THE ASSOCIATION OF INTESTINAL PARASITES WITH CHRONIC DIARRHOEA IN ADULT AIDS PATIENTS IN NORTH-EASTERN TANZANIA

BY

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ABSTRACT

A study on the association of intestinal parasites with chronic diarrhoea among adult AIDS patients was conducted in the North-eastern zone of Tanzania. Diarrhoea was found to be a major problem among the patients. More than 70% of the patients had diarrhoea during their illness.

Intestinal parasites were detected in 123 out of 352 (34.9%) patients. The proportion of patients with intestinal parasites and chronic diarrhoea was 62.6% (77 out of 123). The prevalence of chronic diarrhoea in patients who were in clinical stage III-IV of AIDS was highest among patients harbouring parasites that are known to cause diarrhoea. The association of diarrhoeagenic parasites (combined together) with chronic diarrhoea was found to be statistically significant. The adjusted associations were also found to be statistically significant. Cryptosporidium spp, Isospora belli and Strongyloides stercoralis were only isolated from patients with chronic diarrhoea .

It was concluded that chronic diarrhoea was a major complaint of adult AIDS patients in North-eastern Tanzania. Parasites known to cause diarrhoea considered together were the most likely cause of chronic diarrhoea in such patients. Cryptosporidium spp, Isospora belli, and

Strongyloides stercoralis were the most likely cause of chronic diarrhoea among the patients. Development of clinical infection due to parasites known to cause diarrhoea was found to be related to the immunological status (clinical stage) of the AIDS patients.

It is recommended to strengthen home based care for AIDS patients as well as laboratory facilities at all levels of health care delivery centres for diagnosing specific intestinal parasites in AIDS patients so as to institute specific therapies thereby improving the quality of life of such patients.

A comprehensive case-control study, to establish cause and effect of the various agents isolated from AIDS patients with chronic diarrhoea is proposed. There is a need to determine the type of drugs which will be suitable for the treatment of opportunistic infections which may interact with AIDS in our set up.

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DECLARATION

I HEREBY DECLARE THAT THIS DISSERTATION IS MY ORIGINAL WORK AND HAS NEVER BEEN SUBMITTED IN ANY FORM, FOR A DIPLOMA OR DEGREE IN ANY OTHER UNIVERSITY

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CHAPTER I.0 INTRODUCTION AND LITERATURE REVIEW, STATEMENT OF THE PROBLEM, RATIONALE AND OBJECTIVES

1.1 INTRODUCTION AND LITERATURE REVIEW

The acquired immunodeficiency syndrome (AIDS) is at the most severe end of the clinical spectrum of HIV infection. It is characterized by the presence of several diseases some of which affect the gastrointestinal system. Chronic diarrhoea is one of the gastrointestinal manifestations in patients with AIDS and can be the presenting manifestation or a life threatening complication in such patients when associated with hypovolaemia, severe dehydration and electrolytes imbalance. According to the WHO classification patients with chronic diarrhoea are in clinical stage III or IV of AIDS (Apendix 4). Indeed, persistent or chronic diarrhoea has been one of the major criteria for the clinical diagnosis of the acquired immunodeficiency syndrome. Persistent non-bloody diarrhoea was found to be an outstanding feature of AIDS patients in Africa being associated with profound weight loss (Colebunders *et al.*, 1987; Lucas, 1988; Conlon *et al.*,1990; Dallabeta and Miotti, 1992). Until now, the most common chronic problem in African patients with AIDS is the HIV wasting syndrome accompanied with chronic diarrhoea (Gilks and Ojoo, 1991).

Chronic diarrhoea in AIDS patients can be caused by viruses, bacteria and intestinal parasites (WHO[a],1992; Cegielski *et al.*,1993). The acquired immunodeficiency syndrome (AIDS) is known to predispose patients with the HIV disease to several bacterial, viral, fungal and parasitic infections. Among parasitic infections, only the intracellular coccidia, Isospora belli and Cryptosporidium parvum, are clearly known to be opportunistic infections of the gut (Fleming, 1990). Since the acquired immunodeficiency syndrome is characterized by functional defects in humoral and cell mediated immunity, this status may affect the susceptibility to intestinal parasitic infections and influence the spectrum of severity and clinical manifestations (WHO, 1989).

The downward spiral of poverty and malnutrition predisposes the affected individuals to intestinal parasitic infections which further impoverishes the nutritional status and resistance of the human hosts to infections. This is a classic example of immunocompromisation and parasitic infections which is principally a third world situation where intestinal protozoa and helminths are common. Thus the problems caused by some of the intestinal parasites like malabsorption, malnutrition, diarrhoea and anaemia with the attendant lowered body resistance will be made worse in AIDS patients with chronic diarrhoea (Ministry of

health, Tanzania, 1992).

