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Cryptosporidium homai n. sp. (Apicomplexa: Cryptosporidiidae) from the guinea pig (Cavia porcellus)

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Highlights:
- Morphological and molecular characterisation of a new Cryptosporidium species.
- Phylogenetic relationships of C. homai n. sp.
- Histological analysis of the small intestine of the infected host.

ABSTRACT

The morphological, biological, and molecular characterisation of a new Cryptosporidium species from the guinea pig (Cavia porcellus) are described, and the species name Cryptosporidium homai n. sp. is proposed. Histological analysis conducted on a post-mortem sample from a guinea pig euthanised due to respiratory distress, identified developmental stages of C. homai n. sp. (trophozoites and meronts) along the intestinal epithelium.

Molecular analysis at 18S rRNA (18S), actin and hsp70 loci was then conducted on faeces from an additional 7 guinea pigs positive for C. homai n. sp. At the 18S, actin and hsp70 loci, C. homai n. sp. exhibited genetic distances ranging from 3.1\% to 14.3\%, 14.4\% to 24.5\%, and 6.6\% to 20.9\% from other Cryptosporidium spp., respectively. At the 18S locus, C.
Graphical Abstract

Cryptosporidium parvum
Cryptosporidium erinacei
Cryptosporidium hominis
Cryptosporidium cuniculus
Cryptosporidium tyzzeri
Cryptosporidium meleagridis
Cryptosporidium wrairi
Cryptosporidium fayeri
Cryptosporidium viatorum
Cryptosporidium ubiquitum
Cryptosporidium suis
Cryptosporidium viatorum
Cryptosporidium ubiquitum
Cryptosporidium suis
Cryptosporidium felis
Cryptosporidium canis
Cryptosporidium varanii
Cryptosporidium macropodum

Cryptosporidium homai. n. sp.
*homai* n. sp. shared 99.1% similarity with a previously described *Cryptosporidium* genotype in guinea pigs from Brazil and it is likely that they are the same species, however this cannot be confirmed as actin and *hsp70* sequences from the Brazilian guinea pig genotype are not available. Phylogenetic analysis of concatenated 18S, actin and *hsp70* sequences showed that *C. homai* n. sp. exhibited 9.1% to 17.3% genetic distance from all other *Cryptosporidium* spp. This clearly supports the validity of *C. homai* n. sp. as a separate species.

Keywords: *Cryptosporidium homai*; guinea pig, 18S; actin; *hsp70*

1. Introduction

*Cryptosporidium* spp. are protozoan parasites responsible for gastroenteritis in a wide range of vertebrates including humans, domestic and wild animals and are a common cause of waterborne outbreaks worldwide (Zahedi et al., 2016; Ryan et al., 2016; Efstratiou et al., 2017). The parasite is transmitted via the faecal-oral route with both zoonotic and anthroponotic transmission cycles (Ryan et al., 2014). Currently relatively little is known about the molecular characteristics, host specificity, pathogenicity and zoonotic importance of *Cryptosporidium* spp. in wild and domestic rodents (Appelbee et al., 2005; Ziegler et al., 2007a, b; Ryan et al., 2014; Kváč et al., 2016; Li et al., 2016). To date, of the 33 recognised *Cryptosporidium* spp. (cf. Ryan et al. 2016; Jezkova et al., 2016), 11 species including *C. proliferans*, *C. meleagridis*, *C. tyzerri*, *C. andersoni*, *C. ubiquitum*, *C. wrairi*, *C. parvum*, *C. suis*, *C. meleagridis*, *C. muris* and *C. rubeyi* and over 20 genotypes of unknown species status have been reported in rodents with a prevalence ranging from 1% to 63% (Table 1) (Qi et al., 2015; Song et al., 2015; Stenger et al., 2015; Zahedi et al., 2016; Li et al., 2016).

