
**College of Medicine**

# Spectrum of intestinal pathogens and their clinical presentation in HIV-positive patients with diarrhoea at Queen Elizabeth Central Hospital adult medical wards

**By**

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**(MBBS )**

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## DECLARATION

I, Patrick Nachipo, hereby declare that this dissertation is my original work and has not been presented for any other awards at the University of Malawi or any other University.

Name of Candidate Patrick Nachipo

Signature

Date August, 2021

**CERTIFICATE OF APPROVAL**

The dissertation of Patrick Nachipo is approved by the Dissertation Examination Committee

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## ABSTRACT.

Diarrhoea is one of the most common conditions in people living with HIV (PLWHIV) in Malawi. However, there is limited data on the aetiology of diarrhoea and the specific clinical presentations in this population and setting.

The aim of this study was to identify pathogens associated with diarrhoea in PLWHIV. Specifically, the study wanted to determine the prevalence of cryptosporidiosis and other enteric pathogens and to describe their clinical presentations

This was a cross-section observational study at the Queen Elizabeth Central Hospital (QECH), a referral hospital in Blantyre, nested within a Phase 2A clinical trial of treatment of cryptosporidiosis in PLWHIV. Identification of the infectious agents in stool was done by Gastrointestinal (GI) TaqMan Array testing. We performed descriptive analyses on the prevalence of specific intestinal pathogens in PLWHIV presenting with diarrhoea comparing those with and without cryptosporidiosis. We also described the clinical presentations associated with the pathogens.

We recruited 20 adults with diarrhoea caused by cryptosporidiosis and 10 controls without diarrhoea and cryptosporidiosis. The median CD4+ T-lymphocyte count was 21 cells/microlitre for the cases and 419 cells/microlitre for the controls ($p<0001$). The most common pathogens isolated among the cases were *enteroaggregative Escherichia* coli (EAEC)-65% (95% CI 40.78 to 84.6%), *enterotoxigenic Escherichia coli* (ETEC) (60%), *Shigella* spp. (60%) and *Encephalitozoon bieneusi* (40%). Among the controls, the most common pathogens isolated were *Blastocystis* in 87.5% (95% CI 47.35% to 99.68%), *Shigella* spp. in

75% (95% CI 34.91% to 96.81%), *enteropathogenic Escherichia coli* (EPEC) in 50 % (95% CI 15.70% to 84.30%) and ETEC in 50%. There was statistically significant difference in the prevalence of *Blastocystis*, being more common in the controls than the cases (87.5% and 30% respectively, p=0.0107). There was no statistically significant difference in the median number of pathogens isolated from one individual between the cases and the controls, 6.5 pathogens/person among the cases and 3.5 pathogens/person among the controls (p=0.2714). The most common clinical features identified were anorexia, nausea, abdominal pain and vomiting. 95% of the cases had used antibiotics prior to admission (95% CI 50.90 to 91.34).

Our study showed that PLWHIV and diarrhoea with cryptosporidium, have low CD4 counts. They also have other enteric pathogens including EAEC, EPEC, ETEC, *Shigella* spp., *E. bienuesi* and Giardia. It also showed that *Blastocystis* is a common pathogen in HIV reactive patients, with and without diarrhoea. Anorexia, nausea, abdominal pain and vomiting were the common accompanying symptoms in patients with chronic diarrhoea. We also found that the use of antibiotics in chronic diarrhoea was prevalent.

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| | |
|---|---|
| ART | Antiretroviral therapy |
| QECH | Queen Elizabeth Central hospital |
| CFZ | Clofazimine |
| ETEC | Enterotoxigenic *Escherichia coli* |
| EAEC | Enteroaggregative *Escherichia coli* |
| EPEC | Enteropathogenic *Escherichia coli* |
| HIV | Human immunodeficiency virus |
| PLWHIV | People living with HIV |

# CHAPTER 1: INTRODUCTION

## 1.1 **Background Information**

### 1.1.1. **HIV in the World and Malawi**

HIV/AIDS is still a big problem worldwide. Between 1981 when it was first discovered and 2020, it is estimated that 77 million people have become infected and 35 million have died [1]. Although it is a global epidemic, some regions are affected more than others. Sub-Saharan Africa is the epicenter of the epidemic with over half of all the people living with HIV living in eastern and southern Africa, despite contributing only 6 percent of the world population [2]. In this part of the world, HIV/AIDS and its related opportunistic infections are leading cause of years of life lost [3].

Malawi has one of the highest prevalence of human immunodeficiency virus (HIV) in the world, at 10.6% for people of ages between 15 and 64 years [4]. This contributes to 4% of all HIV cases in sub-Saharan Africa. Over 1 million adults in Malawi were living with HIV in 2020 [5]. Prior to rollout of antiretroviral therapy (ART) in 2004, there has been a high burden of HIV in the adult wards, and in 1999, up to 70% of adults admitted to both medical and surgical wards were HIV reactive, with 45% meeting the WHO definition of AIDS [6].

### **1.1.2 Burden of Diarrhoeal Diseases**

Enteric infections are a global problem. In 2016, 1.77 million deaths resulted from these infections, with over 90% of them having a diarrhoeal illness, and most of the remaining dying from salmonella infections [7]. Diarrhoeal illnesses, despite being the 8$^{th}$ cause of global mortality, affects more people in the low-income countries,

with 90% of the deaths occurring in sub-Saharan Africa and south-Eastern Asia with the majority of the deaths occuring in children before the age of five and in adults after the age of 70 years [8].

In Malawi, the burden of diarrhoea in adult population is relatively unknown. Most studies have focused on children under the age of five years. In a national survey in 2016, the prevalence of diarrhoea in children under the age of five years was 22% [9]. Experience from medical admissions indicate that there is significant burden among in-patients.

Diarrhoeal illnesses pose a big economic burden on households and the health system. In Malawi, in 2014, the cost of in-patient treatment of gastroenteritis ranged from United States Dollar (USD) 65 for children under the age five years to over USD 200 for adults[10,11]. There are a lot of physical and psychological adverse effects from diarrhoeal illnesses [12]. There is marked reduction in the quality of life among the patients [13]. In pregnant women one study showed an association between diarrhoea and small for gestation age births [14].

### 1.1.3 **Literature Review on HIV and Diarrhoea**

Intestinal diseases are a major cause of morbidity and mortality in PLWHIV, and patients can present with both acute and chronic diarrhoea [15-17]. Since the early descriptions of HIV, chronic diarrhoea was identified as one of the complications of HIV, being found in up to 50% of HIV patients in developed countries and up to 100% of those in developing countries [18,19]. Patients who are living with HIV are at an increased risk of intestinal infections at all levels of immune status as compared to HIV nonreactive individuals [20].

Individuals living with HIV are at a risk of acute diarrhoea caused by similar pathogens as in individuals not living with HIV. Common bacterial pathogens causing diarrhoea in PLWHIV include *Escherichia coli, Clostridium difficile, Campylobacter jejuni, Salmonella* species, *Shigella* species and mycobacterial species [21,22]. *C. jejuni* occurs 39 times more commonly in PLWHIV than in HIV negative individuals [23]. There is also a higher risk of invasive disease with longer duration of illness in PLWHIV [24].

Ayele Kedebe and colleagues evaluated the contribution of bacterial pathogens in HIV reactive patients with signs and symptoms of gastrointestinal disease at Hawassa University Comprehensive Specialized Hospital (Ethiopia) from February to May, 2016, using stool cultures. They recruited 215 patients, of whom 47.4% had diarrhoea, 62% were on ART and 63% were not on cotrimoxazole prophylactic therapy. They had positive cultures in 12.6% of the participants. 80% of the isolates were identified in patients reporting to have had diarrhoea. The pathogens isolated were *campylobacter* spp in 6.04% of the participants, *salmonella* spp in 5.1%, *shigella* spp in 1.4% and EHEC in 0.9% of the participants. In terms of antimicrobial susceptibility, the majority of the pathogens were susceptible to norfloxacin, nalidixic acid, gentamicin, ceftriaxone and ciprofloxacin. They were resistant to cotrimozaxole and chloramphenicol. The study was limited by a small sample size [25].

Intestinal parasites are also important causes of diarrhoea, especially at CD4+ T-lymphocyte counts of less than 200cells/mm [17,26,27]. Parasitic causes include *Cryptosporidium* species, *Microsporidia* especially *Enterocytozoon bienneus* and *Encephalocytozoon intestinalis, Cysclospora cayetanensis, Isospora belli, Giardia*

*lamblia, Entamoeba histolytica* and *Strongyloides stercoralis.* In Cameroon, intestinal parasitic infections were found in 46.8% of diarrhoiec and 23% of non diarrhoiec stool samples from PLWHIV [26].

A study was done at Naidu Municipal Corporation hospital in Pune, India, from January 2009 to May 2010. 331 consecutive patients presenting with diarrhoea were recruited, 65 of whom were HIV reactive. The infective causes of diarrhoea in these patients were investigated. Parasites were identified in 60% of the HIV reactive patients, with *cytosospora belli*(28%) and *cryptosporidium parvum* (12%) being the most common pathogens. In the HIV negative group, parasites were isolated in 14.3 % of the patients. Bacterial causes were found in 34% of the HIV reactive patients and 28.2% of the HIV nonreactive patients. They concluded that intestinal pathogens are more common in HIV reactive patients [28].

K. Gupta and his colleagues looked at parasitic infections in patients with HIV in a case-control study in North India from January to December, 2011. They recruited 100 HIV reactive patients with or without diarrhoea, and 100 controls who were HIV non-reactive with diarrhoea. Microscopy was used to identify pathogens. Intestinal parasites were identified in 35% of the cases and 25% of the controls. Intestinal parasites in HIV reactive patients were more likely in patients with diarrhoea than in those without diarrhoea (73% and 25% respectively). Patients with diarrhoea were more likely to have low CD4 cell count as compared to those without diarrhoea (181 cells/$mm^3$ and 352 cells /$mm^3$ respectively). The most common pathogens isolated in HIV reactive patients were *I. belli* (23%) and *G. lamblia* (18%) and c.parvum (8%). In the control group, from the 25 stool samples with an identified pathogen, the most common parasite was *E. histolytica* (28%), *Entamoeba*

*coli* (21%) and *G. lamblia* (15%). This study was limited by a small sample size and inability to do some sensitive tests like PCR to identify pathogens [29].

Other studies from New Delhi, India have shown similar results. B. Uppal and colleagues looked at enteric pathogens in patients with diarrhoea and HIV. It was a case control study with patients with HIV and diarrhoea as the cases and 2 control groups of HIV reactive patients without diarrhoea and HIV negative individuals with diarrhoea. They recruited 50 in each group. The prevalence of parasitosis was 20% in cases, 2% in HIV positive patients without diarrhoea and 18% in HIV negative patients with diarrhoea. In the cases, bacterial pathogens identified were *E. coli* (24%), *C. difficile* (10%) and *salmonella* spp (4%). In those with HIV but without diarrhoea, the most common bacterial pathogens were *E. coli* (10%) and *C. difficile* (4%). It was concluded that enteric pathogens are one of the important health problems in HIV reactive patients [30].