In general, infection with these parasites is through exposure to infective stages in the environment as a result of lack of adequate and safe water supply, poor hygiene and sanitation. Since exposure to infective stages in the environment is independent of any possible alterations of host immunological status by HIV, it follows that once infected, progression to disease will entirely depend on the level of host immunity which is expected to be low in patients with AIDS (Robinson *et al.*,1991).

The most common parasites isolated from African AIDS patients with chronic diarrhoea are Cryptosporidium spp and Isospora belli (Fleming,1990). These are recently recognized pathogenic intestinal parasites that were brought to the attention of the medical community by the recognition of AIDS (Soave and Jhnson, 1988). Indeed, it was the recognition of the increased occurrence and severe clinical manifestations of otherwise rare infections such as Pneumocystis carinii, Toxoplasma gondii and Cryptosporidium spp. that led to the recognition of AIDS. Cryptosporidium spp and Isospora belli are coccidian parasites widely distributed in domestic and farm animals (Cook, 1988). They are the predominant intestinal parasitic infections in immuno-compromised patients whose

immunological defenses are impaired to a degree such that protection against the development of clinical infection is reduced. In immuno-competent human hosts they produce a transient diarrhoeal illness while in immuno-compromised human hosts infected with HIV they may cause transient, intermittent or persistent diarrhoea ranging from loose stools to watery diarrhoea (WHO, 1987; Weller, 1988; Current, 1989).

In Kampala, Uganda, 61% (N=23) of the AIDS patients with chronic diarrhoea had one or both of these opportunistic infections and it was concluded that the aetiology of chronic diarrhoea in the majority of Ugandan adult AIDS patients is coccidian infection. However the sample size of 23 was too small to make such generalisation (Sewankambo *et al.*, 1987). Similar findings were noted in Haiti where coccidia were only found in AIDS patients with chronic diarrhoea (n=110) in whom about 61% had Isospora belli, Cryptosporidium spp or both and 5.3% of the AIDS patients had other enteric parasites Ascaris lumbricoides, Giardia lamblia, hookworms and Strongyloides stercoralis identified in their stools (DeHovitz *et al.*,1986). In a similar study on adult patients with enteropathic AIDS in Lusaka, Zambia;

Cryptosporidium spp, Isospora belli and Strongyloides stercoralis were identified with frequencies of 32%, 16%

and 6% respectively (Conlon *et al.*,1990).

The high prevalence of HIV infection in areas where intestinal parasitic diseases are endemic raises the fear that the impact of the HIV pandemic may be even more serious than has been predicted if there are significant adverse interactions between HIV infections and intestinal parasitic diseases. In this context the presence of the HIV infection/AIDS will make the patients to be more susceptible to parasites that are known to cause diarrhoea thereby increasing the incidence and prevalence of chronic diarrhoea as a result of immune depression.

Although exposure to severe infections with Strongyloides stercoralis and pathogenic strains of Entamoeba histolytica are known to be associated with impaired cell mediated immunity, these infections are not commonly seen in AIDS patients. Thus the frequency of subclinical infections with Strongyloides stercoralis in African patients with AIDS reflects that in the general population (Lucas, 1988). However in Jamaica, a strong association was shown to exist between HIV infection and parasitologically proved Strongyloides stercoralis (Robinson *et al.*,1990).

It was hypothesized that intestinal parasitic infections that are known to be associated with chronic diarrhoea

occur more frequently among adult AIDS patients with chronic diarrhoea than in those without diarrhoea. Since most of these parasites can be readily diagnosed and treated, efforts should be made to search for them and appropriate therapy when available be administered (Smith *et al.*,1988; Glatt *et al.*,1988).

The Kilimanjaro and Arusha regions have had a dramatic increase in the incidence of AIDS over the last seven years such that in a period of one year (1991-1992) about 62.5% (N = 1135) of the persons referred for HIV testing in the two regional hospitals were found to be positive and more than two thirds of those positive had developed AIDS (Mnyika *et al.*,1993; Klepp *et al.*,1993). HIV infection in these regions is more prevalent in urban areas (Arusha and Moshi) than in roadside settlements (townships) and rural villages.

In both regions there is extensive trading across the border to Kenya, very high rates of unemployment and few young people are able to continue their education past primary school. As a result there is a lot of business related travel and increasing number of young men and women are involved in casual commercial sexual practices consequently predisposing them to HIV infection and AIDS. An increasing number of young women rely on commercial sex-

work consequently making them extremely vulnerable to the HIV/AIDS epidemic. In these two regions diarrhoea is the commonest presenting symptom among adult AIDS patients (Howlett *et al.*,1989).

