Molecular epidemiology of *Blastocystis* sp.

By

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Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (Science) at the University of Technology, Sydney
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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Chapter 2:


Chapter 3:


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Chapter 6:

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


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# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AIDS</td>
<td>Acquire immunodeficiency syndrome</td>
</tr>
<tr>
<td>ART</td>
<td>Antiretroviral therapy</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
</tr>
<tr>
<td>CPS</td>
<td>Cat protection society</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ELSIA</td>
<td>Enzyme linked immunosorbant assay</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HM</td>
<td>Haematological malignancies</td>
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<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
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<tr>
<td>IBS</td>
<td>Irritable bowel syndrome</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin alpha</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin gamma</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>kb</td>
<td>Kilobases</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo Dalton</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Matrix-assisted laser desorption/ionisation time-of-flight</td>
</tr>
<tr>
<td>MBD</td>
<td>Modified Boeck and Drbohlav’s</td>
</tr>
<tr>
<td>Mb</td>
<td>Megabase</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MLC</td>
<td>Minimum lethal concentration</td>
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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, paromomycin and ornidazole.
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.