New discovery and ultrastructural description of *Dientamoeba fragilis* cysts and the establishment of an animal model for their study

By

Varuni Subashini Munasinghe

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School of Life sciences,

Ithree Institute,

Faculty of Science,

University of Technology

Australia

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Certificate of original authorship

This study was conducted in the School of Medical and Molecular Biosciences and i3 institute, Faculty of Science, University of Technology, Sydney and in the Microbiology Department, St. Vincent's Hospital Sydney, under the supervision of Professor John T. Ellis and Dr. Damien Stark.

I certify that the work in this thesis are all done in part for the fulfilment of this thesis and has not been submitted as part of requirements for a degree except within this thesis.

Finally, I certify that the thesis has been written by me with editorial support from my supervisors, Professor Michael Wallach, Professor John Ellis and Dr. Damien Stark as acknowledged in individual chapters. I have acknowledged all the help, support and resources that I received in fulfilment of this work. Finally, I certify all literature and information sources used have been indicated in this thesis.

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Table of contents

List of figures

Referred publications arising from this thesis

- **Munasinghe, V.S**., Stark, D., Ellis, J.T., 2012. New advances in the in-vitro culture of Dientamoeba fragilis. Parasitology 139, 864-869.

- **Munasinghe, V.S**., Vella, N.G.F., Ellis, J.T., Windsor, P.A., Stark, D., 2013. Cyst formation and faecal–oral transmission of Dientamoeba fragilis – the missing link in the life cycle of an emerging pathogen. Int J Parasitol. 43, 879-883

Conference Proceedings

■ **Munasinghe, V. S.**, J., Ellis, J. T., and Stark, D. New Advannces in the *in vitro* culture of *Dientamoeba fragilis*, **Poster presentation,** ICOPA, Melbourne, Australia, 15 -19th August, 2010.

■ **Munasinghe, V. S.**, J., Ellis, J. T., and Stark, D. New Advances in the *in vitro* culture of *Dientamoeba fragilis*, **Poster presentation**, 27th RNSH.UTS.USYD. Kolling Scientific Reserach Meeting, Sydney, Australia, 9-10th November, 2010.

■ **Munasinghe, V. S.**, J., Ellis, J. T., and Stark, D. New Advances in the *in vitro* culture of *Dientamoeba fragilis* for further studies on pathogensis and gut immune responses, **Poster presentation**, ,meeting on microbial pathogensis and host response, Cold Spring Harbour Laboratory, New York, USA, 13-17th September, 2011.

■ **Munasinghe, V. S.**, J., Ellis, J. T., and Stark, D. New Advances in the *in vitro* culture of *Dientamoeba fragilis*, **Poster presentation**, ASP Annual Conference, Cairns, Australia, 10-13th July 2011.

■ **Munasinghe, V. S.**, J., Ellis, J. T., and Stark, D. New Advances in the *in vitro* culture of *Dientamoeba fragilis*, **Poster presentation**, "Malnutrition, Gut-Microbial Interactions and Mucosal Immunity to Vaccines." Keystone symposia, New Delhi, India , 7-11th November, 2012

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Abbreviations

Terms:

Ax	Axostyle
ATCC	American Type Culture Collection
BB	Basal Body
Co	Costa
CWP	Cyst wall protein
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
EBSS	Earle's Balanced Salt Solution
Gc	Golgi Complex
HCl	Hydrochloric Acid
IBS	Irritable bowel syndrome
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
Pf	Parabasal Filament
RNA	Ribonucleic Acid
RNase	Ribonuclease
rRNA	Ribosomal RNA
TEM	Transmission Electron Microscopy

Units:

°C	Degree Celsius
G	Relative Centrifugal Force
KDa	Kilo Daltons

Kg	kilogram
M	Molar
μ M	Micromolar
μ m	Micrometre
μ L	Microlitre
mg	Milligram
mL	Millilitre
mM	Millimolar
min	Minute
ng	Nanogram
nm	Nanometer

Abstract

Dientamoeba fragilis is a pathogenic protozoan parasite which causes diarrhoea and gastrointestinal disease in humans with a propensity for chronic infections. Although *Dientamoeba* was discovered over a century ago, its life cycle and mode of transmission are poorly defined. No cyst stage has been described in the scientific literature and no animal models were available for the further study of this parasite in the past. The clinical and pathologic features of Dientmaoebiasis, along with the pathogenic mechanisms of the disease and the nature of the host defence weren't fully elucidated.

In this study the *in vitro* culture for *D. fragilis* was established and further improved, which increased the trophozoite numbers to large and sufficient numbers for further study. A new overlay was designed for the *in vitro* culturing of *D. fragilis* trophozoites which is Earle's balanced salt solution (EBSS) enriched with ferric ammonium citrate and cholesterol. The large trophozoite numbers obtained from the *in vitro* culture using this overlay enabled their use to inoculate experimental animals in order to develop an animal model. A rodent model was developed using BALB/C mice and rats to study the mode of transmission of this parasite, which remained a mystery in the past. This was an important step in this research as attempts to establish an animal model for this parasite have been unsuccessful in the past. Moreover, the animal model s enabled us to fulfil three criteria of Koch's postulates for *D. fragilis*. The most important finding of this study was the discovery of a cyst stage of *D. fragilis,* adding to the evidence on the mode of transmission of *D. fragilis* via cysts. Ultrastructural observations of the cysts were carried out in detail using transmission electron microscopy. These studies of cysts showed a clear cyst wall surrounding an encysted parasite. The cyst wall was double layered with an outer fibrillar layer and an inner layer enclosing the parasite. Hydrogenosomes, endoplasmic reticulum and nuclei were present in the cysts. Pelta-axostyle structures, costa and axonemes were identifiable and internal flagella were present. These cysts shared similar morphological characteristics to those of *Giardia*, *Histomonas,* which belong to the same family as *D. fragilis* showing its phylogenetic relationship with these parasites. This study provides additional novel details and knowledge of the ultrastructure of the cyst stage of *D. fragilis,* that plays an important role in the mode of transmission of this pathogen.

The data support the pathogenic potential of this organism, demonstrates chronic infection and parasite carriage along with prolonged shedding of the organism. The recurrent nature of Dientamoebiasis in human hosts could be attributable to the cysts stage which is more resistant to the environmental conditions than the trophozoite stage. Further research is needed to study the biology and the virulence of the cyst stage of *D. fragilis*. The discovery of the cyst stage and the establishment of an animal model have major implications for the potential control of Dientamoebiasis in humans and in gaining a better understanding of the disease itself.