There was therefore a need to determine whether specific pathogenic intestinal parasites are associated with chronic diarrhoea in adult AIDS patients in Moshi and Arusha so as to be able to select specific palliative therapies which may improve the quality of life of the patients.

1.2 STATEMENT OF THE PROBLEM

The care of AIDS patients comprise of clinical management, nursing and palliative care as well as socio-economic support. The clinical management aims at reducing suffering from conditions caused by the HIV infection itself or by opportunistic diseases thereby improving the quality of life. This is so because until now there is neither effective antiviral agent for the human immunodeficiency virus itself nor treatment for the ensuing immuno-deficiency and therefore the main focus of medical management is the treatment and prevention of the associated conditions like chronic diarrhoea (Pinching and Jeffries, 1988). This can restore the condition of the patients, although temporarily, to a state of good or reasonable health. Before treatment and prevention of

diarrhoea in AIDS patients can be effected, data and information about the associated pathogens including intestinal parasites must be known.

In about 50% of AIDS patients with diarrhoea pathogenic agents can be identified and these include intestinal parasites (Dallabetta and Miotti, 1992). With the limited laboratory facilities in developing countries it is possible to identify intestinal parasites in up to 41% of AIDS patients with chronic diarrhoea (Henry *et al.*,1986; Cegielski *et al.*,1993). In a small study involving 16 patients with chronic diarrhoea, eleven showed improvement when treated specifically for their enteric infections, emphasizing the importance of specific laboratory diagnosis and therapy (Smith *et al.*,1988).

Until now the only available information on diarrhoea and HIV infection in adults in Tanzania is from the study done in Mwanza in which it was concluded that diarrhoea was not common among HIV infected patients and that isolation rates of intestinal parasites were lower among HIV positive patients (N=56) than HIV negative ones (N=291) (Temu *et al.*,1994). It is not possible to make firm conclusion about such findings because the sample of the HIV positive patients was too small compared to the HIV negative ones.

Available epidemiological data shows that the prevalence of intestinal parasites in persons above 15 years old in Kilimanjaro and Arusha is around 30% (MOH, 1992). In the two regions, the prevalence of HIV infection/AIDS has increased dramatically over the last 8 years such that in a period of one year (1991-1992), 62.5% (N=1135) of the persons who were referred for HIV testing in the two regional hospitals were found to be positive and about 42% of those positive had developed signs and symptoms of AIDS (Klepp *et al.*,1993). The Arusha and Kilimanjaro regions are located along the major high way to neighbouring countries and attract a number of tourists. Thus the two regions are thought to be at a high risk for HIV/AIDS transmission and have been found to have a significant number of HIV infected individuals (Mnyika *et al.*,1994).

Several factors may be related to the distribution of intestinal parasites in adult AIDS patients with chronic diarrhoea in North-eastern Tanzania. Since there is a lot of business related travel in Kilimanjaro and Arusha regions it is possible that a person who travels between urban and rural areas seeking for commercial goods both consumable and non consumable, which might have been contaminated through handling may be important in transmitting intestinal parasites between rural and urban areas. Since intestinal parasites are common in rural areas

where favourable geo- ecological conditions exist, persons who travel from such areas may pick up those infections and disseminate them to the urban areas. Patients who stay in rural areas may be more predisposed to intestinal parasites than those staying in urban areas because of poor hygiene and sanitation which is common in rural areas. The risk of exposure to intestinal parasites may differ with the age and sex of the study population because priori knowledge show that age and sex are potential confounders of many associations.

Since AIDS has a clinical spectrum related to the disease progression, it follows that patients with diarrhoea are in clinical stage III or IV which are the most severe stages of the disease (WHO[a],1992). Thus the parasite load, duration and severity of diarrhoea may differ according to the clinical stage of the HIV disease while the prevailing socio-economic factors may be responsible for predisposing the patients to diarrhoea causing intestinal parasite. Such important information is required in order to plan the care of AIDS patients.

The purpose of this study therefore was to address the following questions:- What proportion of adult AIDS patients develop chronic diarrhoea? What are the pathogenic intestinal parasites that can be found in adult AIDS

patients with chronic diarrhoea? Are there specific pathogenic intestinal parasites that are associated with chronic diarrhoea in adult AIDS patients? Are there age and sex differences in the distribution of pathogenic intestinal parasites among adult AIDS patients with chronic diarrhoea? Is there a difference in the distribution of pathogenic intestinal parasites between urban and rural adult AIDS patients with chronic diarrhoea? Is the duration of diarrhoea related to specific intestinal parasites? Is the frequency of bowel movements per day related to specific intestinal parasites? Is the presence of abdominal cramps in the patients with chronic diarrhoea related to specific intestinal parasites?

