



Biology of *Cryptosporidium* from marsupial hosts

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ABSTRACT

The majority of biological data on *Cryptosporidium* has been collected from humans and domestic animal hosts which creates a bias in knowledge on the biodiversity and evolution of this parasite genus. Further to understanding *Cryptosporidium* biology are studies encompassing broad hosts that represent diverse taxa sampled across wide geographic ranges. Marsupials represent a group of wildlife hosts from which limited information on *Cryptosporidium* is available. As marsupial hosts are an ancient mammalian lineage they represent an important group for studying parasite evolution. This review summarises information of the biology, epidemiology and evolution of *Cryptosporidium* in marsupial hosts, and discusses the importance of further understanding interactions in this parasite–host system.

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1. Introduction

Cryptosporidium host occurrence and genetic diversity is an area of rapidly expanding knowledge. The parasite has been identified in greater than 150 vertebrate hosts, with 22 described species and greater than 40 cryptic species (genotypes) identified across a range of taxa (Fayer, in this issue). Despite knowledge on the extent of host distribution of *Cryptosporidium*, much of the biological data of the parasite is derived from *Cryptosporidium* from a limited host range, such as clinically relevant hosts for human and domestic animal health. From an evolutionary perspective this bias is problematic, as sampling from a broad host range is necessary, particularly to prevent biased inferences in phylogenies. Additionally, data on parasites from host species of ancient evolutionary origins may potentially provide information on deep evolution. This review brings together the limited information on *Cryptosporidium* from marsupials, and emphasises the importance of understanding *Cryptosporidium* from marsupial hosts to the understanding the evolutionary history of this genus.

2. Marsupials

Marsupials are divided into two broad groups according to their geographic distributions in Australasia and America. Extant marsupials are classified into seven orders and 19 families, and are represented by some 300 species (Kirsch, 1977). Although the Australasian taxa have been geographically isolated from American

taxa for approximately 40 million years, human influence has seen Australasian and American species dispersed to different continents for zoological displays and for the pet trade, particularly in United States of America. In Australia, marsupials represent natural wildlife species, and hence are naturally present in water catchments across the country. Many marsupial species have also adapted to human settlement, such as possums in urban areas, and kangaroos in agricultural areas. The dispersal of Australian wildlife species into areas dominated by human activities increases interactions between humans and introduced placental mammal species such as cattle, sheep, dogs and cats. Interactions at the wildlife, domestic animal, human interface present risks for pathogen transfer and zoonoses that are conducive to emerging disease (Daszak et al., 2000), and predispose wildlife to parasite species that are atypical in their natural habitats.

3. Occurrence of *Cryptosporidium* in marsupials

Cryptosporidium oocysts have been identified in 16 species of marsupials (Table 1) with 14 hosts from Australia and a single host species from each of North America and South America. The 16 host species represent eight marsupial families indicating a broad host distribution across marsupial taxa. *Cryptosporidium* is also geographically dispersed across the Australian continent as evidenced by reports of marsupial hosts from four states (Tasmania, South Australia, Western Australia and New South Wales). *Cryptosporidium* has also been identified in brushtail possums *Trichosurus vulpecula* in New Zealand (Chilvers et al., 1998). Brushtail possums were introduced into New Zealand from Australia in 1837 to establish a fur trade, they are now considered a pest species (Montague,

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Table 1Reports on the identification of *Cryptosporidium* in marsupial hosts from Australasia, America and New Zealand.