The guinea pig (*Cavia porcellus*) is one of eight species in the genus *Cavia* (Rodentia: Caviidae), and is endemic to South America. Based on available archaeological and molecular data, it has been living in the region since the Miocene-Pliocene boundary, and it has been suggested that *C. porcellus* was initially derived from *Cavia tschudii*, when the
Amerindia peoples of Peru started to domesticate guinea pigs 4500 to 7000 years ago. Eventually, the utility of the domesticated form of guinea pig as a food source or pet and laboratory animal, has resulted in its worldwide distribution including Australia (Dunnum and Salazar-Bravo, 2009).

Currently, *C. wrairi* is the only valid *Cryptosporidium* spp. described in guinea pigs (*Cavia porcellus*), with strong host specificity and no reports of human infection (Vetterling et al., 1971; Chrisp et al., 1990; Spano et al., 1997; Lv et al., 2009, Gressler et al., 2010; Smith et al., 2010). Previous experimental infections indicated that *C. wrairi* was infective to mice, lambs and calves, causing a sparse infection in ruminants, however as genotyping was not conducted, this cannot be confirmed (Angus et al., 1985; Chrisp et al., 1992).

The present study examined the morphological, biological and molecular characteristics of a *Cryptosporidium* sp. detected in the gastrointestinal tract and faeces of guinea pigs. Based on the collective data from the present study, the *Cryptosporidium* spp. detected in these guinea pigs is genetically and biologically distinct from all species of *Cryptosporidium* described previously, and we propose the species name *Cryptosporidium homai* n. sp. For clarity, we herein refer to this novel species by its proposed name.

2. Materials and methods

2.1. Source of sample and sample processing

A guinea pig, which was part of a group of experimental animals held at the University of Western Australia Animal Care Services, Perth, Australia, presented with audible respiratory distress (rattled breathing sounds, difficulty in breathing and chin coated in saliva) and as a result was euthanised. *Post-mortem* examination was performed and intestinal and lung sections were sent to a specialist veterinary laboratory for further histopathological examination, and during routine handling, individual faecal sample were collected and stored at 4°C until required. Further to the initial histopathology and molecular analysis, additional
faecal samples (n=28) were collected either from individual animals or pooled from animals kept in the same enclosure for further molecular analysis (Table 2).

2.2. Histopathology

Sections of intestinal tissue were fixed in 10mg/100 mL phosphate buffered formalin for at least 24 hrs, dehydrated in an ethanol-xylene series and embedded in paraffin wax. Two micrometer tissue sections were dewaxed in xylene, rehydrated in an ethanol series and stained by haematoxylin & eosin (H&E) or Giemsa. Giemsa stock solutions were made up with 0.75 g Giemsa powder, 65 ml methanol and 65 ml glycerol, and diluted 1:10 with tap water immediately prior to use. Giemsa stained tissue sections were acidified with 10% acetic acid for 15 sec, and placed in freshly diluted Giemsa stain, preheated in a microwave (Kambrook model 686LE, 1150W) on medium for 30 sec, followed by microwaving on low for 30 sec. Slides were rinsed in tap water followed by absolute ethanol, before permanent mounting in DPX (Dako).

2.3. DNA isolation

Following five cycles of freeze-thaw, genomic DNA was extracted from 250 mg of each faecal sample (n=29), using a Power Soil Kit (MO BIO, Carlsbad, California, USA) in accordance to the manufacturer’s instructions. An extraction blank (no faecal sample) was used in each extraction group. Purified DNA was stored in -20°C prior to molecular analyses. DNA extraction and post-DNA extraction procedures were performed in separate dedicated laboratories.