In Malawi, Cranendonk et al, in 2001, assessed the importance of *C.parvum* and *I.belli* as causes of diarrhoea in HIV reactive patients admitted to Queen Elizabeth central hospital. They recruited 121 patients with diarrhoea and 122 patients without dairrhoea. *I. belli* and *c.parvum* oocysts were demonstrated by phenol auramine-O-fluorescence staining with fluorescence microscopy. *C. parvum* was also investigated by monoclonal antibodies against Cryptosporidium (Crypto-cel IF test, Cellabs, Brookvale, Australia). HIV seroprevalence was 84 percent among the subjects, 88% in patients with diarrhoea and 79% in patients without diarrhoea. *C.parvum* was found in 11% of those with diarrhoea and 3 percent of those without diarrhoea. *I. belli* was isolated in 12 % of those with diarrhoea and 3% of those without diarrhoea. These pathogens were only isolated from stools of those who

were HIV reactive. This shows that enteric parasitic infections are common causes of diarrhoea in HIV reactive patients in Malawi [31].

In a metanalysis of cryptosporidium infection in HIV reactive patients, Ehsan Ahmadpour et al, looked at 161 studies with a total of 51123 study participants. 53 of these studies were from African region, 53 from south East Asian region, 23 from the region of the Americas, 17 from western pacific region, 10 from the Eastern Mediterranean Region and 5 from the European region. One study from Malawi was included. The pooled prevalence of cryptosporidium enteric infection was 11.2% with 95% confidence interval of 9.4% to 13.0%. The prevalence differed with the method of parasite identification. The prevalence was 26% with antigen detection, 13.3% with molecular methods and 10% with staining methods. The risk of cryptosporidial infection was associated with unsafe water, low CD4 cell count and presence of diarrhoea [32].

In Ethiopia, Haileeyesus Adamu, Teklu Wegayehu and Beyene Petros identified a high prevalence of diarrhoegenic intestinal parasite infections among non-ART HIV patients at fitche hospital. They recruited 214 HIV reactive patients on ART and 164 HIV reactive patients who were not on ART. Among those on ART, 55.1% had diarrhoea, as compared to 77.3% of those not on ART. Modified Zeihl-Neelsen staining was used to identify pathogens. Intestinal parasites were identified in 63.5% of the participants. The isolated parasites included *Cryptosporidium* spp *Blastocystis* spp., *I. belli, E. histolytica, Hymnolepis nana, G. lamblia, Enterobius vermicularis, Schistosoma mansoni, Taenia* spp., *Trichuris trichiura, Ascaris lumbricoids*, hookworms, and *S. stercoralis*. The most common parasite identified was *c.parvum*. Presence of the parasites was associated with low CD 4cell count, with the presence

of pathogens being 5 times more likely at CD 4 cell counts of less than 200 cells/$mm^3$ as compared to CD 4 cell count of more than 500 cells/$mm^3$ [33].

Viral causes include Cytomegalovirus, especially with CD4+ T-lymphocyte counts of less than 50 cells/µL. It has been attributed to cause up to 15% of diarrhoea among PLWHIV [34]. In some of these patients, however, pathogens may not be identified as the aetiological agent. In these patients, attributed causes of diarrhoea have included HIV itself, pancreatic insufficiency, HIV-associated malignancies and ART, especially protease inhibitors [15,35].

Un-published data from a pilot study looking at Cryptosporidium diarrhoea in adult inpatients at the QECH, in Malawi, revealed that bacterial causes are more common than other pathogens. In this study, in which multiple pathogens were identified, multiple infections were very common. The most common pathogen was *E. coli* (different pathovars) [36]. However, these data did not evaluate the differences in clinical presentation among the identified pathogens.

### 1.2 **Justification for the Study**

To our best knowledge, no studies have been done in Malawian adults to describe the spectrum of aetiological pathogens in PLWHIV presenting with diarrhoea and their clinical presentation. This data is relevant and important because it would form the basis for the empirical treatment for diarrhoea in this patient population. Findings from this study will help clinicians to treat patients presenting with diarrhoea, targeting the most likely cause based on the clinical syndrome. This will be helpful in our setting since most of the therapeutic decisions are made without the contribution of microbiological diagnoses.

## 1.3 Objectives of the Study

### 1.3.1 **Broad Objective**

The aim of this study was to identify other pathogens associated with diarrhoea in PLWHIV presenting with diarrhoea in whom a diagnosis of cryptosporidiosis was made and then describe the clinical presentation.

### 1.3.2 **Specific Objectives**

a. To describe the spectra of pathogens isolated in stools of PLWHIV with and without diarrhoea and cryptosporidiosis
b. To compare the prevalence of individual pathogens and number of co-infections between the two above groups.
c. To stratify the type and burden of infectious aetiological agents isolated in stools of HIV reactive patients with diarrhoea and cryptosporidiosis according to the immunological status
d. To describe the clinical presentation of HIV reactive patients with diarrhoea and cryptosporidiois according to specific pathogen profiles isolated in stools

### 1.3.3 **Hypothesis**

The hypotheses for the study were as follows:

- PLWHIV presenting with diarrhoea in whom cryptosporidium is isolated would have a broader spectrum of infectious pathogens and higher prevalence for individual pathogens in stools than asymptomatic PLWHIV in whom *cryptosporidium* is not isolated.

- PLWHIV with CD4 count below 50cells/µl presenting with diarrhoea and in whom *Cryptosporidium* is isolated would have more pathogens isolated on stool by Taqman array, and higher prevalence for individual pathogens, than those with CD4 counts above 50cells/µl.
- Different pathogen profiles would be associated with different clinical presentations

# CHAPTER 2: METHODOLOGY

## 2.1 **Type of Research**

This was an observational cross-sectional study. This study was nested within a larger study, the Cryptofaz study, which was "*A Phase 2A, Randomized, Double-Blind, Placebo-Controlled Evaluation of the Safety, Tolerability, Pharmacokinetics and Efficacy of Clofazimine (CFZ) in Cryptosporidiosis"* [37].

## 2.2 **Study Place**

This study was conducted in the adult medical wards of QECH in Blantyre-Malawi. QECH is a tertiary hospital serving over 7.7 million people with bed capacity of 1200 beds [38,39]. QECH admits over 50000 people a year, and there are over 50 admissions every month with diarrhoeal complaints to the adult medical wards [30].

## 2.3 **Study Population for the Parent Study**

The study recruited adults living with HIV who presented with diarrhoea and had confirmed cryptosporidium in their stools. These were screened for enrollment into Cryptofaz study part A. The study also recruited adults living with HIV without diarrhoea who were screened for enrollment into part B of the Cryptofaz study.

For part A of the Cryptofaz study, subjects were recruited from outpatient clinics including the emergency department at QECH, health centers belonging to the Blantyre District Health office, antiretroviral clinic or from those admitted to the QECH medical wards. Participants were eligible for enrollment if they were on a stable antiretroviral regimen for more than two weeks. Other entry criteria included the following; being 18 years and older, male or female with a history of diarrhoea

for at least 3 days. Diarrhoea was defined as passing three or more loose stools per day regardless of the volume, and willing to provide a signed written informed consent form. For illiterate participants, a legal guardian was asked to provide the consent. Information for the study was provided in Chichewa, the most commonly spoken language in the study area.

Cryptosporidium was detected in the stool using either a rapid diagnostic test (Techlabs inc, Blacksburg, VA) and quantitative polymerase chain reaction (PCR) by a TaqMan

Array Card (Thermo Fisher, Waltham, MA) using a customised design made at the Houpt Laboratory (Charlottesville, VA). For part B of the cryptofaz study, patients presenting to the HIV Clinic at the QECH who did not have diarrhoea were approached. Participants for Part B were matched (age, gender, stage of HIV infection by World Health organization criteria) with the 10 subjects enrolled in Part A. Other than the absence of diarrhoea and Cryptosporidium infection, the eligibility and exclusion criteria were the same as for subjects enrolled in PART A

### 2.4 **Study Period**

Initially, the data collection period was supposed to be from December 2017 to May 2018, but because of difficulties in recruiting patients because of strict inclusion criteria in the parent study, the period was extended to January 2019.

### 2.5. **Inclusion/Exclusion Criteria for the Substudy**

This study was nested within the Cryptofaz study. Therefore, the inclusion/exclusion criteria for pre-screening in the Cryptofaz study applied:

### 2.5.1 **Inclusion Criteria**

- Male or Female, aged 18 to 65 years old, HIV positive, Cryptosporidium positive by qPCR, and on stable anti-retroviral treatment for at least 2 weeks to be included in part A of the Cryptofaz study, and similar adults without diarrhoea and cryptosporidium included in part B.
- Weight >78 lbs/35.4 kg.
- Presents with diarrhoea defined as three or more loose stools per day for at least 3 days (for part A of the Cryptofaz study). (If subjects have blood in their stools, they will be treated with Ciprofloxacin which is the standard of care, but will not be excluded.)
- Willing and able to provide signed written informed consent or witnessed oral consent in the case of illiteracy, prior to undertaking any study-related procedures.

### 2.5.2 **Exclusion Criteria**

Any condition for which participation in the trial, as judged by the Site Investigator, could compromise the well-being of the subject or prevent, limit or confound protocol specified assessments.

- Fever >38.0°C on presentation.
- Is critically ill or in the judgment of the investigator has a prognosis that could lead to imminent mortality within 60 days or compromise participation in the study or endanger the patient by entering the study.
- Use of concomitant medications that markedly prolong the QT/QTc interval or are predicted to have drug-drug interactions with CFZ that

may lead to toxicity from the partner drug including Amiodarone, Amprenavir, Atazanavir, Bedaquiline, Bepridil, Chloroquine, Chlorpromazine, Cisapride, Clarithromycin, Cyclobenzaprine, Darunavir, Delamanid, Disopyramide Dofetilide, Domperidone, Droperidol, Erythromycin, Fosamprenavir, Halofantrine, Haloperidol, Ibutilide, Indinavir, Levomethadyl, Lopinavir, Mesoridazine, Methadone, Nelfinavir, Pentamidine, Pimozide, Procainamide, Quinidine, Ritonavir, Simiprinivir, Sotalol, Sparfloxacin, Thioridazine, or Tiprinivir.

- Pregnant and lactating women (screening pregnancy test for females and pregnancy test at the discharge follow up visit (Study Day 56).

2.6 **Sample Size**

The estimated sample size was based on the number to be recruited in the cryptofaz study, 56 cases and 10 controls.

