Molecular epidemiology of *Blastocystis* sp.

By

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Certificate of Original Authorship

This study was carried out in the Microbiology Department, St. Vincent's Hospital, Sydney under the supervision of Professor John Ellis and Dr Damien Stark. I certify that this thesis has not been submitted previously as part of any course or degree other than in fulfilment of the requirements of a PhD degree at the University of Technology, Sydney. I certify that this thesis has been written by me and the vast majority of work described was completed by me. All other contributors have been acknowledged throughout this thesis as necessary.

I hereby certify that the above statements are true and correct:

Tamalee Roberts (PhD candidate): _____

Date: _____

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Peer reviewed Journal Publications

Chapter 1:

Roberts, T., Stark, D., Harkness, J., Ellis, J. 2014. Update on the diagnostic tools and molecular epidemiology of *Blastocystis* sp. Journal of Medical Microbiology and Diagnosis, **3**:131

Roberts, T., Stark, D., Harkness, J., Ellis, J. 2014. Update on the pathogenic potential and treatment options for *Blastocystis* sp. Gut Pathogens, **6**:17

Chapter 2:

Roberts, **T.,** Barratt, J., Harkness, J., Ellis, J., and Stark, D. 2011. Comparison of Microscopy, Culture, and Conventional Polymerase Chain Reaction for Detection of *Blastocystis* sp. in Clinical Stool Samples. Am. J. Trop. Med. Hyg., 84(2), 308–312

Chapter 3:

Roberts, **T.,** Stark, D., Harkness, J., and Ellis, J., 2013a. Subtype distribution of *Blastocystis* isolates identified in a Sydney population and pathogenic potential of *Blastocystis*. Eur J Clin Microbiol Infect Dis. 32, 335–343

Chapter 4:

Roberts, **T.,** Stark, D., Harkness, J., and Ellis, J., 2013b. Subtype distribution of *Blastocystis* isolates from a variety of animals from New South Wales, Australia. Veterinary Parasitology, 196, 85-89

Chapter 6:

Roberts, **T.,** Ellis, J., Harkness, J., Marriott, D., and Stark, D. 2014. Treatment failure in patients with chronic *Blastocystis* infection. Journal of Medical Microbiololgy, 63, 252–257

Conference Presentations

Roberts, T., Stark, D., Harkness, J., Ellis, J. *Blastocystis* molecular epidemiology and susceptibility patterns from Sydney, Australia. 6th Asean congress on Tropical Medicine and Parasitology 5-7th Mar 2014. Kuala Lumpar, Malaysia. Oral presentation

Roberts, T., Stark, D., Harkness J., Ellis, J. Molecular epidemiology and subtype susceptibility patterns of *Blastocystis* from Sydney, Australia. 8th European Congress on Tropical Medicine and International Health 10- 13 Sep 2013, Copenhagen, Denmark. Oral presentation

Roberts, T., Stark, D., Harkness, J., Ellis, J. *Blastocystis* subtype distribution from a variety of animals including Australian native animals. World Association for the Advancement of Veterinary Parasitology 25-29 Aug 2013, Perth, Australia. Oral presentation

Roberts, T., Stark, D., Harkness, J., Ellis, J. Subtype distribution of *Blastocystis* from a variety of animals including Australian native fauna. International Congress of Protistology XIV 28 July- 2 Aug 2013, Vancouver, Canada. Oral presentation

Roberts, T., Stark, D., Harkness, J., Ellis, J. Pathogenic potential of *Blastocystis sp.*RNSH Scientific Research Meeting, November 2012, Sydney, Australia. Oral presentation

Roberts, T., Stark, D., Harkness, J., Ellis, J. Molecular epidemiology and clinical aspects of *Blastocystis sp.*- an Australian perspective. American Society of Parasitologists 86th Annual Meeting 1-4 June 2011, Anchorage, USA. Oral Presentation

Roberts, T., Stark, D., Harkness, J., Ellis, J. Phylogenetic Analysis and Pathogenic Potential of *Blastocystis sp.* From Sydney, Australia. American Society for Microbiology 111th General Meeting 21-25 May 2011, New Orleans, USA. Oral presentation

Roberts, T., Stark, D., Harkness, J., Ellis, J. Molecular epidemiology of *Blastocystis sp.* from Sydney, Australia. 12th International Congress of Parastiology 15-21 Aug 2010, Melbourne, Australia. Poster presentation

Roberts, T., Stark, D., Harkness, J., Ellis, J. Genotyping of *Blastocystis* sp. from symptomatic patients in Sydney, Australia. ASP and ARC/NHMRC Research Network for Parasitology Annual Conference 12-15 July 2009, Sydney, Australia. Oral presentation

