP216707, KP216709), *C. muris* (Cat 97 - KP216705), *C. ryanae* (Cat 44 -KP216704), *Cryptosporidium* sp. rat genotype III (Cat 81 - KP216708), *Cryptosporidium* sp. rat genotype III-like (Cat 100 - KP216706). For *Giardia*, representative Assemblage F sequences were submitted to GenBank: (*β-giardin* gene - Cat 132 - KP216714, Cat 162 - KP216713; *gdh* gene - Cat 3 - KP216711, Cat 277 - KP216710, Cat 320 - KP216712).

3.3. Cryptosporidium and *Giardia oo/cyst numbers*

The numbers of *Cryptosporidium* oocysts per gram of faeces (g^{-1}) in the 34 PCR positive samples was determined using a qPCR at the actin locus (Yang et al., 2014a). *Cryptosporidium* oocyst numbers ranged from 175 to $1.1x10^5$ oocysts/g^{-1} faeces with a median of $3.5x10^3$ oocysts per g^{-1} (Table 2). *Giardia* cyst numbers were quantified for 25 of the 28 PCR positives using a *gdh* qPCR (Yang et al., 2014b). *Giardia* cyst numbers ranged from 550 to 3.4 x 10^7 cysts/g faeces with a median of 2.0 $x10^4$ cysts/g faeces (Table 3). The three samples with high cyst numbers detected by qPCR had a faecal consistency score of 3 (soft, unformed stools).

3.4. Risk factors associated with Cryptosporidium and Giardia parasitism in domestic cats

None of the cats had symptoms of diarrhoea but statistical analysis revealed a significantly higher prevalence of *Cryptosporidium* in elderly cats (≥ six years) (79.3%; 95% CI 60.3-92.0), $P = 0.009$ compared to younger cats (37.9%; 95% CI 20.7-57.7). A Pearson's Chi-Square (χ^2) test and an odds ratio risk analyses was conducted to determine links between *Cryptosporidium* prevalence and demographic data obtained from the cats in this study. There was insufficient evidence to suggest that the faecal consistency, gender, other pets present in the household, indoor versus outdoor environment and deworming had any influence in the likelihood of detecting *Cryptosporidium*. Clinical symptoms such as blood in stools, vomiting, weight loss and anorexia were not present in cats identified positive for *Cryptosporidium*.

Statistical analysis also revealed a higher prevalence of *Giardia* in elderly cats (≥ six years) (80.0 %, 95.0 %; CI 62.5-92.5), $P = 0.009$ compared to younger cats (64.5 %, 95 %; CI 45.4-80.8). Statistical analysis using Pearson's Chi-Square ($\chi 2$) test and odds ratio risk analyses revealed that female cats were 2.0 times more likely to be parasitised than male cats,

crossbred cats were 3.4 times more likely to be parasitised than purebred cats and households that had other pets besides cats were 2.6 more likely to have *Giardia*-positive cats than households that did not have additional pets. There was insufficient evidence to suggest that the faecal consistency and deworming had any influence in the likelihood of detecting *Giardia*. Clinical symptoms such as vomiting were only detected in two samples (Cat 11 and Cat 41) from the cat refuge centre and a veterinary clinic. Cat 41 also exhibited weight loss, anorexia and liver problems.

4. Discussion

In the present study, the overall prevalence of *Cryptosporidium* spp. detected by PCR (9.9%) was higher than previous Australian studies done by both Sargent et al. (1998) (1.2%) and Palmer et al. (2008b) (2.2%). However, the present result is in agreement with a study by McGlade et al., (2003) who reported a prevalence of 10.0% in cats from the Perth metropolitan area. Studies worldwide have reported prevalences ranging from 0% to 29.4% (cf. Lucio-Foster et al., 2010; Sotiriadou et al., 2013; Scorza et al., 2014). These differences are likely due to different detection techniques employed (Lucio-Foster et al., 2010).

The overall prevalence of *Giardia* by PCR in this study (10.1%) was similar to a previous study in Australia (Palmer et al., 2008a), which reported a prevalence of 9.3%, but lower than another study in WA which reported a prevalence of 80.0% (McGlade et al., 2003). In the present study, the highest prevalence was detected in cats from a cat breeder (60.0%) followed by the cat refuge center (10.6%) and privately owned cats (7.9%). This result was not surprising as the breeder cats had contact with each other and also the environment in which the cats are housed plays a major role in the transmission of *Giardia* (Itoh et al., 2006). This finding was congruent with a previous study in Japan (Itoh et al.,

2006) where suburban cats (42.0%) had a significantly higher prevalence than city center cats (29.0%).