2.4. PCR amplification

A nested PCR approach was used to amplify an approximately 825 bp 18S rRNA fragment using the primers SSU-F2 (5’-TTCTAGAGCTAATACATGCG-3’) and SSU-R2 (5’-CCCATTTCCTTCGAAACAGGA-3’) for the primary PCR and SSU-F3 (5’-GGAAGGGTTGTATTTATAGATAAAG-3’) and SSU-R4 (5’-
AAGGAGTAAGGAACAACCTCCA-3′) for the nested PCR (Xiao et al., 1999). Each 25 µl PCR mixture contained 1µl of genomic DNA, 1x Go Taq PCR buffer (KAPA Biosystems, South Africa), 3.75 mM MgCl₂, 400 µM of each dNTPs, 0.4 µM of forward and reverse primers and 1 U Kapa DNA polymerase (Kapa Biosystems, South Africa). The PCR cycling conditions consisted of an initial denaturation step at 94°C for 3 min followed by 40 cycles of 94°C for 45 sec, 58°C for 90 sec, and 72°C for 1 min, followed by a final extension step at 72°C for 7 min. An approx. ~818bp fragment of the actin gene was amplified as previously described (Ng et al., 2006), with the following modifications; denaturation time was increased from 30 sec to 45 sec, annealing time from 20 sec to 30 sec and extension time from 40 sec to 1 min. PCR amplification of an approximately 325 bp fragment of the hsp70 gene was performed using a nested PCR as previously described (Hong et al., 2014).

No-template and extraction reagent blank controls were included in every PCR run. Positive control DNA (C. macropodum) was also added to every run to validate the PCRs. PCR setup and DNA handling procedure were performed in separate physically contained PCR-hoods, and post-PCR procedures were performed in a separate laboratory.

2.5. Sequence and phylogenetic analysis

Nested PCR products were electrophoresed through 1% agarose gels, and DNA fragments of the expected size (bp) for the 18S, actin and hsp70 assays were excised from the gels and purified for Sanger sequencing using an in-house filter tip method (Yang et al., 2013). Purified PCR products from all three assays, were sequenced independently in both directions using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California, USA) according to the manufacturer’s instructions at 58°C, 58°C and 56°C annealing temperature for the 18S rRNA, actin and hsp70 loci, respectively. Sanger sequencing chromatogram files were imported into Geneious Pro 8.1.6 (Kearse et al., 2012), and the nucleotide sequences of each gene was curated, analysed and aligned with reference
sequences from GenBank using Clustal W (http://www.clustalw.genome.jp). The most suitable nucleotide substitution model was assessed in MEGA7 (Kumar et al., 2015). Distance, Parsimony and Maximum Likelihood (ML) trees were constructed using MEGA version 7 (Kumar et al., 2015)). Bootstrap support for branching was based on 1000 replications. Sequences have been deposited in GenBank under the accession numbers MF499131- MF499151.

3. Results

3.1. Prevalence and histological analysis

In the present study, C. homai n. sp. was detected in 24.1% (7/29 -95% CI: 10.3% - 43.5%) of faecal samples collected from guinea pig enclosures by PCR and sequencing at 3 loci. Histological analysis of the small intestine of one guinea pig (isolate E89), indicated moderate to heavy epicellular infection of the intestinal epithelium by Cryptosporidium, associated with a minimal host inflammatory response. Trophozoites and meronts generally measured < 5 µm, as is typical for Cryptosporidium. There was a predominance of merogony with both type I and type II meronts present (Fig 1). The lamina propria was expanded by mild to moderate predominantly lymphocytic-plasmocytic inflammatory infiltrate with the occasional neutrophils, eosinophils and necrotic cells. An average of 1-2 and up to 3 mitotic figures were observed per intestinal crypt in 40-50% of crypts per high power field (40x objective). Mildly tortuous intestinal glands or crypts and the prominence of mitotic figures are suggestive of intestinal epithelial hyperplasia.

3.2. Sequence and phylogenetic analysis C. homai n. sp. at the 18S, actin and hsp70 loci

Phylogenetic relationships were inferred by Distance, Parsimony and Maximum Likelihood (ML) analyses at 18S, actin and hsp70 loci, based on 825, 818 and 325 bp of nucleotide sequences, respectively, and produced trees with mostly similar topologies with
some exceptions (Fig 2, 3, 4). An ML tree was also inferred from concatenated 18S, actin and 
*hsp70* sequences (Fig 5).