Abbreviations

AIDS Acquire immunodeficiency syndrome

ART Antiretroviral therapy

bp base pairs

CDC Centre for Disease Control

CPS Cat protection society

DNA Deoxyribonucleic acid

ELSIA Enzyme linked immunosorbant assay

HIV Human immunodeficiency virus

HM Haematological malignancies

IBD Inflammatory bowel disease

IBS Irritable bowel syndrome

IFN Interferon

IgA Immunoglobulin alpha

IgG Immunoglobulin gamma

IL Interleukin

kb Kilobases

kDa Kilo Dalton

MALDI-TOF Matrix-assisted laser desorption/ionisation time-of-flight

MBD Modified Boeck and Drbohlav's

Mb Megabase

mg milligram

MIC Minimum inhibitory concentration

MLC Minimum lethal concentration

ml millilitre/s

MLO Mitochondria like organelle

MLST Multilocus sequence typing

NCBI National center for Biotechnology Information

NJ Neighbour joining

NSW New South Wales

PBS Phosphate buffered saline

PCR Polymerase chain reaction

PCV Peace corps volunteers

rDNA ribosomal Deoxyribonucleic acid

RFLP Restriction fragment length polymorphism

RNA Ribonucleic acid

rRNA ribosomal Ribonucleic acid

qPCR Quantitative polymerase chain reaction

SAF Sodium acetate acetic acid formalin

SNPs Single nucleotide polymorphisms

SSU Small subunit

ST Subtype

SUPAMAC Sydney University Prince Alfred Macromolecular Analysis Centre

TMP-SMX trimethroprim-sulfamethoxazole

TNF Tumor necrosis factor

TYGM-9 Tryptose, yeast extract, glucose, methionine 9

µl microlitre

µg microgram

w/v weight per volume

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Abstract

Blastocystis sp. is the most common enteric protist of the human gastrointestinal tract. There has been continual controversy over the role *Blastocystis* plays in causing gastrointestinal disease in humans. It has been suggested to be a pathogen or an opportunistic commensal and it has also been suggested that pathogenicity could be related to subtype (ST) determined by molecular methods. Until recently there was little known about this protist in terms of epidemiology, pathogenicity and treatment. Clinical diagnosis has traditionally been based on microscopy of wet preparations or permanent stains but there has recently been a push towards more sensitive techniques such as culture and polymerase chain reaction (PCR). The correct diagnosis of *Blastocystis* is necessary for epidemiological and clinical studies which will aid in determining the actual role of this parasite in the gut and in producing disease. Due to the lack of knowledge on the pathogenicity of this parasite, research into treatment options is limited. Metronidazole is a commonly used anti-parasitic drug that has frequently been used for *Blastocystis* treatment. There is evidence that this drug may not actually have much efficacy at all on *Blastocystis* and therefore be the incorrect treatment option.

This project was designed to address some of the shortcomings in the literature surrounding this parasite. The overall aim of the project was to describe the molecular epidemiology of *Blastocystis sp.* from Australia and comment on the pathogenicity of *Blastocystis* in humans. To be able to determine the molecular epidemiology, it was necessary to use the correct diagnostic method and therefore the first aim of this study was to determine the best diagnostic technique used for the detection of *Blastocystis* (aim 1 of this study). Five different techniques were tested for their sensitivity for detecting *Blastocystis* and it was found that microscopy of a permanent stain was the least sensitive at detecting *Blastocystis* and that PCR was the most sensitive technique. Once the most sensitive diagnostic technique was established it was then possible to determine the prevalence of *Blastocystis* within the Sydney population from clinical samples (aim 2 of this study). It was found that there was a 19% incidence of *Blastocystis* in this population and seven subtypes (STs) were identified by sequencing- ST1, ST2, ST3, ST4, ST6, ST7 and ST8. ST3 was found to be the most common ST in this population.

This study then investigated the prevalence of *Blastocystis* in animals and determined the STs present (aim 3 of this study). There were 38 different species of animal from seven different locations investigated for the presence of *Blastocystis* using PCR. There were 80 (18%) positive isolates from 18 species, and nine different STs 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 has expanded current knowledge on the host range of *Blastocystis*.

Blastocystis is associated with symptoms in humans similar to irritable bowel syndrome (IBS) such as bloating, diarrhoea and abdominal pain and therefore this study aimed to look at the relationship between *Blastocystis* and IBS (aim 4 of this study). This study showed that though there was not a significantly higher percentage of *Blastocystis* seen in the IBS group compared to the control group, there was a difference in the STs present with ST4 only present in the IBS group. This study also highlighted the need for full microbiological work-up before a diagnosis of IBS can be given as *Blastocystis,* along with other microbes, may actually be a contributor to the disease process.

The final part of this study was to look at treatment options for *Blastocystis.* Due to the lack of knowledge on the pathogenicity of *Blastocystis* there have only been a few studies on treatment options and much more information is needed (aim 5 of this study). This study followed 18 patients with chronic *Blastocystis* infection who were treated with a variety of antimicrobials. It was seen that the most common drug treatment of choice, metronidazole, was not effective for the clearance of *Blastocystis.* This study also highlighted the chronic nature of *Blastocystis* infection in the absence of any other infectious agents. This study also carried out *in vitro* testing for four common human *Blastocystis* STs (ST1, ST3, ST4 and ST8) against 12 commonly used antimicrobials- metronidazole, paromomycin, ornidazole, albendazole, ivermectin, trimethoprim- sulfamethoxazole (TMP-SMX), furazolidone, nitazoxonide, secnidazole, fluconazole, nyastatin and itraconazole. Cultures were maintained in media that was determined the best for *Blastocystis* growth from aim 1 of this study. From this *in vitro* study the lack of efficacy of commonly used antimicrobials for the treatment of *Blastocystis* was shown in particular metronidazole, paromomycin and a triple therapy combination of furazolidone,

nitazoxanide and secnidazole. This study did show the efficacy of two drugs- TMP-SMX and ivermectin and suggested the use of these treatments instead of metronidazole.

Each of these studies aims has furthered the knowledge on *Blastocystis* epidemiology, pathogenicity and treatment options. This is the largest molecular epidemiological study to be completed in Australia and also the largest animal study to be undertaken thus far. Overall, this PhD project has contributed significantly by enhancing and extending current knowledge on *Blastocystis* and will hopefully encourage future research on this fascinating protist.