1.3 RATIONALE

A hospital based descriptive study on intestinal parasites and HIV infection among Tanzanian children in Dar es Salam by Cegielski *et al* (1993) showed the presence of pathogenic intestinal protozoa and helminths in paediatric AIDS patients with chronic diarrhoea. Parasites known to cause diarrhoea and those which do not cause diarrhoea were identified. The proportions of these parasites did not differ significantly between paediatric AIDS patients with chronic diarrhoea and those without chronic diarrhoea. It is not known whether the proportions differ in adults given that the clinical course of AIDS in adults differs from

that in children under five years of age (WHO[b],1992). It is not known which intestinal parasites are found in adult AIDS patients when they have chronic diarrhoea and whether specific intestinal parasites are associated with the ensuing chronic diarrhoea in Moshi and Arusha.

This study was therefore set out to investigate these factors in the in the North-eastern zone of Tanzania. The study was also intended to provide a comprehensive description of such parasites in terms of the type and their distribution in relation to the socio-demographic characteristics, clinical stage of AIDS and the association of specific intestinal parasites with chronic diarrhoea in patients with AIDS. This information will give some guidance for the development of guidelines for the management of chronic diarrhoea in adult AIDS patients, their families and the environment in Moshi and Arusha.

1.4 OBJECTIVES

1.4.1 BROAD OBJECTIVE

To determine the association between chronic diarrhoea and specific intestinal parasites in adult AIDS patients (age of 15 years or more) in North-eastern Tanzania.

1.4.2 SPECIFIC OBJECTIVES:

1.4.2.1 To determine the prevalence of chronic diarrhoea among adult AIDS patients in Moshi and Arusha.

1.4.2.2 To determine the prevalence of chronic diarrhoea among adult AIDS patients in relation to their socio-demographic and clinical characteristic in Moshi and Arusha.

1.4.2.3 To determine the prevalence of intestinal parasites in adult AIDS patients with and without chronic diarrhoea in Moshi and Arusha.

1.4.2.4 To determine the prevalence of chronic diarrhoea among adult AIDS patients harbouring parasites that are known to cause diarrhoea in Moshi and Arusha.

1.4.2.5 To determine the prevalence of chronic diarrhoea among adult AIDS patients harbouring parasites that are known to cause diarrhoea in relation to their socio-demographic and clinical characteristics in Moshi and Arusha.

1.4.2.6 To determine the associations of diarrhoea causing intestinal parasites with chronic diarrhoea among adult AIDS patients in Moshi and Arusha.

CHAPTER 2.0 METHODOLOGY

2.1 DESCRIPTION OF THE STUDY AREA

The study was carried out in hospitals and homes in Kilimanjaro and Arusha regions. The two regions are located in the North-eastern zone of Tanzania. Kilimanjaro and Arusha have favourable geo-ecological conditions for intestinal helminths and protozoa such that parasites such as Ascaris lumbricoides, Trichuris trichiura, hookworms, Giardia lamblia and Entamoeba histolytica are quite common (Hospital based laboratory data) and may interact with AIDS or worsen AIDS related conditions. This study was linked to an ongoing Tanzanian Norwegian AIDS Project (MUTAN) in the Kilimanjaro and Arusha regions. The project had established well functioning hospital and community based counselling programmes in the two regions for pre-HIV testing, post-test and follow-up counselling for HIV/AIDS subjects. The investigator took opportunity of the existence of such services to identify the HIV infected patients at hospitals and homes in Arusha and Moshi.

2.2 STUDY DESIGN AND STUDY POPULATION

A cross-sectional study was carried out on patients aged 15 years or more who had been identified as having AIDS. This study design was chosen on the basis of the objectives and hypothesis of the study. The study sought to investigate adult AIDS patients on diarrhoea and intestinal parasites

simultaneously thereby obtaining the estimated prevalence of diarrhoea and intestinal parasites in such patients.

In order to determine whether specific intestinal parasites were associated with chronic diarrhoea, comparison was made between subjects with chronic diarrhoea and those without chronic diarrhoea regarding exposure to intestinal parasites. Thus the measure of effect adopted in this study is that of a case-control design on prevalent cases, the odds ratio. This was an ideal study design in view of the prevailing time and financial constraints. For this analysis cases were defined as AIDS patients aged 15 years or more who at interview had diarrhoea lasting for two or more weeks while the controls were AIDS patients aged 15 years or more who at interview had no diarrhoea.