At the 18S locus, all *C. homai* n. sp. (*n=7*, which included 6 faecal samples and the 
intestinal sample, E89) were identical and grouped in a separate clade, sharing 99.1% identity 
with a novel genotype of *Cryptosporidium* spp. reported in guinea pigs (*C. procellus*) from 
Brazil (7 and 6 SNPs difference over 676 bp of submissions DQ885337 and DQ885338, 
respectively) (Huber et al., 2007). *C. homai* n. sp exhibited 3.1% genetic distance from the 
closest species, *C. felis*, 3.6% genetic distance from *C. wrairi*, and 3.2% (*C. suis*) to 15.4% 
(*C. scophthalmi*) genetic distance from all other *Cryptosporidium* spp.

At the actin locus, *C. homai* n. sp. again grouped separately and exhibited 14.4% genetic 
distance from the closest species, *C. varanii*, 18.4% genetic distance from *C. wrairi* and 
genetic distances ranging from 15.7% (*C. suis*) to 24.5% (*C. scophthalmi*) from all other 
*Cryptosporidium* spp.

Phylogenetic analysis of the *hsp70* gene, also confirmed the genetic distinctness of *C. 
homai* n. sp., where it exhibited 6.6% genetic distance from the closest species, *C. suis*, 7% 
genetic distance from *C. wrairi*, and 7.1% (*C. varanii*) to 20.9% (*C. serpentis*) genetic 
distance from all other *Cryptosporidium* spp.

An ML tree inferred from concatenated 18S, actin and *hsp70* sequences grouped *C. 
homai* n. sp. with *C. varanii* and *C. macropodum*, with 9.1% and 10% genetic distance 
respectively. Based on phylogenetic analysis using concatenated sequences, *C. homai* n. sp. 
exhibited 10.8% genetic distance from *C. wrairi*, the only valid *Cryptosporidium* sp. 
described in guinea pigs, and exhibited between 17.3% (*C. proliferans*) and 9.7% (*C. suis*) 
genetic distance from all other *Cryptosporidium* spp.

3.3. Taxonomic summary and species description

Order: Cryptogregarida (Cavalier-Smith, 2014).
Family: Cryptosporidiidae
Species name: *C. homai* n. sp.
Type host: Guinea pigs (*Cavia porcellus*)
Other natural hosts: Unknown
Type locality: Perth, Western Australia
Site of infection: Intestine
Prepatent period: Unknown
Patent period: Unknown
Material deposited: partial sequences of 18S, actin and *hsp70* genes were submitted to GenBank under accession numbers MF499131-MF499151.
Etymology: This species is named *C. homai* n. sp. in honor of my late aunt, Ms. Homa Hoorfar.

4. Discussion

In the present study, post-mortem analysis of a guinea pig euthanised due to respiratory distress, identified an intestinal infection with a *Cryptosporidium* species, which on the basis of molecular analysis is a new species, named *C. homai* n. sp. The new species was detected in 24.1% of faecal samples from guinea pigs held at an experimental animal facility. The exact prevalence is difficult to determine as pooled faecal samples were obtained from enclosures, however the high prevalence is likely due to the close proximity of animals to each other, which would facilitate transmission. Very little is known about *Cryptosporidium* in guinea pigs. Surveys of pet guinea pigs in Italy (n=80) and Ecuador (n=40) failed to detect *Cryptosporidium* (d'Ovidio et al., 2015; Vasco et al., 2016). Another study in Brazilian guinea pigs (*Cavia aperea apera*), detected *Cryptosporidium* in 3 of 5 faecal samples by microscopy, and *Cryptosporidium* was also detected in one guinea pig on a farm in the UK, but no genotyping was conducted in either study (Gressler et al., 2010; Smith et al, 2010).
Other studies have identified *C. wrairi* in guinea pigs (Lv et al., 2009; Feng et al., 2011), and until recently this was the only *Cryptosporidium* spp. identified in guinea pigs.