From a previous study, the prevalence of pathogens isolated in stools of patients with diarrhoea ranged from 1% to 33%. [8] We calculated the expected margins of error for patients with diarrhoea and the control as listed in the table below for estimated prevalence between 1% and 40%

**Table 1; Expected margins of error**

| estimated prevalence | e(patients with diarrhoea n=56) | e(controls n=10) |
|---|---|---|
| 0.01 | 0.0261 | 0.0671 |
| 0.05 | 0.0571 | 0.1351 |
| 0.1 | 0.0786 | 0.1859 |
| 0.2 | 0.1048 | 0.2479 |
| 0.3 | 0.1200 | 0.2840 |
| 0.4 | 0.1283 | 0.3036 |

2.7 **Data Collection**

College of Medicine Research ethics committee granted a permission to carry out this study (study number: P.12/17/2326). Permission was also sought from Queen Elizabeth medical hospital director.

Participants recruited in the Cryptofaz were approached for written consent to be enrolled in this sub-study. Participants that gave consent were assessed. Detailed assessment which included medical history and physical examination was carried out. The emphasis was on the ART regiment and the duration of treatment, use of cotrimoxazole and Isoniazid preventive therapies and other medications, the duration of diarrhoea and the stool frequency, any other associated symptoms: fever, vomiting, nausea, anorexia, fatigue/malaise, urgency of defecation, abdominal pains, weight loss, presence of bloody diarrhoea or flatulence.

Data was collected using a questionnaire (see appendix) A. Results of the investigations done in the cryptofaz study were obtained.

Investigation results obtained were full blood count and CD4 T-lymphocyte count, Renal function and liver function tests, HIV viral load and stool PCR analysis by Taqman assay. These were results of samples taken on the day of enrollment into the cryptofaz study.

### 2.8 **Stool Analysis**

#### 2.8.1 **Nucleic Material Extraction**

DNA and RNA were extracted from the fecal specimen using a modified QIAamp Fast DNA Stool Mini Kit procedure. 180-200 mg (200 µL if liquid) of stool sample was used. The fecal sample underwent a lysate preparation process that included mechanical disruption by bead beating for two to three minutes, removal of inhibitors, and finally purification and elution of DNA and RNA using spin columns. Extrinsic controls MS2 phage, and Phocine herpes virus PhHV were added to each sample during lysate preparation for later evaluation of extraction and amplification efficiencies. The extracted total nucleic acid (TNA) was stored at -80°C prior to testing

#### 2.8.2 **Detection of Pathogens**

20µL of total nucleic acid extract was tested using TAQ Man Array test. The TaqMan Array Card (TAC) is a 384-well real-time PCR format for TAC-compatible instrument platforms. The Houpt Lab at the University of Virginia developed a custom enteric TAC capable of detecting multiple targets on a single card for 8

samples. The enteric TAC targets included viruses, bacteria, fungi, protozoa, and helminths.

TAQ Man master mix was prepared for one card at time by mixing Ag-Path-ID 2X RT-PCR buffer (425µL), Ag-Path-ID Enzyme mix (34µL) and Nuclease free water (221µL). 80 µL of the master mix was combined with 20 µL of total nucleic acid extract in 1.5mL microcentrifuge tubes and was centrifuged to eliminate air bubbles. Each 100 µL of the PCR reaction mix from the eight microcentifuge tubes was put on the TAC, and this was centrifuged at 1200rpm for a total of two- 1 minute spins. The cards then were run on ViiA 7 PCR system using PCR cycling conditions comprising 20 minutes at 45°C, 10 minutes at 95°C and 40-two step cycles of 15 seconds at 95°C and 1 minute at 60°C.

These tests were done at the Malawi-Liverpool Wellcome Trust laboratory.

### 2.9 **Data Management**

Every study participant was assigned a unique study number, which was different from the one in the cryptofaz study. The case record forms had only the study number. The filled case record forms were stored in a locked cabinet accessible only by the principal investigator.

Data was entered and analyzed using Microsoft Excel and EPI info.

For descriptive statistics, we reported proportions (respectively medians for the number of co-infections), ranges and 95% confidence intervals (respectively inter quartile ranges for the number of co-infections). Specifically, for objective a, we calculated exact binomial confidence intervals.

We used Fisher's exact test to compare prevalences of individual pathogens in HIV reactive patients with and without diarrhoea.

We used non-parametric Mann-Whitney U tests to compare the number of co-infections between HIV reactive patients with and without diarrhoea.

We defined clinical profile as specific, recurrent patterns of pathogen co-infections. We defined recurrent profiles as those found in at least 5 study subjects.

### 2.10 **Ethical Consideration**

#### 2.10.1 **Ethical Clearance**

Ethical Clearance was sought from the National health science research committee for the Cryptofaz study. For this study, clearance was sought from College of Medicine Research and Ethics Committee.

Approval was also sought from the head of department of medicine and the hospital director of Queen Elizabeth Central hospital to conduct the study in the medical wards.

#### 2.10.2 **Informed Consent**

Written informed consent was obtained from the study participants (appendix B/C)

#### 2.10.3 **Confidentiality**

All patient data collected during the study was confidential. Case record forms were kept in a secured locker in the medical annex. Electronic data was password protected with access restricted to study investigator.

#### 2.10.4 **Risks to the Patients**

This sub-study was deemed minimal risk by the institutional review board.

# CHAPTER 3: RESULTS

## 3.1 **Participant Recruitment**

Participants were recruited from among those recruited in parent cryptofaz study. Cryptofaz study had recruited 23 cases and 11 controls. Out of these, 22 cases were approached for the study and 20 gave consent. All 11 controls were approached and 10 gave consent. The cases were patients with diarrhoea and PCR detected cryptosporidium. The controls were matched to the cases, and these were individuals living with HIV but without diarrhoea and *Cryptosporidium*.

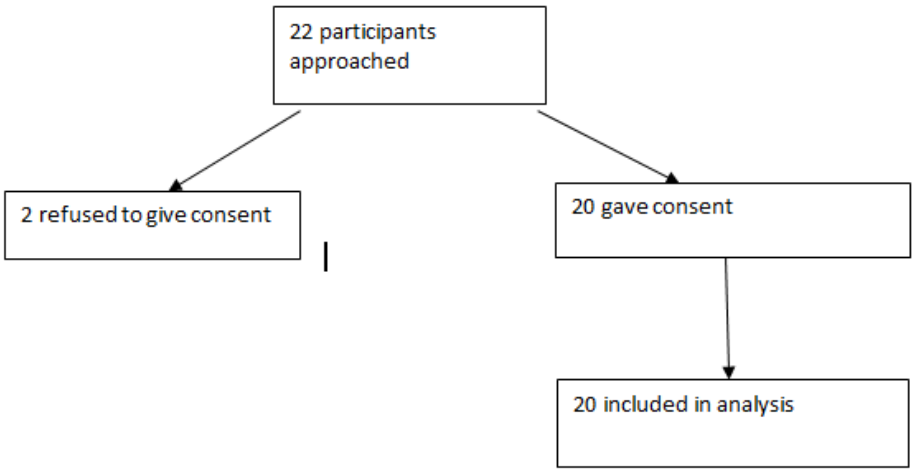


**Figure 1; Case recruitment**

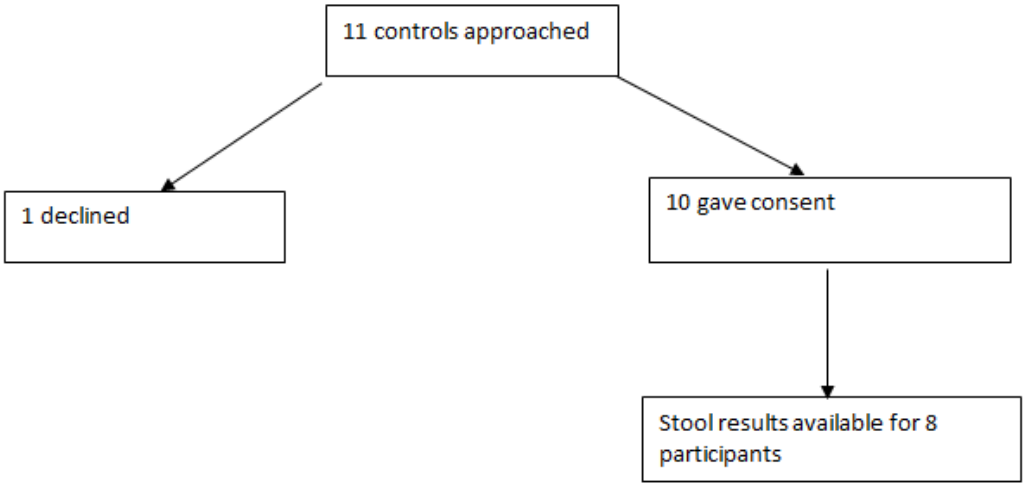


**Figure 2; Control recruitment**

### 3.2 **Baseline Characteristics**

The basic characteristics are outlined in table 2 below.

There were 10/20 (50%) males in the case group and 7/10 (70%) males in the control group. The mean age in the cases group was 38.9 years with the youngest being 22 and the oldest was 59 years. In the control group, the mean age was 44 and the range was from 30 to 64 years. There was no significant difference in the mean age groups, with a p value of 0.170597.

The mean body weight of the case and control groups were 46.45 Kg (range 35.1 kg to 59 kg) and 47.1 (range 36kg to 56kg). There was no significant difference in the mean body weights between the two groups, with a p value of 0.7797.

The median duration of time on antiretroviral medications was 28.4 months for cases (IQR: 74.25) and 14 months for the controls (IQR: 36.5). There was no statistical difference between the two groups (P-value 0.1728).

All the p-values for the baseline characteristics were calculated using student's *t*-test.

**Table 2. baseline characteristics**

| | case group (n=20) | control group (n=10) | p-values |
|---|---|---|---|
| males | 10 (50%) | 7 (70%) | |
| Mean Age in years (range) | 38.9 (22-59) | 44 (30-64) | 0.170597 |
| Mean weight in kilogram (range) | 46.45 (35-59) | 47.1 (36-56) | 0.7797 |
| Median duration on ART in months (IQR) | 28.4 (74.25) | 14 (36.5) | 0.1728 |

### 3.3 **Clinical Features**

The median duration of diarrhoeal symptoms was 18 weeks, with a median stool frequency of five times per day (IQR= 1). 6 participants reported associated vomiting, 8 reported abdominal pain, and 5 had some abdominal tenderness elicited

on physical examination. 12, 4 and 1 participants reported anorexia, feeling fatigued and blood in stool respectively.

