The molecular epidemiology of *Dientamoeba fragilis* isolates in an Australian population

by

Damien Stark

BSc (Syd) MSc (UTS)

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Department of Cell and Molecular Biology, Faculty of Science,

University of Technology, Sydney

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Certificate

This study was carried out in the Microbiology Department, St. Vincent's Hospital, Sydney, under the supervision of Professor John T. Ellis, Associate Professor John Harkness, Associate Professor Deborah Marriott, and Dr Nigel Beebe. I certify that no part of this work has been submitted to any other university or institute. I also certify that this thesis has been written by me and that all help received and all sources used have been acknowledged in this thesis.

Production Note:
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Damien Stark

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Refereed Publications arising from this Thesis

4. Stark D, Beebe N, Marriott D, Ellis JT, Harkness J. 2006. Evaluation of three diagnostic methods, including real-time PCR, for the detection of *Dientamoeba fragilis* in stool specimens. J Clin Microbiol. 44(1):232-235.

3. Stark D, Beebe N, Marriott D, Ellis JT, Harkness J. 2006. Dientamoebiasis: a review on the clinical importance and recent advances. Trends in Parasitol. 22(2):92-96.

2. Stark D, Beebe N, Marriott D, Ellis JT, Harkness J. 2005. A prospective study on the prevalence, genotyping and clinical relevance of *Dientamoeba fragilis* infections in an Australian population. J Clin Microbiol. 43(6):2718-2723.

1. Stark D, Beebe N, Marriott D, Ellis JT, Harkness J. 2005. Detection of *Dientamoeba fragilis* in fresh stool specimens using PCR. Int J Parasit. 35(1):57-62.

Conference and Symposia Presentations arising from this Thesis

3. Stark D, Beebe N, Marriott D, Ellis JT, Harkness J. *Dientamoeba fragilis* – clinical significance and diagnosis. Oral presentation. Australasian College of Tropical Medicine, Hobart, November, 2005.

2. Stark D, Beebe N, Marriott D, Ellis JT, Harkness J. 2005. A prospective study on the prevalence, genotyping and clinical relevance of *Dientamoeba fragilis* infections in an Australian population. Oral presentation. Australian Society of Microbiology, Canberra, Australia, September, 2005.

1. Stark D, Beebe N, Marriott D, Harkness J, Ellis JT. Detection of *Dientamoeba fragilis* in fresh stool samples by PCR. Oral presentation .Australian Society of Microbiology, Sydney, Australia, September, 2004.

Abbreviations

ANGIS	Australian National Genomic Information Service
bp	base pair
BSA	bovine serum albumin
DNA	Deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EDTA	ethylenediamine tetraacetic acid
EtBr	ethidium bromide
ICT	Immunochromatographic
kb	kilobases
PBS	phosphate buffered saline
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
RFLP-PCR	restriction fragment length polymorphism – PCR
rDNA	ribosomal DNA
RNA	ribonucleic acid
SAF	sodium acetate acetic acid formalin
SSU rDNA	small-subunit ribosomal DNA
SSU rRNA	small-subunit ribosomal RNA
TBE	tris borate/EDTA
Tris	tris[hydroxymethyl]aminomethane
V	volts

Abstract

Dientamoeba fragilis is a trichomonad parasite that causes human gastrointestinal disease. It has been reported from most parts of the world in both rural and cosmopolitan areas and is a 'neglected cause of diarrhoea' and dysentry with chronic infections common. Current diagnosis of dientameobiasis is by microscopic identification of the trophozoite in stool. However this method is time-consuming and relatively insensitive while PCR technology offers an attractive alternative to conventional diagnosis. A conventional PCR assay based on the small-subunit ribosomal RNA gene of *D. fragilis* for the specific detection of *D. fragilis* DNA in fresh unpreserved stool samples was developed. The *D. fragilis* PCR was positive in 29/31 samples with positive microscopy and did not cross-react with other protozoan parasites. The PCR protocol showed a specificity of 100% and a sensitivity of 93.5% and the entire procedure can be performed in one day. A prospective study was also conducted over a 30 month period, in which 6,750 faecal samples were submitted to the Department of Microbiology at St. Vincent's hospital Sydney, Australia. Trophozoites of *Dientamoeba fragilis* were detected in 60 (0.9%) patients by permanent staining and confirmation was performed by PCR. Gastrointestinal symptoms were present in all patients, with diarrhoea and abdominal pain the most common symptoms. Thirty-two percent of patients presented with chronic symptoms. The average age of infected patients was 39.8 years. No correlation was found between *D. fragilis* and *Enterobius vermicularis*, a proposed vector of transmission for *D. fragilis*. The genetic diversity of 50 *D. fragilis* isolates was examined by PCR and the PCR products were analysed for the presence of a restriction fragment length polymorphism. These results showed no variation in the small subunit rRNA gene and demonstrated a single genotype for all Australian isolates. This study indicates the potential pathogenic properties of *D. fragilis*, and the need for all laboratories to routinely test for this organism. I also developed a 5' nuclease (TaqMan) based real-time PCR assay, targeting the small-subunit ribosomal RNA gene, for the detection of *D. fragilis* in human stool specimens and compared its sensitivity and specificity to the conventional PCR and microscopic examination by a traditional modified iron-haematoxylin staining procedure. Tests were

performed using all three techniques on 200 stool specimens referred for screening on the basis of diarrhea. The real-time PCR assay exhibited 100% sensitivity and specificity compared with microscopy. The detection limit of both PCR tests was compared; real-time PCR was 100 times more sensitive than conventional PCR, with a detection limit of 0.01 trophozoites. In conclusion, all three methods for the detection of *D. fragilis* were highly specific, with real-time PCR being the most sensitive. The use of the real-time assay in a diagnostic laboratory provides a superior sensitive and specific method for the diagnosis of *D. fragilis*.

Table of Contents

List of Figures

List of Tables