A previous study identified a novel *Cryptosporidium* genotype in guinea pigs (*C. procellus*) obtained from an indoor public market, in Rio de Janeiro, Brazil (Huber et al., 2007), which shared 99.1% similarity with *C. homai* n. sp. at the 18S locus (7 and 6 SNPs difference over 676 bp of submissions DQ885337 and DQ885338, respectively). Phylogenetic analysis grouped them in a clade together with high bootstrap support, suggesting that they are likely the same species. Unfortunately, sequences at the actin and *hsp70* loci were unavailable for this genotype to confirm this.

Trophozoites and meronts of *C. homai* n. sp. measured < 5 µm, but oocysts were not observed. However, it is widely accepted that morphology is not a useful criterion for delimiting *Cryptosporidium* spp. (Fall et al., 2003). Phylogenetic analysis at the 18S, actin and *hsp70* loci confirmed the genetic distinctness of *C. homai* n. sp. which exhibited genetic distances ranging from 3.1% to 15.4%, 14.4% to 24.5%, and 6.6% to 20.9% from all other *Cryptosporidium* spp., respectively. Phylogenetic analysis of concatenated 18S, actin and *hsp70* sequences also exhibited 9.1% to 17.3% genetic distances between *C. homai* n. sp. and other *Cryptosporidium* spp. This clearly supports the species status of *C. homai* n. sp., as these differences are greater than between many currently accepted species. For example, the genetic distance at the 18S and actin loci between *C. hominis* and *C. cuniculus* is 0.4% and 1.6%, respectively (Kvác et al., 2014), and the genetic distance between *C. muris* and *C. andersoni* at the 18S, actin, *hsp70* loci is 0.7%, 3.5% and 2.2%, respectively (Holubová et al., 2016).

In the present study, *C. homai* n. sp. did not group with *C. wrairi* (the only currently valid species in guinea pigs) and exhibited 3.6%, 18.4% and 7.0% genetic distance from this species at 18S, actin and *hsp70* loci, respectively and is clearly a separate species from *C.*
wrai. The phylogenetic relationship of *C. homai* n. sp. to other *Cryptosporidium* spp. is however still ambiguous; at the 18S locus, it was most closely related to *C. felis*, while at the actin locus, it was closest to *C. varanii* (14.4% genetic distance), at the hsp70 locus, it grouped most closely with *C. suis* (6.6%), and a concatenated analysis of all 3 loci, grouped it most closely with *C. varanii* (9.1%). Analysis at additional loci or whole genome analysis will shed more light on the evolutionary relationships between *C. homai* n. sp. and other *Cryptosporidium* spp.

The pathogenic potential of *C. homai* n. sp. is unknown. Histopathological analysis indicated minimal host inflammatory responses, with the lamina propria expanded by mild predominantly lymphocytic-plasmocytic inflammatory infiltrate with the occasional neutrophils, eosinophils and necrotic cells. Intestinal mucosal cells are usually replaced from germinal cells in the crypts, as the older epithelial cells are sloughed at the tips of villi. The occasional mitotic figure is expected in the normal healthy animal as renewal of cells. But large numbers indicate a response to the infection. Histopathological analysis of the infected guinea pig in the present study revealed large numbers of mitotic figures which suggest increased replacement of intestinal cells from hyperplasia. More structured studies are required to clearly define the clinical signs (if any) caused by *C. homai* n. sp.

The host range of *C. homai* n. sp. and its zoonotic potential are also currently unknown, but it has not been previously reported in any other host, suggesting that it may be host specific, however, further analysis is required to determine this.

**Acknowledgements**

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**References**


Angus, K.W., Hutchison, G., Munro, H.M., 1985. Infectivity of a strain of *Cryptosporidium* found in the guinea-pig (*Cavia porcellus*) for guinea-pigs, mice and lambs. J. Comp. Pathol. 95, 151-165.