The study population consisted of known adult AIDS patients attending clinics or admitted in the wards or recuperating in their homes. It also consisted of patients newly diagnosed as having AIDS. Outpatients or inpatients clinically suspected by clinicians as having AIDS were referred to counsellors for pre-test counselling and subsequent HIV testing if informed verbal consent was obtained. In the Kilimanjaro and Arusha regions, blood for HIV testing was drawn only by counsellors for the sake of maintaining confidentiality. After drawing the blood samples the counsellors arranged to see their clients for

the second time when the test results are out. The blood specimens were assigned code numbers by the counsellors according to the Tanzanian Ministry of Health guide lines and then sent to the laboratory for HIV testing. In each hospital there was a technician who could perform HIV serological tests on blood specimens drawn from patients clinically suspected as having AIDS. The coded test results were entered into the laboratory register book and then delivered to the counsellors for subsequent post test and follow-up counselling.

Counsellors in the two regions kept a list of names and contact addresses of their clients. The clients would first see their counsellors if they had any problem and if admitted in the hospital, information was to be sent to the counsellors. Counsellors were making regular follow-up visits to known clients in order to give social support. Thus, through the counsellors it was possible to identify the study subjects at hospitals and home places.

Recruitment of patients at hospitals was done under the assistance of the counsellors. The counsellors introduced the known or newly diagnosed adult AIDS patients to the investigator either as outpatients or inpatients. For the home-based patients the investigator took opportunity of the routine follow-up visits by the counsellors to visit and interview them. Thus the investigator arranged to work

with the counsellors on hospital patients and do home visits on specific days of the week.

The clinical case definition for AIDS in adults in Moshi and Arusha was used and compared with the WHO clinical case definition for AIDS in adults. All patients had two or more conditions whose presence is a good indicator of AIDS in Moshi and Arusha; and such conditions are used in the WHO clinical staging of the HIV infection/AIDS (See appendix 3). The standard HIV tests done on the patients were recorded. The study involved prevalent as well as incident adult AIDS patients. To ensure that the study subjects were a representative sample of all adult AIDS patients, outpatients or inpatients with AIDS in the respective hospitals as well as known patients in their home places were included in the study. In Moshi study subjects were recruited at the Mawenzi, TPC, Kibosho, Machame, Kilema and Huruma hospitals while in Arusha subjects were recruited at the Mount Meru, Ithnaasheri, Saint Elizabeth and Selian hospitals.

2.3 SAMPLE SIZE ESTIMATION

About 40% of adult AIDS patients develop chronic diarrhoea (WHO, 1992). Pathogenic intestinal parasites can be identified in up to 41% of AIDS patients with chronic diarrhoea (Henry *et al.*,1986; Cegielski *et al.*,1993). Thus

the proportion of subjects with pathogenic intestinal parasites and chronic diarrhoea among adult AIDS patients is a product of the two proportions i.e 16.4%. This proportion was used to estimate the desired sample size. The sample size was obtained from the formular:

$$n = \frac{Z^2Pq}{d^2}$$

where n = the desired sample size

Z = the standard normal deviate set at 1.92 which corresponds to the 94.5 confidence interval.

P = proportion of subjects with pathogenic intestinal parasites and chronic diarrhoea among adult AIDS patients.

q = 1 - P

d = degree of accuracy desired and was set at 5.5% (0.055) to reduce the sample size to a manageable level given the limited resources of time and money.

Thus $$n = \frac{1.92^2 \times 0.164 \times 0.836}{0.055^2}$$

$$= 167$$

The minimum sample size estimated for the study was 167 subjects. Because the data were divided into two strata for a stratified analysis to control for potential confounding variables, the minimum sample size required was 167 x 2 which corresponds to 334 study subjects.

2.4 DATA COLLECTION PROCEDURES

2.4.1 DEVELOPMENT OF QUESTIONNAIRE, TRANSLATION AND PRETESTING

The questionnaire was developed in accordance with the objectives of the study (Lutz *et al.*,1992). The important aims of the study were listed down followed by a list of the information directly relevant and necessary to meet the aims of the study. A decision was made on the main sections within the questionnaire and information required. The main sections consisted of questions to establish personal identification and address, socio-demographic characteristics, diarrhoea status and its characteristics, defaecation habits, counselling services with type of HIV test(s) done and the clinical stage of the HIV disease. Questions which were thought appropriate for obtaining the required information from the respondents were listed down. These questions were checked against the aims of the study to ensure that they would obtain and record the information needed for the study. The questions were framed to be as clear and unambiguous as possible.

A decision was made to leave most of the questions close ended while a few questions were open ended. The closed questions were provided with a minimum number of options which were exhaustive and mutually exclusive. For the open ended questions ample space was left for recording the respondents' answers. The questionnaire was translated from English to Kiswahili by the investigator who speaks both English and Kiswahili fluently. The Swahili questionnaire was then pre-tested by the investigator on five adult AIDS patients admitted in a medical ward so as to make the wording and sequence of the questions logical and clear. The final versions of the questionnaire are appended (Appendices 1 & 2). The closed ended questions were pre-coded before the study while the open ended ones were left to be coded after data collection. A special form was designed for recording parasitological findings of stool examinations (Appendix 3).

