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Subtype distribution of *Blastocystis* isolates from a variety of animals from New South Wales, Australia

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ABSTRACT

A total of 438 stool samples from 38 different species of animal from seven different locations were studied for the presence of *Blastocystis.* PCR analysis was completed on all samples and DNA sequence data from the rDNA were submitted to subtype allocation. There was a total of 80 (18%) sequences from 18 species, and nine different subtypes were identified – ST1, ST2, ST3, ST4, ST5, ST7, ST11, ST12 and ST13. This is the first report of *Blastocystis* from the eastern grey kangaroo, red kangaroo, wallaroo, snow leopard and ostrich. This study highlights the need for further investigation into the genetic diversity of *Blastocystis* which could help show the zoonotic potential of *Blastocystis.*

1. Introduction

Blastocystis is a single celled enteric parasite that is commonly found in humans and a number of different animal species (Stenzel and Boreham, 1996; Tan et al., 2002; Yoshikawa et al., 2004). *Blastocystis* has shown to exhibit extensive genetic diversity and through many recent studies on the small subunit (SSU) ribosomal (r)RNA gene, 13 subtypes (ST) have been identified from humans and a variety of animals including non-human primates, other mammals and birds. (Abe et al., 2003a; Noel et al., 2003; Stensvold et al., 2007, 2009a; Parkar et al., 2010). There are some subtypes that seem to be more host specific such as ST3 which is the most common subtype isolated in humans (Ozyurt et al., 2008; Wong et al., 2008; Souppart et al., 2009), whereas other subtypes appear to show low host specificity which raises the possibility of zoonotic transmission. The transmission of *Blastocystis* from animals living in a community based environment is feasible via the faecal–oral route and there is supporting evidence that this does occur. For example, a higher incidence of infection rates was reported in zoo keepers who came in close contact with animal enclosures (Parkar et al., 2010). Though there has been a reasonable number of animal species studied for the identification of *Blastocystis*, more studies need to be carried out to understand the possible zoonotic transmission of this parasite and to identify whether other subtypes of *Blastocystis* do exist.

The aim of this study was to investigate the presence of *Blastocystis* in a variety of animals from several different locations and to genetically characterise them by SSU rDNA sequencing. This data aims to expand our knowledge on the role of animals in the epidemiology of *Blastocystis*.

2. Materials and methods

2.1. Animal samples

A total of 438 stool samples were collected from 38 different species of animal from seven different locations over a two year period – a farm in western Sydney, a Sydney area veterinary practice, Cat Protection Society (CPS), a rural Aboriginal community (King et al., 2012) a rural field

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Table 1
Location and number of animal species included in this study and the number of *Blastocystis* positive samples for each species.

Location	Host	Scientific name	Samples (*n*)	Positive (*n*)
Farm	Horse	*Equus ferus caballus*	1	0
	Guinea Pig	*Cavia porcellus*	2	0
	Chicken	*Gallus gallus domesticus*	25	1
	Rabbit	*Oryctolagus cuniculus*	1	0
	Guinea fowl	*Numida meleagris*	2	2
Vet/CPS	Cat	*Felis catus*	43	0
	Dog	*Canis lupus familiaris*	11	0
	Possum	*Trichosurus vulpecula*	1	0
	Monkey	*Macaca* sp.	1	1
	Frog	*Litoria ewingii*	1	0
Rural	Dog	*Canis lupus familiaris*	45	0
Field	Deer	*Cervus elaphus*	50	1
Piggery	Pig	*Sus scrofa domesticus*	83	13
Bushland, NSW	Eastern Wallaroo	*Macropus robustus*	3	3
	Swamp Wallaby	*Wallabia bicolor*	1	0
Taronga zoo	Asian Elephant	*Elephas maximus*	20	11
	Tiger	*Panthera tigris*	10	0
	Lion	*Panthera leo*	10	0
	Ostrich	*Struthio camelus*	10	6
	Chimpanzee	*Pan troglodytes*	10	7
	Orang Utan	*Pongo abelii*	10	9
	Gorilla	*Gorilla gorilla*	10	10
	Snow Leopard	*Panthera uncia*	6	1
	Meerkat	*Suricata suricatta*	10	0
	Kodiak Bear	*Ursus arctos middendorffi*	5	0
	Francois Langur	*Trachypithecus francoisi*	6	5
	Giraffe	*Giraffa camelopardalis*	6	1
	Zebra	*Equus burchellii*	4	0
	Cassowary	*Casuarius casuarius*	10	2
	Brazillian Tapir	*Tapirus terrestris*	3	0
	Southern Hairy Nosed Wombat	*Lasiorhinus latifrons*	3	0
	Common Wombat	*Vombatus ursinus*	2	0
	Western Grey Kangaroo	*Macropus fuliginosus*	2	1
	Eastern Grey Kangaroo	*Macropus giganteus*	4	3
	Red Kangaroo	*Macropus rufus*	4	3
	Short beaked echidna	*Tachyglossus Aculeatus*	1	0
	Long beaked echidna	Zaglossus bartoni	2	0
	Koala	*Phascolarctos cinereus*	10	0
	Tasmanian Devil	*Sarcophilus harrisii*	10	0

(Cinque et al., 2008), a piggery (Armson et al., 2009), western New South Wales (NSW) bush area and Taronga Zoo, Sydney.

2.2. DNA extraction and PCR

Stool samples were frozen immediately after collection and DNA was extracted using the Bioline Isolate fecal DNA kit as per manufacturer's instructions. DNA was then submitted to PCR for the detection of *Blastocystis* sp. using a previously described method (Stensvold et al., 2007) targeting the SSU rDNA specific to the *Blastocystis* region. PCR reactions of 25 μl were performed using PuReTaq Ready-To-Go (Amersham Pharmacia Biotech) PCR beads (each containing – 1.5 units Taq DNA polymerase, 10 mM Tris–HCl pH9, 50 mM KCl, 1.5 mM MgCl, 200 μM of each dNTP and stabilizers, including BSA), 2 μl of genomic DNA extract and 0.5 μm of each PCR primer (F1 and BHCRSeq3).

2.3. Sequencing analysis

DNA sequence analysis was performed on all PCR positive samples. PCR products were purified using the QIAquick™ PCR purification Kit (Qiagen) as per the manufacturer's instructions and sent to the Australian Genome Research Facility (Westmead Millennium Institute, Sydney) for sequencing in both directions. The SSU rDNA sequences were then compared to those available in the GenBank database using the BLASTN program run on the National Centre for Biotechnology Information server (http://www.ncbi.nlm.nih.gov/BLAST).

3. Results

A total of 438 stool samples were collected from 38 different species of animal over a two year period from seven different locations within NSW, Australia (Table 1). Eighty samples (18%) from a total of 18 species of animal were positive for *Blastocystis* sp. from PCR. All PCR positive samples were sequenced and nine different subtypes were identified by Blast searching – ST1, ST2, ST3, ST4, ST5, ST7, ST11, ST12 and ST13 (Table 2). There were four mixed infections seen in the chimpanzees with them found to harbour both ST1 and ST11. All of the primate species noted positives as well as four of the five marsupial macropod species. ST1 was the most common subtype isolated from this group of