Fig 1. (A-D) Giemsa-stained section of intestinal epithelium showing moderate to heavy epicellular infection by *Cryptosporidium*, associated with minimal host inflammatory response (isolate E89). Trophozoites (T) and meronts (Me) generally measured less than five micrometres as is typical for *Cryptosporidium*. There was a predominance of merogony with both type I (MeI) and type II meronts (MeII) present. Scale bar: 5 μm.

Fig 2. Evolutionary phylogenetic relationship between *C. homai* n. sp. and *Cryptosporidium* species described to date as inferred by maximum likelihood (ML) analysis of 18S rRNA locus. Percentage support (>50%) from 1000 pseudoreplicates from ML analyses is indicated at the left of the supported node. Scale bars indicate the number of substitutions per nucleotide position.

Fig 3. Phylogenetic relationships between *C. homai* n. sp. and other *Cryptosporidium* species inferred by ML analysis of actin gene. Percentage support (>50%) from 1000 pseudoreplicates from ML analyses is indicated at the left of the supported node. Scale bars indicate the number of substitutions per nucleotide position.

Fig 4. Phylogenetic relationships between *C. homai* n. sp. and other *Cryptosporidium* species inferred by ML analysis of partial hsp70 gene sequences. Percentage support (>50%) from 1000 pseudoreplicates from ML analyses is indicated at the left of the supported node. Scale bars indicate the number of substitutions per nucleotide position.

Fig 5. Phylogenetic relationships between *C. homai* n. sp. and other *Cryptosporidium* species inferred ML analysis of concatenated sequences constructed from partial DNA sequences of 18S, actin and hsp70 loci. Percentage support (>50%) from 1000 pseudoreplicates from ML analyses is indicated at the left of the supported node. Scale bars indicate the number of substitutions per nucleotide position.
Table 1. *Cryptosporidium* species and genotypes reported in rodents.

<table>
<thead>
<tr>
<th>Species/genotype</th>
<th>Major host</th>
<th>Report in rodents</th>
<th>References</th>
<th>Reports in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. rubeyi</em></td>
<td>Rodents</td>
<td>Golden-mantled ground squirrels (<em>Callospermophilus lateralis</em>), Belding's ground squirrels (<em>Urocitellus beldingi</em>), California ground squirrels (<em>Otospermophilus beecheyi</em>), Black-tailed prairie dogs (<em>Cynomys ludovicianus</em>)</td>
<td>Atwill et al., 2004; Pereira et al., 2010; Li et al., 2015; Stenger et al., 2015</td>
<td>No reports in humans to date</td>
</tr>
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<td><em>C. scrofarum</em></td>
<td>Pigs</td>
<td>Asian house rat (<em>Rattus tanezumi</em>), Brown rat (<em>Rattus norvegicus</em>)</td>
<td>Ng-Hublin et al., 2013</td>
<td>Occasionally reported in humans. Kváč et al., 2009a; Kváč et al., 2009b</td>
</tr>
<tr>
<td><em>C. tyzzeri</em></td>
<td>Rodents</td>
<td>Mice (<em>Mus musculus</em>), Brown rats (<em>Rattus norvegicus</em>), Large-footed bat (<em>Myotus adversus</em>), Yellow-necked mouse (<em>Apodemus flavicollis</em>), Bank vole (<em>Myodes glareolus</em>), Common vole (<em>Microtus arvalis</em>)</td>
<td>Morgan et al., 1999; badjer et al., 2003; Karanis et al., 2007; Lv et al., 2009; Ren et al., 2012; Kváč et al., 2013</td>
<td>Occasionally reported in humans. Rasková et al., 2013</td>
</tr>
<tr>
<td><em>C. ubiquitum</em></td>
<td>Ruminants, rodents, primates</td>
<td>Deer mouse (<em>Peromyscus</em>), Eastern grey squirrels (<em>Sciurus carolinensis</em>), Red squirrel (<em>Sciurus vulgaris</em>), Eastern chipmunk (<em>Tamias striatus</em>), Large Japanese field mouse (<em>Apodemus speciosus</em>), Prehensile-tailed porcupines (<em>Coendou prehensilis</em>), Wood chuck (<em>Marmota monax</em>)</td>
<td>Perz and Le Blancq, 2001Feng et al., 2007; Ziegler et al., 2007; Fayer et al., 2010; Murakoshi et al., 2013; Li et al., 2014; Song et al., 2015; Stenger et al., 2015; Qi et al., 2015; Li et al., 2016</td>
<td>Commonly reported. Gatei et al., 2002; Tiangtip and Jongwutiwes 2002; Gatei et al., 2003; Palmer et al., 2003; Gatei et al., 2006; Leoni et al., 2006; Muthusamy et al., 2006; Azami et al., 2007; Al-Brikan et al., 2008; Neira et al., 2012;</td>
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Hasajová et al., 2014; Li et al., 2014; Chappell et al., 2015; Petrincová et al., 2015; Spanakos et al., 2015.