2.4.2 INTERVIEW AND SPECIMEN COLLECTION

Permission to work in the respective hospitals was obtained from the Regional Medical Officers of the two regions. In each region, counsellors of AIDS patients and HIV infected individuals were identified. The Tanzanian Norwegian AIDS Project (MUTAN) had counselling units in the Moshi and Arusha offices. Each unit was headed by a counsellor who was responsible for supervising and monitoring counselling

activities in his or her region. For this study, the heads of the counselling units were asked to introduce the counsellors of the respective regional hospitals to the investigator.

In each regional hospital there were five counsellors while in each of the other hospitals there was only one. The counsellors were asked to assist the investigator to interview and collect stool specimens from the study subjects. They were therefore trained for two days by the investigator to pre-test and administer the questionnaire at their respective work places. Each study subject was individually informed about the purpose of the study and informed verbal consent for participation in the interview and provision of stool specimens for parasite detection obtained. A questionnaire was administered to every study subject by a counsellor. After the interview the investigator performed a clinical examination and determined the clinical stage of the disease in the patients according to the WHO criteria (Appendix 4). The clinical stage of the HIV disease of each study subject was recorded.

During the interview, information was obtained on socio-demographic characteristics, history of chronic diarrhoea (defined as passage of three or more; watery or loose

motions per day occurring at least for 14 days), frequency of diarrhoea per day and presence of abdominal cramps. Patients with chronic diarrhoea at interview were further asked for history of taking any drug during the illness and if so the name of the drug recorded; similarly if a laboratory stool test had been done, the findings were recorded. Patients who at interview had no diarrhoea were asked if they had diarrhoea in the preceeding three months and any drug taken was recorded. Patients known to be HIV positive whether outpatients, inpatients or home-based patients were interviewed and examined.

Every study subject interviewed was provided with a stool container for collecting a fresh morning stool specimen on the following day. Individuals who did not provide stool specimens in the first visit were revisited once and those who still failed to provide specimens were classified as non-responders.

2.4.3 PARASITOLOGICAL WORK

Fecal specimens from study subjects were submitted to the laboratory as soon as they were produced and preliminary laboratory examination done as soon as possible to avoid death of motile trophozoites of amoebae and giardia. Labelling of specimens was made such that each stool container with a specimen had an identification number

orresponding to that on the questionnaire.

Preliminary examination of the specimens entailed:-

1. Reporting the appearance of the specimen, noting the colour, consistency, presence of blood or mucus and worms.
2. Normal fresh wet microscopic mounts in fresh physiological saline and iodine were done on each specimen. The saline preparation was meant for identification of motile parasites such as amoebae, flagellates, larvae and ciliates as well as helminthic eggs. The iodine preparation was meant for identification of cysts.
3. Microscopic examination of the entire saline and iodine preparations was systematically carried out using the 10X objective and the 40X objective was used to identify cysts. Morphological characteristics were used for the identification of specific parasites (Cheesebrough, 1987). The preliminary examination was carried out in a laboratory within the hospital where interview was conducted. Specimens from domiciliary patients were preliminarily examined microscopically as soon as possible at a nearby health care facility with a microscope.

To avoid or reduce selection bias (unequal microscopic surveillance for parasites from diarrhoeic and non-diarrhoeic stool samples) macroscopic examination and preparation of the wet mount smears were done by a qualified technician. The labelled slides containing faecal smears were then sent to the investigator for microscopy. In this way the investigator could not know the source of the smears (whether from diarrhoeic or non-diarrhoeic stool samples) therefore minimizing chances differential searching for parasites in the stool samples.

After the preliminary examination, the remaining fecal specimens were preserved in 10% formol-saline and sent to the KCMC research laboratory where the formol-ether concentration technique was performed on every specimen. The formol-ether concentration technique has been recommended as the best overall technique for the concentration of parasites in faeces (Cheesbrough, 1987). The formalin fixes the parasites thus preserving their morphology. Each fecal sample was emulsified in 10% formol-saline water and thoroughly mixed. The suspension was strained through cotton gauze to remove large fecal particles and the fine suspension was transferred into a centrifuge tube and ether was added. Centrifuge tubes with fecal specimens were balanced in the centrifuge machine and

centrifuged at 3000 rpm for one minute. Cysts, ova and larvae (non-motile) were fixed and sedimented. Fecal debris was separated in a layer between the ether and the formol water. The fecal fat dissolved in the ether. Each centrifuge tube was rapidly inverted to discard the ether, faecal debris and formol water, leaving behind the sediment containing the concentrated parasites. Using a plastic bulb pipette, the sediment was thoroughly mixed and transferred to a glass slide containing a drop of physiological saline and iodine on two different sites, then covered by cover slips. The entire preparation was examined microscopically using the 10X objective with the condenser iris closed sufficiently to give good contrast. The 40X objective was used to detect cysts.