Sequence analysis identified five *Cryptosporidium* species/genotypes in the present study; *C. felis (n=8), C. muris (n=1), C. ryanae (n=1), Cryptosporidium rat genotype III (n=5) and a novel genotype most closely related to Cryptosporidium rat genotype III in one isolate. Cryptosporidium felis* was the most common species identified and is the main *Cryptosporidium* species infecting cats (Lucio-Foster et al., 2010). *Cryptosporidium muris* has been found in a wide range of species including rodents, marsupials (bilbies) and other mammals (Lv et al., 2009; Ryan and Xiao, 2014) and has occasionally been reported in cats (Pavlasek and Ryan, 2007; Lucio-Foster et al., 2010). It has also been identified in a few humans in developing countries but *C. muris* is not common in the human population (Ryan and Xiao, 2014). *Cryptosporidium felis* has a much more restricted host range and has been confirmed using molecular techniques in cats, immunocompetent and immunocompromised humans and a cow (Bornay-Llinares et al., 1999; Lucio-Foster et al., 2010; Ryan and Xiao, 2014). In children in developing countries, *C. felis* is responsible for as much as 3.3% of overall cryptosporidiosis cases (Lucio-Foster et al., 2010). To date, *C. felis* has not been identified in immunocompetent humans in Australia. This is the first report of *C. ryanae* in cats. *Cryptosporidium ryanae* was first described in cattle in 2008 and was previously identified as the *Cryptosporidium* deer-like genotype (Fayer et al., 2005). This species has only previously been reported in cattle and has not been identified in humans. This is also the first report of rat genotype III in cats and the novel rat genotype III-like isolate which exhibited 98.8% genetic similarity with rat genotype III. *Cryptosporidium* rat genotype III has previously been described from brown rats and Asian house rats from China (Lv et al., 2009) and rats from the Philipines (Ng-Hublin et al., 2013). The detection of rodent associated *Cryptosporidium* (*C. muris* and rat genotype III) in these cats may be the result of

mechanical transmission due to consumption of rodents and not an actual infection. However at least one of the cats (Cat94), in which rat genotype III was detected, had oocyst counts of $1.1x10^5$ oocysts/g^{-1} faeces as determined by the actin qPCR, which suggests this is a real infection. Further studies are required to confirm this. As most human cases of cryptosporidiosis worldwide are associated with *C. hominis* and *C. parvum* (Xiao, 2010; Ryan et al., 2014), the identification of *C. muris, C. felis, C. ryanae* and rat genotype III in cats are likely to be of low zoonotic risk to humans.

For *Giardia*, sequence analysis identified the majority of the positives as *G. duodenalis* assemblage F (cat genotype), with one isolate (Cat 164) identified as assemblage A1, suggesting that the cat population in WA is unlikely to be a major zoonotic reservoir for human infection. This study is one of the first to report oo/cyst numbers per gram of faeces in cats for *Cryptosporidium* and *Giardia*. Overall oo/cyst numbers were low for most positives with median oo/cyst shedding of $3.5 x10^3$ and $2.0 x10^4$ for *Cryptosporidium* and *Giardia* respectively.

The prevalence of both *Cryptosporidium* and *Giardia* was higher in older cats (more than six years) and in outdoor cats, similar to a previous study where outdoor cats had a higher prevalence of *Giardia* (53%) than indoor cats (33%) (Itoh et al., 2006), but in contrast to another study which reported that cats at increased risk of *Giardia* species infection were under 4 years of age (De Santis-Kerr et al., 2006). No other risk factors were identified for *Cryptosporidium* prevalence in cats but for *Giardia*, crossbred cats were 3.4 times more likely to be parasitised than purebred cats. This is in contrast to a previous study, which reported that purebred cats had an increased prevalence of *Giardia* compared with mixed breed cats (De Santis-Kerr et al., 2006).

In conclusion, this is the first report of *Cryptosporidium ryanae* and *Cryptosporidium* rat genotype III in cats and is also the first study to analyse oo/cyst shedding in cats using qPCR. Further studies are required to determine the range of *Cryptosporidium* and *Giardia* species infecting cats.

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No.	Sample code	Sample source	Oocysts per g faeces

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482 **Table 1.** Prevalences of *Cryptosporidium* and *Giardia* by PCR in cat faecal samples from
483 four different sources (with 95 % confidence intervals).