**Table 3. Clinical features**

| CLINICAL FEATURE | YES (%) | NO (%) |
|---|---|---|
| Abdominal pain | 8 (40) | 12 (60) |
| Abdominal tenderness | 5 (25) | 15 (75) |
| anorexia | 12 (60) | 8 (40) |
| vomiting | 6 (30) | 14 (70) |
| Blood in stools | 1 (5) | 19 (95) |
| fatigue | 4 (20) | 16 (80) |

### 3.4 **Blood Tests Results**

The blood tests results are shown the table 4 below.

#### 3.4.1 **Full Blood Count**

There were statistical differences in some haematological parameters, with controls showing higher mean haemoglobin and lymphocyte counts than the cases. There was no statistical difference in mean white cell, neutrophil and platelet counts.

The mean haemoglobin for cases was 11.55g/dl with a range of 7.3 to 19.5g/dl while mean haemoglobin for the controls was 13.98 with a range of (12.2 to 17g/dl). There

was a statistically significant difference in the mean haemoglobin in the two groups (P=0.014)

The mean total white cell count was 4.23 $x10^9$ cells/l and 5.04 $x10^9$ cells/l for the cases and the controls respectively. The neutrophil counts were 2.65 and 2.56 $x10^9$ cells/l for the two cohorts. The lymphocyte counts were 0.96 and 2.01 for the cases and controls respectively. This showed a statistically significant difference with a p value of 0.0001.

The mean platelet count for the cases was 323 $x10^9$ cells/l. For the controls, the mean platelet count was 291 $x10^9$ cells/l. There was no statistically significant difference in the platelet counts with a p-value of 0.34.

### 3.4.2 **Renal Function and Electrolytes**

There was no difference in the renal function tests between cases and controls. The mean creatinine and blood urea nitrogen for the cases were 72.4 µmol/L and 4.4 mmol/l respectively and for the controls, the mean creatinine and blood urea nitrogen of 65.5 µmol/L and 3.6 mmol/l respectively. These results are captured in table 4

The cases had a mean sodium level of 138.0 mmol/l and the controls had a mean sodium level of 138.1 mmol/l. The potassium levels for the two groups were 3.9mmol/l for the cases and 4.8 mmol/l for the controls. This difference was statistically significant with a p-value of 0.005. The levels of chloride were 107 for the cases and 104 for the controls.

### 3.4.3 **Liver Function Tests**

Aminotransferases were assessed in this study. The mean alanine aminotransferase level for the cases was 38, and so was the level in the control group. The Aspartate aminotransferase was 56 for the cases and 50 for the controls. There were no statistically significant differences in the levels of alanine and aspartate aminotransferases with p-values of 0.9753 and 0.4743 respectively.

**Table 4. Blood tests results (student's *t*-test used to calculate the p-values)**

| **PARAMETER** | **CASES** | **CONTROLS** | **p-value for the differences between cases and controls** |
|---|---|---|---|
| Haemoglobin (g/dl) | 11.55 | 13.98 | 0.014 |
| White cell count($10^9$ cells/L) | 4.23 | 5.04 | 0.474 |
| Neutrophils($10^9$ cells/L) | 2.65 | 2.56 | 0.922 |
| Lymphocytes($10^9$ cells/L) | 0.96 | 2.01 | < 0.001 |
| Platelets($10^9$ cells/L) | 323 | 291 | 0.338 |
| Sodium (mmol/l) | 138.0 | 138.1 | 0.940 |
| Potassium (mmol/L) | 3.9 | 4.8 | 0.005 |
| Chloride (mmol/l) | 107 | 104 | 0.094 |
| Creatinine (µmol/L) | 72.4 | 65.4 | 0.590 |
| Blood urea Nitrogen (mmol/l) | 4.4 | 3.6 | 0.362 |
| Alanine aminotransferase(IU/l) | 38 | 38 | 0.975 |
| Aspartate aminotransferase (IU/L) | 56 | 50 | 0.474 |

### 3.4.4 **CD4 Counts**

Cases were more severely immunodepressed compared with controls. The median CD4 cell count for cases and controls were 21 cells/µl and 419 cells/µl respectively. This was statistically significant with a p-value of 0.00013. Out of 20 cases, 16 had CD4 cell counts which were less than 50 cells/ µl, and only 2 participants had CD 4 count above 100. In contrast, all the controls had CD 4 count above 100 cells/ µl. This is shown in the figure 3 below.

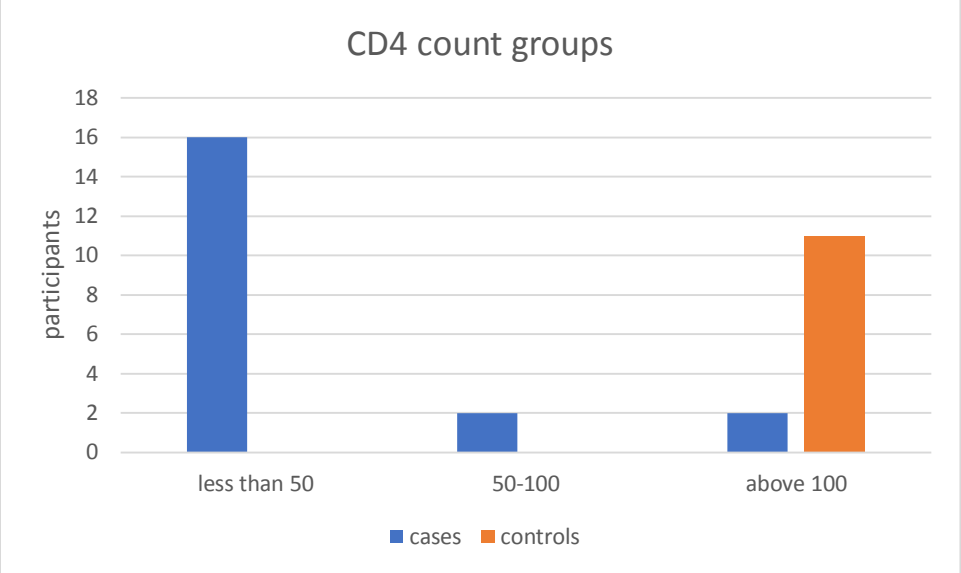


**Figure 3; CD4$^{+}$ T-lymphocyte cell count**

3.4.5 **Viral Load**

90% (18 cases) were failing on the ART regimen with a mean HIV viral load of 503932 copies/mL. Only 2/20 cases were virally suppressed (less than 40 copies/mL). All controls except one were virally suppressed. The difference in proportion of cases and controls with viral load suppressions were statistically significant with a (p-value <0.0001). This is shown in figure 4 below

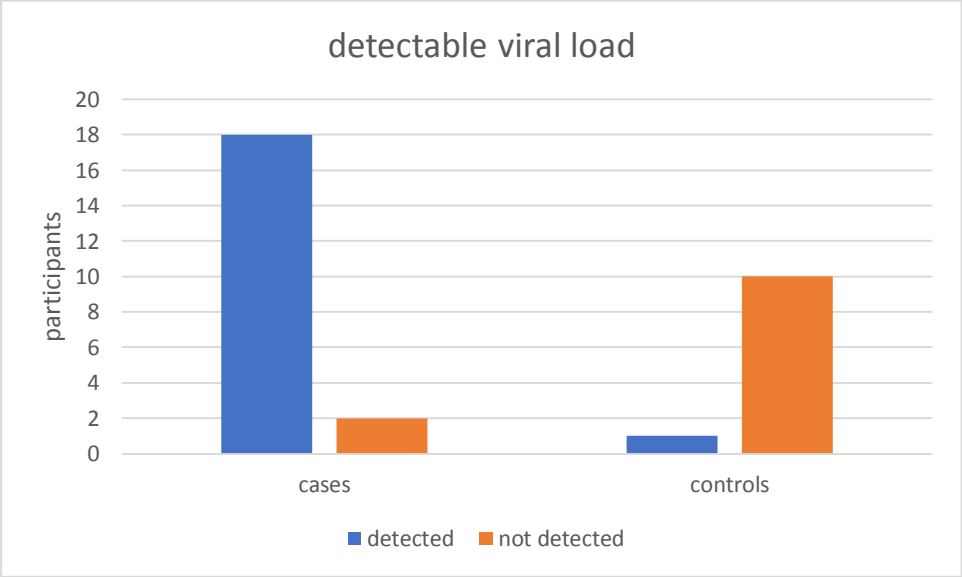


**Figure 4; Detectable viral load**

## 3.5 **Pathogens Isolated**

### 3.5.1 **Pathogens in Stool Samples from Cases**

Taqman PCR was done on the stool samples from both the cases and the controls

The most frequent isolate identified was *cryptosporidium* spp, which was positive in 15 stool samples. Specific *cryptosporidium* species, *C. parvum* and *C. hominis* were identified in 5 and 2 stool samples respectively.

*Escherichia coli* species were also identified. Enteroaggregative *E.coli* (EAEC) species were identified in 13 stool samples (65%, 95% CI 40.78% to 84. 6%), and Enterotoxigenic *E.coli* (ETEC) were identified in 12 samples (60%, 95% CI 36.05% to 80.88%). Enteropathogenic *E.coli* (EPEC) were found in 7 stool samples (35%, 95% CI 15.39% to 59.22%).

The stool PCR also identified *shigella* species. *Shigella* species were identified in 12 stool samples (60%, 95% CI 36.05% to 80.88%). *Shigella flexneri* was identified in 7 stool samples (35%, 95% CI 15.39 to 59.22%) and *shigella sonnei* in 3 samples (15%, 95% CI 3.21% to 37.89%).

Eight stool samples were positive for *microsporidium* (40%, 95% CI 19.12% to 63.95%). All eight of them were identified as *enterocytozoon bienuesi*. No *Encephalitozoon intestinalis* species were identified.

*Blastocystis* was identified in six stool samples (30%, 95% CI 11.89% to 54.28%). *Giardia* was also identified in 6 stool samples (30%, 95% CI 11.89% to 54.28%).

Viral pathogens identified were *adenovirus* in 4 stool samples (20% 95% CI 5.73% to 45.66%), *norovirus* in 2 stool samples (10%, 95% CI 1.23% to 31.70%), *astrovirus* in one stool sample (5%, 95% CI 0.13% to 24.87%) and *sapovirus*, also in two stool samples (10% 95% CI 1.23% to 31.70%)

The following pathogens were not isolated: *Clostridium difficile*, *Cyclospora*, *Entamoeba histolytica*, *Isospora*, *Rotavirus*, *Salmonella* and *Strongyloides*. Figure 5 below shows the number of pathogens isolated.

### 3.5.2 **Pathogens in Stool Samples from Controls**

Stool samples from eight control subjects were processed. The most common pathogen isolated was *Blastocystis*. This was isolated in seven stool samples (87.5%, 95% CI 47.35 to 99.68). *Shigella* spp was isolated in 6 stool samples (75%, 95% CI 34.91% to 96.81%), while *Shigella flexneri* was found in 2 stool samples (25%, 95% CI 3.19% to 65.09%).

