University of Venda

MOLECULAR CHARACTERIZATION OF *ENTAMOEBA HISTOLYTICA* tRNA GENES

A dissertation submitted in fulfillment of the requirements for the award of Master`s Science degree in Microbiology

BY

DAVHANA NDIVHUDZANNYI CAROLINE

(11560723)

Submitted to the Department of Microbiology

SCHOOL OF MATHEMATICAL AND NATURAL SCIENCES

UNIVERSITY OF VENDA

Supervisor: Prof. Amidou Samie

Co-supervisor: Prof. Peter Mbati

ABSRACT

BACKGROUND: *Entamoeba histolytica* is a eukaryotic protozoan parasite responsible for the disease called amoebiasis. Amoebiasis is a major cause of morbidity and mortality in the developing world. It is well established that about 500 million people are infected worldwide, resulting in up to 100,000 deaths annually. It is not completely understood why some individuals once infected with *E. histolytica,* develop clinical amoebiasis while others remain asymptomatic. Very few studies have been conducted in order to determine the potential role of the parasite genomic features on the outcome of the infection. No studies have been done in South Africa to show how parasite genotypes play a role in determining the outcome of infection. Therefore, the present study determined the molecular characteristics of tRNA genes of *E. histolytica* in relation to the occurrence of diarrhea.

METHOD: In this study, patients were recruited from rural primary health care clinics in Giyani, Limpopo Province and private clinics in Pretoria, Gauteng Province. The participants were supplied with a consent form and their information was kept confidential. Diarrheal and non-diarrheal stool samples were collected. All the stool samples were observed under a light microscope for the presence of *E. histolytica* cysts and trophozoites. The Techlab *E. histolytica* II kit was used to detect the antigen against *E. histolytica.* Genomic DNA was extracted from 78 stool samples that were positive by ELISA using ZYMO RESEARCH fecal DNA mini Prep kit from inqaba biotech. A multiplex PCR protocol was used for the identification of *E. histolytica.* Specific primers for the different loci (NK, RR, AL, DA and S^{TGA}-D) of the tRNA genes of *E. histolytica* were used for genotyping. In this study, 15 stool samples from the 42 positive

samples identified by ELISA and confirmed by PCR were amplified for the NK locus us tRNA specific primers NK-3 and NK-5. The genotyping as well as the data were analyze the Statistical Package for Social Sciences (SPSS for WINDOWS version 18.0) program i to determine the potential implications of *E. histolytica* infection.

RESULTS: A total of 774 stool samples were collected and it was found that, out of 77 samples examined by wet mount microscopically 16.7% were infected with *E. histolytic* and trophozoites. The TechLab ELISA based antigen detection kit specific only for *E. hist* in stool samples revealed that 10.1% were positive for *E. histolytica*. The highest prevale *E. histolytica* was found in Pretoria with 10.5%, as compared to Giyani which was 5.4% a difference was not statistically significant (X^2= 1.491; P= 0.222). *Entamoeba histolytic* more common in males (12%) than in females (8.4%), but the difference was not statis significant (X^2= 2.653; P= 0.103). Most of the participants who were infected were aged b 26-45 years with 21.2% followed by those who were in the age group 1-25 years with The least infected were of the age group 49-90 years with 8.2%. However, the difference w statistically significant (X^2= 3.341; P= 0.188). According to samples consistency, the h prevalence was found in watery stool samples with 13.6%, followed by soft with 11.1% a least was formed with 5.9%, but the difference was not statistically significant (X^2= 5.7 0.056).

Forty-two samples showed positive for *E. histolytica* small-subunit rRNA gene. Ni different banding patterns were obtained for NK locus. The ratio of the profile and the num samples tested was therefore three profiles for every 5 samples. Two hundred base pairs w most common band that occurred five times in different samples as compared to 150

250bp that occurred twice. Other bands observed included 120bp, 300bp, 350bp, 500bp, 600bp and 750bp which occurred only once each. Out of the 42 positive samples identified by ELISA and confirmed by PCR the RR locus was amplified in 30 samples using the tRNA specific primers RR-3 and RR-5. This gave a success rate of 71%. The product sizes of 150, 200, 250, 300, 400,450, 500, 550, 600 and 750bp were obtained. It was observed that 150bp and 250bp occurred only once. A total of 16 profiles were obtained giving a ratio of 0.53.

Out of the 42 positive samples identified by ELISA and confirmed by PCR, the AL locus was amplified in 25 samples using the tRNA specific primers AL-3 and AL-5. This gave an amplification success of 59.5%. The product size of 150, 180, 200, 220, 300, 350, 400,450, 500, 550, 600 and 1000bp were obtained. A total of 15 profiles were obtained for a ratio of 0.6. The band size of 200bp was seen in most of the samples, followed by 150, 180, 220, 300, 350, 400,450, 500, 550, 600 and 1000bp. It was observed that 150bp and 550bp occurred only once.

Thirteen (31%) stool samples from the 42 positive samples identified by ELISA and confirmed by PCR were amplified for the DA locus using the tRNA specific primers DA-3 and DA-5. The product sizes of 150, 200, 280, 300, 500 and 1200bp were obtained. One hundred and fifty base pair was seen in most of the samples, followed by 300 and 500bp bands. It was observed that bands of 200bp, 280bp, and 1200bp occurred only once.

Nine (21%) stool samples from the 42 positive samples identified by ELISA and confirmed by PCR were amplified for the S^{TGA}-D locus using the tRNA specific primers S^{TGA}-D -3 and S^{TGA}-D -5. The product size of 150, 180, 200, 220, 300, 350, 400,450, 500, 550, 600 and 1000bp were obtained. From the 9 samples that amplified for the S^{TGA}-D locus, bands of 150, 200, 280 and

400bp was seen in most of the samples. It was observed that 100, 240, and 300bp occurred only once. All samples were from Pretoria.

CONCLUSION: The present study indicates that infection caused by *E. histolytica* was prevalent in diarrheal samples obtained from Pretoria. *Entamoeba histolytica* infection was more prevalent in males than in females, and in the age group of 26- 45 years. The possible cause of infection in the stools of patients in this study could possibly be due to various factors such as drinking water from unprotected source or by direct contact with infected animals. According to our results, icroscopy is a simple method and it should be combined with other methods such as ELISA and PCR for identification of the species to avoid false and/or insufficient diagnosis and treatment applications.

Loci NK, RR, AL, DA and S^{TGA}-D of the tRNA genes identified in this study show promise as surrogate markers for prediction of infection outcome of *Entamoeba histolytica*. The findings of this study therefore suggest that further studies are needed to evaluate the prevalence, heterogeneity and combination of virulence-related genes in *E. histolytica* infection as well as their association with diarrhea and non-diarrhea.

Key words: *Entamoeba histolytica*, Genotyping, South Africa