For the identification of Cryptosporidium spp and Isospora belli oocysts, a modified Ziehl-Neelsen (Kinyoun) stain was performed on the fecal concentrate (Cheesbrough, 1987; Current, 1989; Girdwood, 1989). In this method thin smears of the concentrated faecal samples were prepared, dried in air then fixed in methanol. The fixed smears were stained with cold carbol-fuchsin for about 10 minutes and washed off with tap water.The stained smears were decolourized using acid-alcohol and rinsed with tap water then counter stained with malachite green for about 30 seconds, washed off and dried. The dry smears were examined microscopically

for oocysts, using the 40X objective to identify the oocysts. Labels on glass slides and centrifuge tubes were counter checked to ensure that they corresponded with container labels and the identification numbers on the questionnaire. To avoid or reduce selection bias (differential searching for parasites in diarrhoeic and non-diarrhoeic stool samples), macroscopic examination and preparation of fecal specimens on slides were done by a qualified technician thereby blinding investigator about the identity of the specimens (that is whether they were diarrhoeic or not). Microscopic examination of the specimens was done independently by the investigator and a qualified technician and the results compared. Where the findings differed the specimens were re-examined so as to reach a common agreement. The final results were reported in this study. The results were communicated to the counsellors and relevant prescriptions given so that the patients could get treatment.

2.5 DATA ANALYSIS

Having returned from the field, the investigator arranged and assigned serial numbers to the questionnaires. The responses to the open ended questions were reviewed, categorized and coded. With the assistance of an experienced computer operator, the data were entered into the computer using the Statistical Package for Social

Scientists (SPSS/PC and Soft ware package). The data were cleaned and validated. Finally data analysis was carried out using the EpiInfo 5.0 computer programme. Frequency distributions and cross tabulations were drawn. Infection rates of intestinal parasites among adult AIDS patients in relation to diarrhoea status were calculated. Chi-square tests were conducted to analyse the prevalence of chronic diarrhoea in relation to the socio-demographic and clinical characteristics as well as the presence of intestinal parasites. Statistical significance was assessed using the p-value which was set at the 0.05 significance level. To assess the association between diarrhoea causing intestinal parasites and chronic diarrhoea, odds ratios and the 95% confidence limits for the odds ratios were computed. Stratified analysis was conducted in order to control for potential confounding variables and the Mantel-Haenszel weighted odds ratios with the 95% confidence limits were computed.

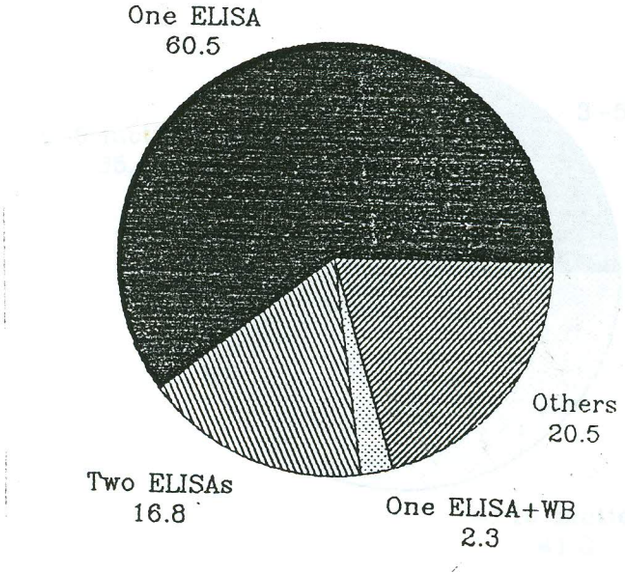
CHAPTER 3

3.0 RESULTS

3.1 GENERAL DESCRIPTION

A total of 362 AIDS patients aged 15 years or more were interviewed but complete data were available for 352 who could produced stool specimens for examination. Ten patients did not produce stool specimens and were therefore excluded from the analysis. All the 352 study subjects were positive for one or more HIV tests as shown in Figure 1. The majority of patients, 213 out of 352 (60.5%) were tested for HIV infection using a single ELISA test.