*E. coli* species were also isolated. ETEC was isolated in four stool samples (50%, 95% CI 15.70% to 84.3%), EPEC was also isolated from four stool samples (50%, 95% CI 15.7% to 84.3%) and EAEC was found in three stool samples (37.5%, 95% CI 8.52% to 75.51%).

*Giardia* was identified in 3 stool samples (37.5%, 95% CI 8.52 to 75.51%), and the other identified pathogens were *E. bienuesi* (one stool sample), Sapovirus (one stool sample) and *Schistosoma* (one stool sample) (12.5%, 95% CI 0.32% to 52.65%). Figure 6 below shows a graph of the isolated pathogens.

### 3.5.3 **Comparison of The Prevalences of the Pathogens Between Cases and Controls**

There was a statistically significant difference in the prevalence of the Blastocystis between cases and controls with a p-value of 0.017. For the rest of the pathogens, there was no statistically significant differences in the prevalence between the cases and the controls. Table 5 below shows the prevalence.

**Table 5. Prevalence of the pathogens**

| Pathogen (%) | Prevalence among cases- percentage (n=20) | Prevalence among controls- percentage (n=8) | Fisher's exact test p-value |
|---|---|---|---|
| *EAEC* | 65 | 37.5 | 0.2309 |
| *ETEC* | 60 | 50 | 0.6908 |
| *Shigella spp* | 60 | 6 | 0.6692 |
| *E. bieneusi* | 40 | 12.5 | 0.2143 |
| *EPEC* | 35 | 50 | 0.6715 |
| *Blastocystis* | 30 | 87.5 | 0.0107 |
| *Giardia* | 30 | 37.5 | 1 |
| *S. flexneri* | 35 | 25 | 1 |

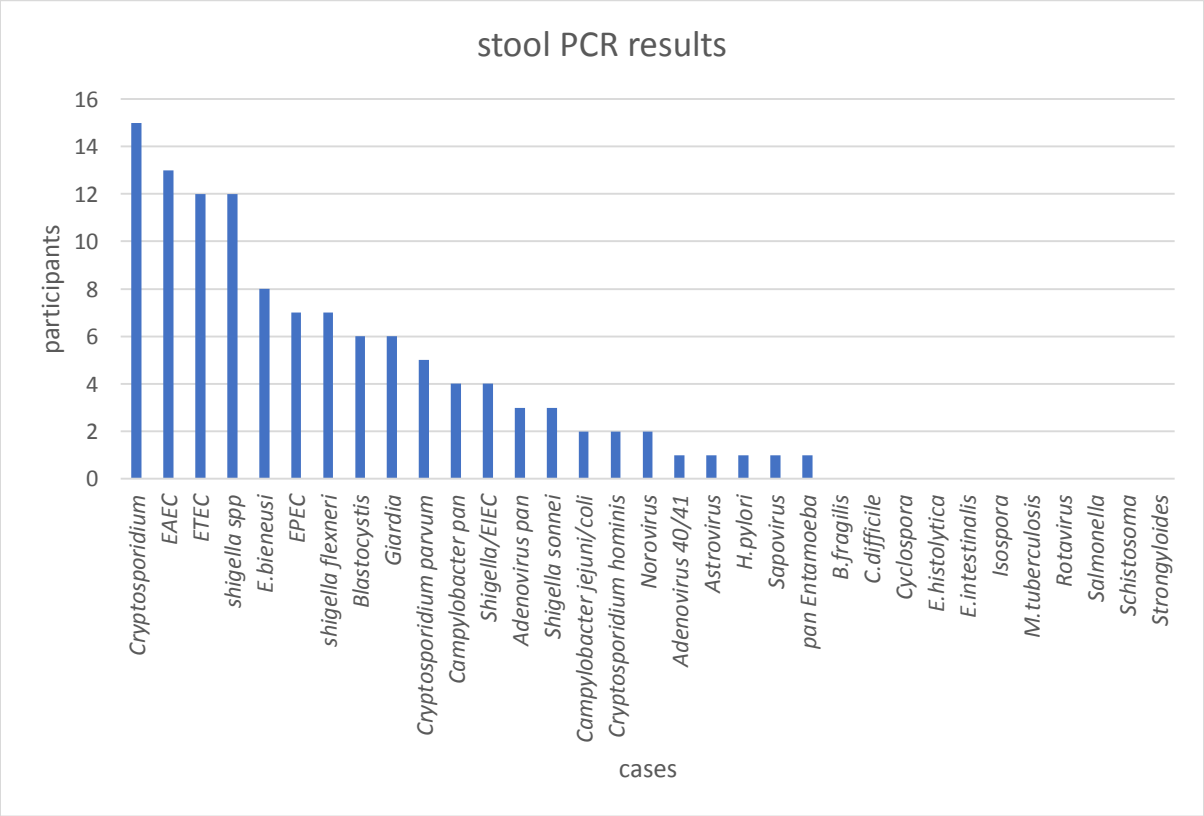


**Figure 5: Stool PCR results -cases**

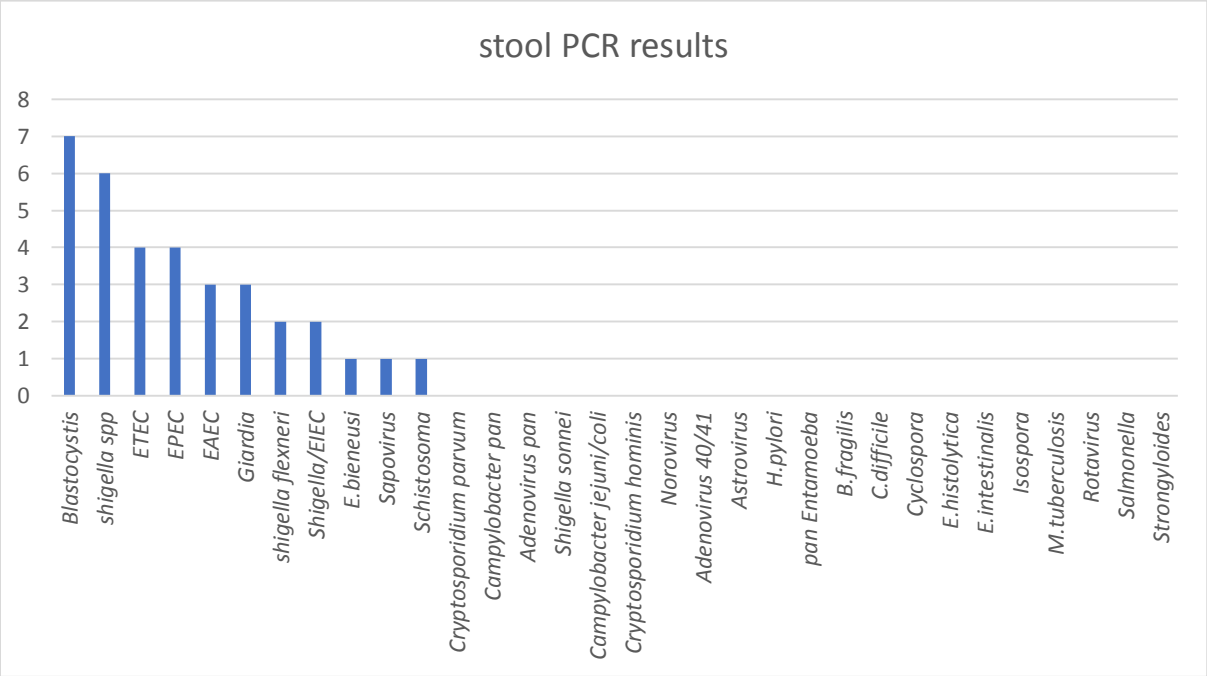


**Figure 6: Stool PCR results-controls**

### 3.5.4 Co-Infections

In total, there were 116 pathogens identified from 20 stool samples from the cases, and 35 pathogens from 8 stool samples from the controls. The median number of pathogens identified from one stool sample was 6.5 from the cases, and 3.5 from the controls. The number of isolated pathogens in one stool ranged from one to 12 from the cases, and 2 to 10 in the control group. There was no statistically significant difference in the median number of pathogens isolated between the cases and the controls with a p-value of 0.2714 (mann-whitney u test- U value 58)

### 3.5.5 **Symptoms and Stool Results**

The three most common symptoms identified in the cases were anorexia, nausea and abdominal pains. Anorexia was reported by 12 out of the 20 cases. 11 participants reported feeling nauseous. Abdominal pain was a complaint in 8 people, and 6 people reported having at least one episode of vomiting. 5 percent of the cases reported at least one episode of bloody diarrhoea and 10 percent reported flatulence. (see table 5)

From the participants who had EAEC identified in the stool (among other pathogens), eight reported feeling anorexic, five had abdominal pains and 4 had vomiting and 7 reported nausea. From the participants who had ETEC isolated, eight participants reported feeling anorexic, six had nausea, five had abdominal pains and four reported at least one episode of vomiting. 12 patients had *shigella* clade 1 identified from their stools. Of these, 7 had reported having anorexia, 6 having abdominal pains,5 having had episodes of vomiting and 6 participants had nausea.