Host species and common name	Detection method	Wild or captive	Genotyping and species	Reference
<i>Antechinus stuartii</i> Brown antechinus	Intestine Zeihl Neelson	Wild	No	Barker et al. (1978)
<i>Phascogale cinereus</i> Koala	Faeces smear	Captive (hand raised)	Yes <i>C. fayeri</i>	Morgan et al. (1997)
<i>Macropus giganteus</i> Eastern grey kangaroo	Faeces smear Faeces IMSFC Faeces IMSFC	Captive Captive Wild	No No Yes <i>C. fayeri</i> and <i>C. macropodum</i>	Jacob (1992) Power et al. (2003) Power et al. (2004)
<i>Isoodon obesulus</i> Southern brown Bandicoot	Intestine Zeihl Neelson	Wild	No	O'Donoghue (1995)
<i>Macropus rufous</i> Red kangaroo	Intestine Zeihl Neelson	Captive (hand raised)	Yes <i>C. fayeri</i>	O'Donoghue (1995)
<i>Thylogale billardieri</i> Tasmanian pademelon	Faeces smear	Captive	No	O'Donoghue (1995)
<i>Didelphis virginiana</i> Virginia opossum	Faeces PCR	Captive	Yes Opossum genotypes I and II	Xiao et al. (2003)
<i>Thylogale thetis</i> Pademelon	Faeces IMSFC	Captive	No	Power et al. (2003)
<i>Trichosurus vulpecula</i> Brushtail possum	Faeces IMSFC	Captive Wild	No Yes Brushtail possum genotypes I and II	Power et al. (2003) Hill et al. (2008)
<i>Petrogale xanthopus</i> Yellow footed rock wallaby	Faeces IMSFC	Captive	Yes <i>C. fayeri</i>	Power et al. (2003) Power et al. (2009)
<i>Macropus rufogriseus</i> Red necked wallaby	Faeces IMSFC	Wild	No	Power (2002)
<i>Vombatus ursinus</i> Wombat	Faeces IMSFC	Wild	No	Power (2002)
<i>Macrotis lagotis</i> Bilby	Faecal smear	Captive	Yes <i>C. muris</i>	Warren et al. (2003)
<i>Macropus fuliginosus</i> Western grey kangaroo	Faeces PCR	Wild	Yes <i>C. fayeri</i> <i>C. macropodum</i>	McCarthy et al. (2008)
<i>Didelphis albiventris</i> Mouse opossum	Faeces Zinc floatation	Wild	No	Zanette et al. (2008)
<i>Peremeles bougainville</i> Western-barred bandicoot	Faeces PCR	Wild	Yes <i>C. fayeri</i>	Weilinga et al. (unpublished)
<i>Wallabia bicolor</i> Swamp wallaby	Faeces PCR	Wild	Yes <i>C. macropodum</i>	Ryan et al. (unpublished)

2000). Nine of the *Cryptosporidium* identifications in marsupials were prior to the current understanding of the complexity of the genus *Cryptosporidium*, and the need for molecular tools to identify species. Therefore samples were either not identified to species level, or classified as *Cryptosporidium parvum*, in line with past taxonomy. In light of current knowledge regarding the necessity for molecular tools for determining *Cryptosporidium* species, the identifications in the brown antechinus, southern brown bandicoot, pademelon, red neck wallaby, wombat and the mouse opossum are only to the level of genus as no molecular typing was performed in these studies.

4. Biodiversity of *Cryptosporidium* from marsupials

The first *Cryptosporidium* isolate to be genetically characterised was from a Koala in 1997 (Table 1) (Morgan et al., 1997). Analysis showed the isolate to be genetically distinct from other *Cryptosporidium* species, and it was referred to as the *Cryptosporidium* marsupial genotype (Morgan et al., 1997; Sulaiman et al., 2000a,b; Xiao et al., 1999a,b, 2000). A study on eastern grey kangaroos resulted in identification of a second genetically distinct *Cryptospori-*

dium isolate from marsupials, named marsupial genotype II (Power et al., 2004). Since the identification of *Cryptosporidium* marsupial genotypes I and II, analyses at multiple loci including 18S rDNA, internal transcribed spacer 1, heat shock protein 70 (HSP70), *Cryptosporidium* oocyst wall protein (COWP) and actin for both genotypes, and dihydrofolate thymidylate reductase (Morgan et al., 1999) and glycoprotein 60 (Power et al., 2009) for *C. fayeri*, has confirmed that marsupial genotypes I and II are genetically distinct from each other and from described species and strains. As a result of this the two marsupial genotypes were recently named as new *Cryptosporidium* species, *Cryptosporidium fayeri* (marsupial genotype I) (Ryan et al., 2008) and *Cryptosporidium macropodum* (Marsupial genotype II) (Power and Ryan, 2008). Further diversity in *Cryptosporidium* isolates from marsupials were identified in isolates from opossums undergoing rehabilitation in captivity (Xiao et al., 2003). One of these isolates (opossum genotype I) was greater than 99% similar to *C. fayeri* (marsupial genotype I) at three loci, and has since been included as a subtype of this species (Ryan et al., 2008). The second isolate, opossum genotype II, represents a cryptic species (Xiao et al., 2003) that differs from *Cryptosporidium* described in other vertebrate hosts. More recently, novel *Cryptosporidium* marsupial genotypes (brushtail possum genotype I)

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