	Cat Refuge Centre (*n*=179)	**Privately owned (*n*=127)**	**Pet Shops (*n*=29)**	**Breeder (*n*=10)**	**Overall Prevalence (*n*=345)**
***Cryptosporidium* spp.**	13.4 % 24/179 (8.4-18.4)	7.1 % 9/127 (2.6-11.5)	3.4 % (1/29) (0.0-10.1)	0	9.9 % (34/345) (6.7-13)
***Giardia* spp.**	10.6 % 19/179 (6.1-15.1)	7.9% 10/127 (3.2-12.6)	0	60 % (6/10) (29.6-90.4)	10.1 % (35/345) (7-13.3)
Total prevalence	24% 43/179 (17.8-30.3)	15% 19/127 (8.8-21.2)	3.4 % (1/29) (0.0-10.1)	60 % (6/10) (29.6-90.4)	20% (69/345) (15.8-24.2)

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486 **Table 2.** *Cryptosporidium* oocyst load g^{-1} in 34 cat faecal samples quantified by qPCR at the
487 actin locus.

1	Cat 20	Refuge Centre	$3.3x10^4$
2	Cat 44	Privately owned	$1.4x10^3$
3	Cat 60	Privately owned	188
4	Cat 61	Privately owned	$1.4x10^3$
5	Cat 62	Privately owned	$3.3x10^3$
6	Cat 63	Privately owned	$1.1x10^3$
7	Cat 68	Privately owned	910
8	Cat 73	Privately owned	$2.7x10^3$
9	Cat 78	Privately owned	$3.6x10^3$
10	Cat 79	Privately owned	$1.0x10^3$
11	Cat 80	Refuge Centre	$6.9x10^3$
12	Cat 81	Refuge Centre	$9.6x10^3$
13	Cat 85	Refuge Centre	$2.0x10^4$
14	Cat 86	Refuge Centre	$5.1x10^3$
15	Cat 89	Refuge Centre	175
16	Cat 93	Refuge Centre	$1.2x10^3$
17	Cat 94	Refuge Centre	$1.1x10^5$
18	Cat 95	Refuge Centre	$4.6x10^4$
19	Cat 97	Refuge Centre	$1.7x10^4$
20	Cat 98	Refuge Centre	840
21	Cat 100	Pet Shop	$6.2x10^4$
22	Cat 101	Refuge Centre	336
23	Cat 103	Refuge Centre	687
24	Cat 105	Refuge Centre	$9.4x10^3$
25	Cat 106	Refuge Centre	660
26	Cat 109	Refuge Centre	$8.5x10^3$
27	Cat 110	Refuge Centre	$7.5x10^3$
28	Cat 111	Refuge Centre	$6.6x10^3$
29	Cat 115	Refuge Centre	$4.4x10^3$
30	Cat 118	Refuge Centre	880
31	Cat 143	Refuge Centre	279
32	Cat 161	Refuge Centre	$1.1x10^3$
33	Cat 321	Refuge Centre	$6.4x10^3$
34	Cat 322	Refuge Centre	$1.6x10^4$
Median			$3.5x10^3$
max			$1.1x10^5$
Minimum			175

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490 **Table 3.** *Giardia* cyst load g^{-1} in 25 cat faecal samples quantified by qPCR at the *gdh* locus.

No.	Sample code	Sample source	Cysts per g faeces
1	Cat 3	Refuge	$7.8x10^3$

2	Cat 16	Refuge	$9.2x10^3$
3	Cat 17	Refuge	$3.7x10^3$
4	Cat 20	Refuge	$1.3x10^5$
5	Cat 22	Refuge	$3.6x10^3$
6	Cat 39	Privately owned	$2.4x10^6$
7	Cat 40	Privately owned	$1.8x10^4$
8	Cat 44	Privately owned	$1.4x10^4$
9	Cat 46	Privately owned	$2.0x10^4$
10	Cat 48	Privately owned	550
11	Cat 127	Refuge	$3.7x10^4$
12	Cat 132	Refuge	$5.5x10^6$
13	Cat 137	Refuge	$2.8x10^4$
14	Cat 138	Refuge	$1.3x10^7$
15	Cat 161	Refuge	$3.4x10^7$
16	Cat 162	Refuge	$3.3x10^4$
17	Cat 164	Refuge	$5.9x10^4$
18	Cat 277	Privately owned	$7.8x10^3$
19	Cat 279	Privately owned	$4.4x10^4$
20	Cat 284	Privately owned	750
21	Cat 286	Privately owned	$1.6x10^3$
22	Cat 313	Breeder	600
23	Cat 314	Breeder	780
24	Cat 320	Breeder	$5.4x10^4$
25	Cat 321	Refuge Centre	29,800
Median			**$2.0x10^4$**
Max			**1.3x107**
Minimum			**550**

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