Out of 8 patients with *E. bieneusi*, 4 had reported anorexia, 3 reported nausea, 3 had reported vomiting and two had reported abdominal pains. For patients with giardia, five out of six reported feeling anorexic. Three reported having abdominal pains, reported episodes of vomiting, and 5 reported nausea. Six people had blastocysts isolated in from their stool specimen. Five of these reported nausea, 3 reported having anorexia, 2 people had abdominal pains, and 2 people reported having episodes of vomiting. (see figure 7)

**Table 6- symptoms associated with diarrhoea**

| **symptoms** | frequency % (n=20) |
|---|---|
| anorexia | 60 (12) |
| nausea | 55 (11) |
| Abdominal pain | 40 (8) |
| vomiting | 30 (6) |
| flatulence | 10 (2) |
| bloody diarhoea | 5(1) |

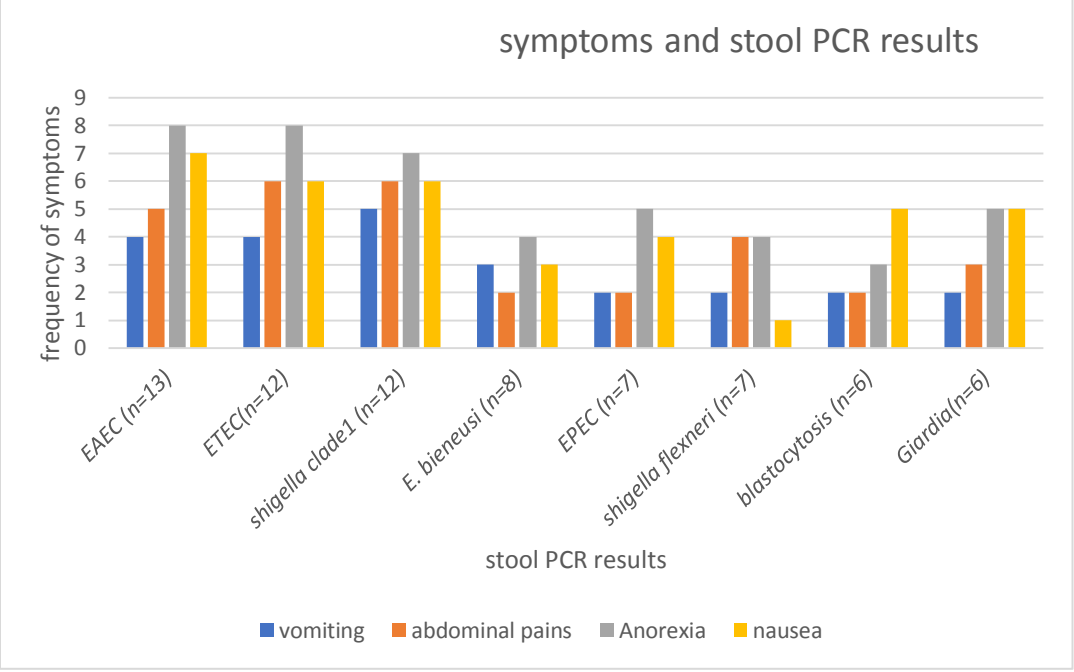


**Figure 7 symptoms according to pathogens**

3.6 **Antibiotics Use**

95% (95% CI 75.13% to 99.87%) of the study subjects with diarrhoea had used antibiotics at some point in time within six weeks of presenting to the hospital. 75% (95% CI 50,90% to 91.34) of the subjects had taken metronidazole, 10% (95% CI 1.23% to 31.70%) had taken ciprofloxacin, 5% (95% CI 0.13% to 24.87%)had had ceftriaxone and another 5% had taken doxycycline. (See figure 8)

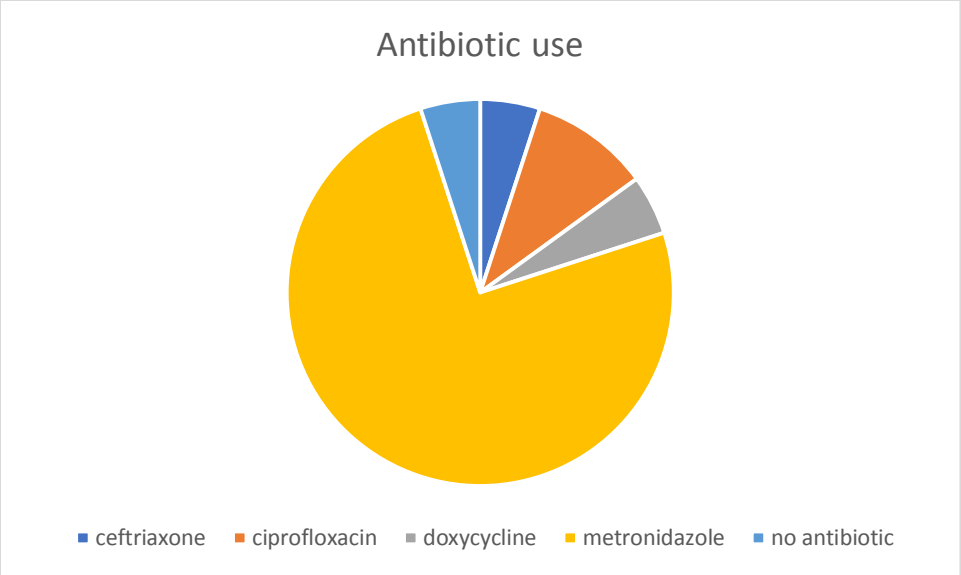


**Figure 8;Antibiotics use within 6 weeks of admission**

# CHAPTER 4: DISCUSSION

## 4.1 **General Discussion**

This study designed to investigate the spectrum of pathogens associated with chronic diarrhoea in PLWHIV. The entry point to the study was a confirmed diagnosis of cryptosporidium and diarrhoea. The 2005 revised WHO clinical staging of HIV categorized chronic cryptosporidiosis as a stage 4 HIV disease. (40) Since all the participants had been on ART for over six months, all participants would clinically be classified as ART clinical failure. Among cases, only 2 out of 20 participants had CD4 counts above 100, and only 1 participant had undetectable viral loads. Therefore, 19 out of 20 participants had virological failure and all of them had immunological failure. This is in keeping with findings in multiple studies that have shown that cryptosporidiosis is associated with low CD 4+ cell counts, and worsening immunosuppression [15,40-45]. In a study looking at intestinal parasites and CD 4+ cell count in Addis Ababa, Ethiopia, the rate of infection of cryptosporidium was higher in those with CD 4 counts less than 200, and they were more likely to present to hospital with diarrhoea as well [40]. This was also seen in another study in Nigeria, where the presence of cryptosporidium in stools was also associated with lower CD 4+ cell counts and presence of diarrhoea [44].

In the control group, the mean CD4+ cell count was 419, and only one participant had a detectable viral load (2683 copies/ml). This was a group of people without cryptosporidium and without diarrhoea. In our study, pathogens were also isolated from the asymptomatic participants. Multiple studies have shown presence of enteric pathogens in asymptomatic people living with HIV. In one study in Peru, looking at

*E. coli,* diarrhoegenic EAEC, ETEC and EPEC were isolated in individuals living with HIV without diarrhoea. Enteric parasites have also been found in PLWHIV without diarrhoea [43].

In this study, all the cases were identified with cryptosporidiosis. This was so because all the patients recruited as cases were first screened for *cryptosporidium* and only recruited if positive. EAEC was isolated in 65% of the cases (13 people), ETEC was isolated in 60% (12 people) of the cases, shigella spp. was isolated in 60 % of the cases, *E. bienuesi* was isolated in 40% of the cases, EPEC, *shigella flexineri, blastocystis spp* and *giardia* were isolated in 35%, 35%, 30 % and 30% of the cases respectively.

EAEC usually causes acute self-limiting watery diarrhoea with mucus and some fever. In immunocompromised individuals, protracted diarrhoea can occur [46]. A strong link between EAEC and HIV infection has been shown in previous studies [47]. In a meta-analysis looking at causes of acute and chronic diarhoea, EAEC was associated with diarrhoea in individuals living with HIV in developing countries with an odds ratio of 6.43 [48]. In Peru, in one study looking at adults living with HIV with and without diarrhoea in Lima, EAEC was isolated in in 7 % of people with diarrhoea and 11 percent of people without diarrhoea. In our study, EAEC was isolated in 65% of the cases and 37.5% (3 participants) of the controls. Similar to our findings, a high rate of EAEC isolation from stools of PLWHIV with diarrhoea was also found in Ethiopia, using molecular methods. In that study, EAEC was isolated from 59% of the participants [49].

ETEC is one of the common causes of diarrhoea in developing countries, especially in children. It has also been associated with diarrhoea in adults living with HIV. The pathogen has been isolated in PLWHIV without diarhoea as well. In Iran, one study isolated ETEC in 57% of PLWHIV without diarrhoea [50]. In our study 60% of the cases and 50% of the controls had ETEC. The rate of ETEC isolation has varied widely in different studies. This might be partly because of different isolation methods used and variation in the study populations. Using molecular techniques, a study in Ethiopia found ETEC in 73.3% of HIV reactive patients with diarrhoea [49]. This is in contrast to a study in Peru where ETEC was isolated in 13% of HIV reactive patients with diarrhoea [47].

Enteropathogenic *E. coli* has been described as a common cause of diarrhoea in infants and children, with the incidence decreasing into adulthood [51]. However, it is being recognized as an important cause of diarrhoea in adults living with HIV in developing countries. Okoror et al isolated EPEC in 75% of PLWHIV with diarrhoea in South West Nigeria [52]. Our study found EPEC in 35% of the cases and 50% of the controls. In contrast, EPEC was isolated in only 8.7% of HIV reactive patients with diarrhoea and 8.9% of patients without diarrhoea in Peru [47]. Our study suggests that EPEC is a common enteropathogen in HIV reactive patients with and without diarrhoea.

In our study, a significant proportion of participants had *shigella* species isolated. Among the cases, 60 percent had shigella spp isolated, 35 percent had *shigella flexneri*, 15 percent had *shigella sonnei* and 20% had *shigella/EIEC*. In the control group, 75% had *shigella spp*, 25 percent had *S. flexneri*, and 25 percent had *shigella/EIEC*. Shigellosis is well known to be a common pathogen in HIV reactive

patients. Arago et al in a study in san Francisco USA identified HIV as a risk factor for shigellosis [52]. In Northeast Ethiopia, Belay et al, identified *shigella* in 2% of HIV patients with diarrhoea [53]. In contrast to this Ngalani et al, in Cameroon, identified *shigella* in 68% of HIV positive patients with diarrhoea. In that study, antibiotic resistance was prevalent. Our study confirms high shigella prevalence in HIV reactive patients [55].

Enteric parasites are a leading cause of chronic diarrhoea in HIV reactive patients. In our study, the parasites identified were *E. bieneusi* (in 40% of the cases, and 12.5% of the controls), *Blastocystis* (in 30% of the cases and 87.5% of the controls) and *giardia* (in 30% of the cases and 37.5% of the controls). As stated already, all the cases were cryptosporidium positive. We did not identify *isospora* or *cyclospora.*

*Blastocystis* is one of the most prevalent parasites in both humans and animals [56,57]. It has been isolated in HIV reactive patients with and without diarrhoea. The prevalence of *blastocystis* in HIV reactive patients has varied from as low as 3 percent, to as high as over 70%, depending on detection method [41,42,58-66].High prevalences have been documented in senegal (100%) and Liberia (70%) [67,68]. using molecular methods. In our study, the prevalence of *blastocystis* was higher in controls (who had higher CD4+ counts) as compared to the cases (p-value 0.0107). This has been observed in previous studies as well. In Ghana, among patients with HIV, *blastocystis* was more prevalent at higher CD4 counts. Some authors have hypothesized that *blastocystis* might be part of normal gut microbiota [64]. Therefore, its role as a cause of diarrhoea is still debatable.