Occasionally reported in humans. Xiao et al., 2002; Leoni et al., 2006; Cama et al., 2007; Wang et al., 2013; Bodager et al., 2015

Xiao et al., 2002; Leoni et al., 2006; Cama et al., 2007; Wang et al., 2013; Bodager et al., 2015

No reports in humans to date.

Leoni et al., 2006; Morse et al., 2007; Waldron et al., 2011; Agholi et al., 2013; Jiang et al., 2014; Liu et al., 2014; Hussain et al., 2017

Commonly reported in humans

Chalmers et al., 1997; Matsui et al., 2000; Torres et al., 2000; Perz and Le Blancq, 2001; Bajer et al., 2003; Lv et al., 2009; Ng-Hublin et al., 2013; Qi et al., 2015; Zhao et al., 2015; Li et al., 2016

Commonly reported in humans.

Morgan et al., 2000; Cama et al., 2003; Gatei et al., 2006; Muthusamy et al., 2006; Leoni et al., 2006; Berrilli et al., 2012; Elwin et al., 2012; Neira et al., 2012; Silverlås et al., 2012; Kurniawan et al., 2013; Sharma

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<td>Lv et al., 2009; Wang et al., 2012</td>
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<td>Ruminants</td>
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<td>Chalmers et al., 1997; Matsui et al., 2000; Torres et al., 2000; Perz and Le Blancq, 2001; Bajer et al., 2003; Lv et al., 2009; Ng-Hublin et al., 2013; Qi et al., 2015; Zhao et al., 2015; Li et al., 2016</td>
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<td>C. meleagridis</td>
<td>Birds and humans</td>
<td>Deer mouse (<em>Peromyscus</em>)</td>
<td>Feng et al., 2007; Bodager et al., 2015</td>
</tr>
<tr>
<td>Species</td>
<td>Hosts</td>
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<td>--------------------------------------------</td>
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<tr>
<td>C. wrairi</td>
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<td>Many reports - Gatei et al., 2006; Leoni et al., 2006; Muthusamy et al., 2006; Azami et al., 2007; Al-Brikan et al., 2008; Neira et al., 2012; Hasajová et al., 2014; Petrincová et al., 2015; Spanakos et al., 2015</td>
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<td>Chipmunk genotype</td>
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<td>Jiang et al., 2005; Feltus et al., 2006; Feng et al., 2007; Kváč et al., 2008; Insulander et al., 2013; Lebbad et al., 2013; Guo et al., 2015; Song et al., 2015</td>
<td>Emerging human pathogen - Feltus et al., 2006; ANOFEL, 2010; Lebbad et al., 2013; Guo et al., 2015</td>
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<td>Genotype</td>
<td>Order</td>
<td>Species</td>
<td>Authors, Year(s)</td>
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<td>Siberian chipmunk (<em>Tamias sibiricus</em>)</td>
<td>Lv et al., 2009</td>
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<td>Deer mouse genotype I</td>
<td>Rodents</td>
<td>Deer mouse (<em>Peromyscus</em>)</td>
<td>Feng et al., 2007</td>
</tr>
<tr>
<td>Deer mouse genotype II</td>
<td>Rodents</td>
<td>Deer mouse (<em>Peromyscus</em>)</td>
<td>Feng et al., 2007</td>
</tr>
<tr>
<td>Deer mouse genotype III</td>
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<td>Deer mouse (<em>Peromyscus</em>)</td>
<td>Feng et al., 2007</td>
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<td>Deer mouse genotype IV</td>
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<td>Deer mouse (<em>Peromyscus</em>)</td>
<td>Feng et al., 2007</td>
</tr>
<tr>
<td>Ferret genotype</td>
<td>Rodents</td>