*Microsporidium* is another common parasite identified in HIV reactive patients with chronic diarrhoea. In our study, 40% of the cases and 12.5% of the controls had *E. bieneusi*, and no *Encephalitozoon intestinalis* was identified. This is in keeping with previous observations that *E. bieneusi* is more commonly isolated in HIV patients with chronic diarrhoea than *E. intestinalis*. From one meta-analysis, the prevalence of *microsporidium* was calculated to be 14.4% in sub-Saharan Africa [43]. Higher prevalences have been reported in other studies. In Mexico, *Microsporidium* was isolated in 43% of HIV infected individual. In that study, it was the most common parasite in HIV reactive patients [62]. *Microsporidium* is usually isolated at low CD4 counts [44,45,70,71].

In our study, we found high rate of coinfection. The median number of pathogens isolated per person in the cases was 6.5 and 3.5 in the control group. However, there was no statistically significant difference between the median number of pathogens isolated in cases and the controls. Presence of multiple enteric pathogens in HIV reactive patients with chronic diarrhoea is a common finding. In Southern Ethiopia, in one study, 2 or more enteric pathogens were isolated in over 33% of the subjects. One participant had up to 5 pathogens isolated [40]. In India, Dwivedi et al isolated multiple pathogens in 44% of patients with HIV and chronic diarrhoea. In patients with HIV but without diarrhoea, only 9% had multiple pathogens. Our study suggests high burden of pathogens in people with chronic diarrhoea.

In our study, the most common symptoms reported were anorexia in 60 percent of the participants, nausea, reported in 55% of the participants, abdominal pain, reported in 40 percent and vomiting in 30%. Two out of the 20 participants reported bloody diarrhoea and flatulence. All these are common symptoms in enteric

infections regardless of the specific aetiological pathogen. Some specific pathogens have been associated with some symptoms. For example, Lactose intolerance with flatulence has been documented to be associated with giardia infection [72]. Despite that, clinical features have generally failed to distinguish between coccidial causes of chronic diarrhoea [73]. In this study all the pathogens were associated with all the common symptoms.

Ninety five percent of cases had used some antibiotic within 6 weeks of admission. The most commonly used antibiotic was metronidazole, which was used by 15 out of 19 patients. Two individuals had ciprofloxacin, and one had ceftriaxone and the other one had doxycycline. Antibiotic use is very common in both acute and chronic diarrhoea. In one study in Nigeria, antibiotics were prescribed in 85% of patients with acute diarrhoea despite lack of clear indications [74]. In Ethiopia, antibiotics were used in 72% in patients presenting with acute diarrhoea regardless of age of the patients [75].

The Malawi treatment guidelines for management of HIV associated chronic diarrhoea advocate a step wise approach to treating patients with chronic diarrhoea [76]. It recommends starting with cotrimoxazole to target *I. belli*, *Cyclospora* and bacterial causes. In our study, the most common pathogens isolated were bacterial; EAEC, ETEC, EPEC and *shigella spp*. The antibiotic sensitivity of these bacterial pathogens in our setting is not known. Various studies have looked at the antibiotic sensitivity patterns of diarrhoeagenic *E.coli* in sub-Saharan Africa. In south Africa, from Eastern Cape, up to 82%-96% of diarrhoeagenic *E.coli* isolates from patients with diarrhoea were resistant to cotrimoxazole [77,78]. Another study from Ethiopia identified cotrimoxazole resistance in 62% of diarrhoeogenic *E.coli* from stool

samples of underfive children with diarrhoea [79]. Our study could not answer if cotrimoxazole is still the best option for bacterial causes of diarrhoea in HIV reactive patients at Queen Elizabeth central hospital. Based on the above studies, it might be imperative to identify antimicrobial susceptibility patterns in diarrhoeogenic *E.coli* isolates from our population.

Cotrimoxazole is also the first line treatment option for *I.belli* and *cyclopora* [80-83]. This study did not identify any *I. belli* or *Cyclospora* in our study subjects. Since our sample size was small, we cannot conclude with confidence that *I. belli* and *Cyclospora* are not important causes of chronic diarrhoea in our setting.

The second step in managing chronic diarrhoea according to Malawi treatment guidelines is metronidazole, targeting *giardia, clostridium, amoeba* and *microsporidium*. *Giardia lamblia, amoeba and clostridium* are known to be susceptible to metronidazole [84]. However, metronidazole is known to be inactive against *microsporidium* in vitro or in animal models and most guidelines do not recommend it as anti-microsporidial treatment [85,86]. The recommended treatment options for *microsporidium are* albendazole for *E. intestinalis* and oral fumagillin for *E. bieneusi* [87,88]. Despite the above recommendation it has been shown that albendazole has some benefit in patients with *E. bieneusi*. In one study, treatment with albendazole for *E. bieneusi* led to a statistically significant reduction in bowel movements despite failure to eradicate the infection [89]. In our study, we isolated E. bieneusi only which might not respond very well to albendazole.

Albendazole is on the third step in managing HIV reactive patients with chronic diarrhoea. It targets microsporidium and helminths [84]. In our study, we did not identify any helminths in patients with chronic diarrhoea.

In our study, no participant was prescribed cotrimoxazole or albendazole for chronic diarrhoea. This might be a sign of lack of knowledge or poor adherence to the guideline.

### 4.2 Limitations

Our study was limited by the small number of participants recruited. This study was nested within a bigger study which closed before they recruited the targeted number of participants because mid-term analysis did not show positive results. Initially, 56 cases and 10 controls were expected to be recruited. But by the time the big study closed, we had recruited only 20 cases and 10 controls. The small sample size meant that our study was not powered enough to answer some of the objective set out initially.

# CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

## 5.1 **Conclusion**

With the limitations described, our study showed that patients with HIV and diarrhoea with *cryptosporidium*, have low CD4 counts and high viral loads. They also have other enteric pathogens including *EAEC, EPEC, ETEC, shigella spp, E. bienuesi and giardia*. It also showed that *blastocystis* is a common pathogen in HIV reactive patients, with and without diarrhoea. Anorexia, nausea, abdominal pain and vomiting were the common accompanying symptoms in patients with chronic diarrhoea. We also found that the use of antibiotics in chronic diarrhoea is prevalent, though not according to Malawi treatment guidelines.

## 5.2 **Recommendations**

We have shown that patients presenting with diarrhoea on the background of living with HIV have a high rate multiple enteric pathogens. A diagnosis of cryptosporidiosis diarrhoea is an indication that other pathogens are likely present. There is need for a larger sample size to assess this finding with confidence. A larger sample size is also needed to assess if specific symptoms could be used to identify specific pathogens in people living with HIV who present with diarrhoea. We also recommend assessment for ART failure in HIV reactive patients with chronic diarrhoea, as 90% of the patients in our study had virological failure. We recommend front line clinicians to follow the recommended approach to treat chronic diarrhoea to avoid unnecessary use of antibiotics which can lead to antibiotic resistance.

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**APPENDICES**

**Appendix A**

**Case Record Form (English)**

**Spectrum of intestinal pathogens and their clinical presentation in HIV-positive patients with diarrhoea at QECH adult medical wards**

study id ☐ date of ☐

recruitment ☐

☐

Age ☐ sex

Residence:

1. History

| | yes | No |
|---|---|---|
| Anteretroviral medications | | |
| If yes what regiment | | |
| Duration on ART (years) | | |
| Cotrimoxazole preventive therapy | | |
| Isoniazid preventive therapy | | |
| Any other medications | | |

CURRENT ILLNESS

| | Yes | No |
|---|---|---|
| Duration of diarrhoea (in days) | | |
| Stool frequency(per day) | | |
| Vomiting | | |
| Nausea | | |
| Anorexia | | |
| Fatigue/malaise | | |
| Urgency of defecation | | |
| Abdominal pains | | |
| Fever | | |

| | | |
|---|---|---|
| Weight loss | | |
| bloody diarrhoea | | |
| flatulence | | |
| Antiobiotics (within 6 weeks) | | |
| If yes, what antibiotic | | |

2. Examination on recruitment:

weight BP PR RR

Temperature

| | Yes | No |
|---|---|---|
| abdominal tenderness | | |
| hepatomegaly | | |
| splenomegaly | | |
| conjunctival pallor | | |
| jaundice | | |

3. investigations

| ***Hematology*** | |
|---|---|
| Hemoglobin (g/dl) | |
| Platelets ($x10^9$ /$mm^3$) | |
| WBC ($x10^3$/$mm^3$) | |
| Neutrophils ($x10^3$/$mm^3$) | |
| Lymphocytes ($x10^3$/$mm^3$) | |
| CD4 (cells/µl) | |
| ***biochemistry*** | |
| Sodium (mmol/l) | |
| Potassium (mmol/l) | |
| Chloride (mmol/l) | |
| Bicarbonate (mmol/l) | |
| Creatinine (mg/dl) | |
| BUN (mg/dl) | |
| Alanine Aminotransferase (ALT) (u/l) | |

| | |
|---|---|
| Aspartate Aminotransferase (AST) (u/l) | |
| Alkaline Phosphatase (u/l) | |
| Glucose (mg/dl) | |
| ***Microbiology*** | |
| HIV viral load | |
| Taqman assay result | |

**Appendix B**

**Consent form (English)**

| Centre | Study Number | Patient Identification Number |
|---|---|---|
| | | |

## PATIENT OR GUARDIAN CONSENT FORM

### STUDY TITLE

**Spectrum of intestinal pathogens and their clinical presentation in HIV-positive patients with diarrhoea at Queen Elizabeth Central Hospital-adult medical wards**

□ Statement by patient;

**Please circle Yes or No to confirm whether you agree or do not agree with each of the points below:**

- I have read the information sheet or it has been read to me
  Yes / No
- I have had the chance to ask questions and I am satisfied with the answers that I have been given
  Yes / No
- I voluntarily agree to take part but I know that I can change my mind at a later date
  Yes / No
- I agree that my results from the Cryptofaz study can be used for this substudyYes / No

| Name of participant | Date | Signature/thumbprint |
|---|---|---|
| | | |
| *Name of guardian | Date | Signature/thumbprint |
| | | |
| **Name of witness | Date | Signature |
| | | |
| Name of patient administering consent | Date | Signature |
| | | |

*****If participant cannot give consent on their own e.g. children, mentally ill, unconscious patients

**If both participant and guardian cannot read or write.

Guardian relationship: _____

## Appendix C
## Patient information (English)

PATIENT INFORMATION DOCUMENT

**STUDY TITLE:**

Spectrum of intestinal pathogens and their clinical presentation in HIV-positive patients with diarrhoea at Queen Elizabeth Central Hospital-adult medical wards

### Introduction
Diarrhoea is a common problem in HIV reactive patients regardless of the level of immunity. There are many causes of chronic diarrhoea in this population. In our local setting, the various causes of chronic diarrhoea have not been described properly.

### what is this research for?
This is a substudy within the Cryptofaz study. This study wants to find out the specific infectious causes of chronic diarrhoea in HIV reactive patients and how they present, and to compare the causes in people with different levels of immunity. It will also compare the pathogens found in HIV reactive people that do not have diarrhoea.

### Why am i being asked to take part?
You are being asked to take part in this substudy because you have been found with cryptosporidium in stools, or you are a possible match for another subject with cryptosporidium in their stool

### what will happen to me?
If you consent to take part in this study, we will take some of the results from the tests taken for the cryptofaz study for analysis. No further tests will be done on you.

### what will happen to my samples?
No other samples will be collected for this substudy. We will only use some of the results from the samples collected for the Cryptofaz study.

### what impact will the study have on me?
BENEFITS: At the end, you would have contributed to new medical knowledge on causes of chronic diarrhoea in HIV reactive patients

RISKS: there are no risks for you if you participate in this substudy.

### WHO WILL hAVE ACCESS TO THE INFORMATION YOU COLLECT ABOUT ME?
The researcher team who will collect the samples, will have access to the information. We may also send samples abroad for testing. The records of this study will be kept private and will be protected to the fullest extent provided by law. In any sort of report we might publish, we will not include any information that will make it possible to identify you as a subject. Research records will be stored securely and only the researcher team will have access to the records

## WHAT HAPPENS IF I DON'T WANT TO TAKE PART?

Your participation in this substudy is purely voluntary. There are no any negative consequences that you will face if you do not want to take part in this substudy. If you consent to participate in this substudy, and later on change your mind, you are free to stop. This will not affect the usual daily hospital care you will receive at Queen Elizabeth Central Hospital.

## WHO CAN I GO TO FOR MORE INFORMATION ABOUT THIS?

Dr Patrick Nachipo (MMED student)
College of Medicine)
P/Bag 360
Chichiri, Blantyre 3
Tel: +265 0999041515

COMREC Secretariat
College of Medicine
P/Bag 360,
Chichiri, Blantyre 3
Tel: 01 871 911 ext. 334

**Appendix D**

CASE RECORD FORM-chichewa version

**Spectrum of intestinal pathogens and their clinical presentation in HIV-positive patients with diarrhoea at QECH adult medical wards**

study id ☐ date of ☐

recruitment ☐

☐

Age ☐ sex

Residence:

1. History

| | inde | ayi |
|---|---|---|
| Mankhwala a HIV (ARV) | | |
| Ngati ndichoncho, tchulani mtundu wamankhwala | | |
| Mwakhala mukumwa ma ARV kwa nthawi yayitali bwanji (zaka) | | |
| Kodi mukumwa mankhwala oteteza matenda a bactrim | | |
| Kodi mukumwa mankhwala oteteza chifuwa chachikula cha TB (isoniazid) | | |
| Aliponso mankhwala ena amene mukumwa ndipo tchulani mtundu wamankhwalawo | | |

Zokhudza matendawa (otsegula mmimba)

| | Inde | Ayi |
|---|---|---|
| Mwatsekula mmimba kwa nthawi yayitali bwanji (masiku) | | |
| Mukutsekula mmimba kangati pa tsiku | | |

| | | |
|---|---|---|
| mukusanza | | |
| Mukuchita nseru | | |
| Chilakolako chachakudya chathawa | | |
| Kodi mukukhala ofooka | | |
| Kodi mukumafuna kupita kuchimbudzi mwachanguchangu (urgency of defecation) | | |
| Kodi mukumamva kupweteka mmimba | | |
| Mukutentha thupi | | |
| Mukuona kuti mwaonda | | |
| Kodi mukutsekula zamagazi | | |
| Mukuphwisa pafupipafupi | | |
| Mwamwako mankhwala othana ndi tizilombo toyambitsa matenda mmasabata asanu ndi imodzi apitawa (Antiobiotics within 6 weeks) | | |
| Ngati ndi choncho, tchulani mtundu wa mankhwalawo | | |

2. Examination on recruitment:

weight BP PR RR

Temperature

| | Yes | No |
|---|---|---|
| abdominal tenderness | | |
| hepatomegaly | | |
| splenomegaly | | |
| conjunctival pallor | | |
| jaundice | | |

3. investigations

| | |
|---|---|
| ***Hematology*** | |
| Hemoglobin (g/dl) | |
| Platelets ($x10^9$ /$mm^3$) | |
| WBC ($x10^3$/$mm^3$) | |
| Neutrophils ($x10^3$/$mm^3$) | |
| Lymphocytes ($x10^3$/$mm^3$) | |
| CD4 (cells/µl) | |
| ***biochemistry*** | |
| Sodium (mmol/l) | |
| Potassium (mmol/l) | |
| Chloride (mmol/l) | |
| Bicarbonate (mmol/l) | |
| Creatinine (mg/dl) | |
| BUN (mg/dl) | |
| Alanine Aminotransferase (ALT) (u/l) | |
| Aspartate Aminotransferase (AST) (u/l) | |
| Alkaline Phosphatase (u/l) | |
| Glucose (mg/dl) | |
| ***Microbiology*** | |
| HIV viral load | |
| Taqman assay result | |

**Appendix E**

consent form (Chichewa)

| Centre | Study Number | Patient Identification Number |
|---|---|---|
| | | |

## CHIKALATA CHOPEMPHERA CHILOLEZO CHOTENGA NAWO MBALI KWA ODWALA KAPENA OYANG'ANIRA ODWALA

## MUTU WA KAFUKUFUKU

Spectrum of intestinal pathogens and their clinical presentation in HIV-positive patients with diarrhoea at Queen Elizabeth Central Hospital-adult medical wards

□ Mau olankhula Odwala

**Chonde lembani mozungulira mau oti Inde kapena Ayi kuti mutsimikize kuti mukugwirizana kapena simukugwirizana ndi mfundo zomwe zili m'munsimu:**

- Ndawerenga chikalata chopemphera chilolezo chotenga nawo mbali kapena andiwerengera chikalata chopemphera chilolezo chotenga nawo mbali.
  Inde / Ayi
- Ndapatsidwa mwayi wofunsa mafunso ndipo ndakhutitsidwa ndi mayankho amene ndapatsidwa.
  Inde / Ayi
- Ndavomera kutenga nawo mbali mosakakamizidwa koma ndikudziwa kuti ndikhoza kusintha maganizo anga mtsogolo.
  Inde /Ayi
- Ndavomereza kuti zotsatira za zoyeza zanga (*tchulani mtundu wa zoyeza apa)* / zokhudza ine zikhoza kutengedwa kuti akagwiritsidwe ntchito pa kafukufukuyu
  Inde / Ayi

| Dzina la wotenga nawo mbali | Deti | Siginitchala/chidindo cha chala |
|---|---|---|
| | | |

| *Dzina la oyang'anira odwala | Deti | Siginitchala/chidindo cha chala |
|---|---|---|
| | | |
| **Dzina la mboni | Deti | Siginitchala |
| | | |
| Dzina la odwala amene akupereka chilolezo chotenga nawo mbali. | Deti | Siginitchala |
| | | |

***** Ngati odwala sangathe kupereka chilolezo chotenga nawo mbali pa okha, monga ana, odwala misala, odwala koma achikomokere

**Ngati onse otenga nawo mbali ndi oyang'anira sangathe kulemba kapena kuwerenga.

**Appendix F**

Patient information (Chichewa)

PATIENT INFORMATION DOCUMENT

**MUTU WA KAFUKUFUKU:**

Spectrum of intestinal pathogens and their clinical presentation in HIV-positive patients with diarrhoea at Queen Elizabeth Central Hospital-adult medical wards

Mawu oyamba

Matenda otsekula mmimba amagwira anthu ambiri omwe ali ndi kachilombo ka HIV, komwe kamayambitsa matenda a edzi. Zinthu zimene zimayambitsa matenda otsekula mmimba amgonagona zilipo zambiri. Kwathu kuno, tizilombo timene timayambitsa matendawa sitinadziwike mwatsatanetsatane.

kafukufukUyu ndi wa chani?

Kafukufukuyu ali pansi pa kafukufuku wa CRYPTOFAZ. Tikufuna kupeza tizilombo timene timayambitsa matenda otsekula mmimba amgonagona mwa anthu amene ali ndi kachilombo ka HIV, ndipo tidzaona kuti zimasiyana bwanji molingana ndi chitetezo cha mthupi. Tidzafananizanso ndi anthu ena omwe ali ndi kachilombo ka HIV koma sakutsekula mmimba.

Chifukwa chani ndikupemphedwa kutenga nawo mbali?

Mukupemphedwa kutenga nawo mbali chifukwa mwapezeka ndi kachilombo koyambitsa matenda ostekula m'mimba ka cryptosporidium kapenanso mwapimidwa kuti kuti mutha kulowa nawo mu gawo B la kafukufuku wa Cryptofaz

CHITACHITIKE NDI CHANI KWA INE?

Mukavomera kutenga nawo mbali mu kafukufukuyu, tidzatenga zotsatira za zoyesa zimene atenga kale a kafukufuku wa cryptofaz. Mukafukufukuyu, simudzatengedwanso zoyeza zina zilizonse.

kafukufukuyu abweretsa chani ku moyo wanga?

PHINDU: mudzathandiza kupereka mzeru zatsopano zokhuza tizilombo timene timayambitsa matenda otsekula mmimba mwamgonagona mwa anthu amene ali ndi kachilombo ka HIV

ZIOPYEZO: Palibe zoopsa zinazilizonse kwa inu mukalowa mu kafukufukuyu.

Kodi ndi ndani amene ali ndi chilolezo chowona uthenga umene mutatenge kuchokera kwa ine?

Gulu loyendetsa kafukufuku ndilimene likhale ndi mwayi owona zimenezi. Zolembedwa zonse za kafukufuku uyu zidzasungidwa pazokha ndipo zidzatetezedwa kwambiri moyendera malamulo avomerezeka ndi boma. Pazolemba zimene tingatsindikidze, sitidzalembamo uthenga umene ungapangitse kuti inu mudziwike kuti uthengawo ndi wanu. Malipoti onse a kafukufuku uyu adzasungidwa motetezedwa ndipo a gulu lochititsa kafukufuku adzakhala ndi mwayi owona malipotiwa.

## Chichitika ndi chani ngati sindikufuna kutenga nawo mbali?

Kutenga nawo mbali kwanu ndi kudzipereka kwanu mosakakamizidwa. Palibe zotsatira zilizonse zoipa zimene mukumane nazo chifukwa simukufuna kutenga nawo mbali mu kafukufuku uyu. Ngati mwavomera kutenga nawo mbali mu kafukufuku uyu, muli ndi ufulu kusiya kutenga nawo mbali patsogolo mukaganizo kusintha maganizo anu. Izi sizidzasintha chisamaliro cha kuchipatala chimene mukulandira ku Queen Elizabeth Central Hospital.

## Kodi ndingadziwe zambiri kuchokera kwa ndani zokhudzana ndi kafukufuku uyu?

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