<td>Siberian chipmunk (<em>Tamias sibiricus</em>), River otters (Lontra canadensis), Red squirrel (<em>Sciurus vulgaris</em>), Guinea pig (<em>Cavia porcellus</em>), Hamster (<em>Phodopus sungorus</em>)</td>
<td>Kváč et al., 2008; Lv et al., 2009; Feng et al., 2011; Li et al., 2016</td>
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<td>Ground squirrel genotype I</td>
<td>Rodents</td>
<td>Thirteen-lined ground squirrel (<em>Ictidomys tridecemlineatus</em>)</td>
<td>Stenger et al., 2015</td>
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<td>Ground squirrel genotype II</td>
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<td>Ground squirrel genotype III</td>
<td>Rodents</td>
<td>Thirteen-lined ground squirrel (<em>Ictidomys tridecemlineatus</em>)</td>
<td>Stenger et al., 2015</td>
</tr>
<tr>
<td>Hamster genotype</td>
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<td>Siberian hamster (<em>Phodopus sungorus</em>)</td>
<td>Lv et al., 2009</td>
</tr>
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<td>Mouse genotype II</td>
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<td>House mouse (<em>Mus musculus</em>)</td>
<td>Foo et al., 2007; Silva et al., 2013</td>
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<td>Mouse genotype III</td>
<td>Rodents</td>
<td>House mouse (<em>Mus musculus</em>)</td>
<td>Silva et al., 2013</td>
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<td>Muskrat genotype I</td>
<td>Rodents</td>
<td>Muskrat (<em>Ondatra zibethicus</em>), Boreal red-backed vole (<em>Myodes rutilus</em>).</td>
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<td>Rodents</td>
<td>Muskrat (<em>Ondatra zibethicus</em>), Deer mouse (<em>Peromyscus maniculatus</em>), Meadow vole (<em>Microtus pennsylvanicus</em>)</td>
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<td>Kimura et al., 2007; Chalmers et al., 2010; Ng-Hublin et al., 2013</td>
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<tr>
<td>Rat genotype II</td>
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<td>Lv et al., 2009; Ng-Hublin et al., 2013; Silva et al., 2013</td>
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<td>Tanezumi rat <em>(Rattus tanezum)</em>, Asian house rat <em>(Rattus tanezum)</em>, Brown rat <em>(Rattus norvegicus)</em></td>
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<td>Skunk/ skunk-like genotype</td>
<td>Skunk</td>
<td>Eastern grey squirrel <em>(Sciurus carolinensis)</em>, American red squirrels <em>(Tamiasciurus hudsonicus)</em></td>
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<td>Vole genotype</td>
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<td>Novel genotype</td>
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<td>Guinea pig <em>(Cavia porcellus)</em></td>
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Table 2. List of faecal samples collected for this study, from guinea pigs, held at the University of Western Australia Animal Care Services, Perth, Australia.

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<td>12/12/2016</td>
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<tr>
<td>Z3</td>
<td>12/12/2016</td>
<td>Faeces - Individual</td>
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<tr>
<td>Z4</td>
<td>12/12/2016</td>
<td>Faeces – Pooled</td>
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<td>Z5</td>
<td>12/12/2016</td>
<td>Faeces – Pooled</td>
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Cryptosporidium homai n. sp.