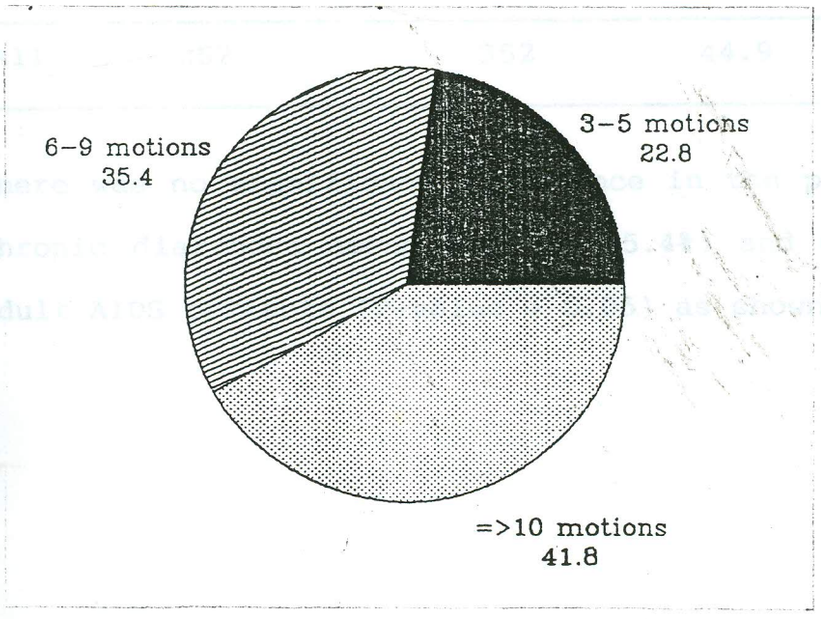
Figure 1: Distribution of HIV sero-positive adult AIDS patients by type of HIV test done.



3.2 SOCIO-DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

A total of 158 out of 352 adult AIDS patients (44.9%) had chronic diarrhoea at interview while 105 out of 352 (29.8%) had diarrhoea for some time during the preceeding three months. A high proportion of the patients with chronic diarrhoea, 134 out of 158 patients (84.8%) had received some form of treatment during their illness. Of the patients with chronic diarrhoea, 66 out of 158 patients (41.8%) had more than ten bowel motions per day as shown in Figure 2.

Figure 2: Distribution of adult AIDS patients with chronic diarrhoea in relation to the number of bowel motions per day.



The age group 25-34 constituted the highest proportion (47.7%) of the study population (Table 1). However, the prevalence of chronic diarrhoea in this age group was found to be siginificantly lower than in the other age groups. (P-value < 0.05).

Table 1: Age specific prevalence of chronic diarrhoea among adult AIDS patients.

Age Group	Total number examined	No with diarrhoea	Percent prevalence
15-24	62	36	58.1
25-34	168	55	32.7
35-44	87	50	57.5
45-64	35	17	48.6
All	352	352	44.9

There was no significant difference in the prevalence of chronic diarrhoea among female (45.4%) and male (44.3%) adult AIDS patients (P-value > 0.05) as shown in Table 2.

Table 2: Sex specific prevalence of chronic diarrhoea among adult AIDS patients

Sex	Total number examined	No with diarrhoea	Percent prevalence
Males	158	70	44.3
Females	194	88	45.4
All	352	158	44.9

Adult AIDS patients who were single constituted the highest proportion (37.8%) of categories of marital status among the patients (Table 3). However there was no significant difference in the prevalence of chronic diarrhoea among the various categories of marital status (P-value > 0.05).

Table 3: Prevalence of chronic diarrhoea among adult AIDS patients according to marital status.

Marital Status	Total number examined	No with diarrhoea	Percent prevalence
Single	133	60	45.1
Married monogamous	105	45	42.9
Married polygamous	26	10	38.5
Divorced/separated	46	18	39.1
Widow/widowed	21	10	47.1
Cohabiting	21	15	71.4
All	352	158	44.9

Regarding the residence of the patients (Table 4) it was found that more adult AIDS patients lived in urban areas (56.0%) than in rural areas (40.0%). The prevalence of chronic diarrhoea was not found to differ significantly between adult AIDS patients who lived in urban areas and those who lived in rural areas (P-value > 0.05).

Regarding the occupation of the patients, peasants and persons doing petty business constituted the majority (57.7%) (Table 6). However the prevalence of chronic diarrhoea was not found to differ significantly among the different occupational groups (P-value > 0.05).

Table 6: Prevalence of chronic diarrhoea among adult AIDS patients by occupational status .

Occupation	Total number examined	Number with diarrhoea	Percent prevalence
Peasant	109	48	44.0
Petty business	94	47	50.0
Civil servant	59	21	35.6
Employed in business	47	20	42.6
Own business	32	14	43.8
Unemployed	11	8	72.7
	352	158	44.9

When the defaecation habits of the adult AIDS patients were analysed the majority of the patients (64.5%) reported using water, than using toilet paper or other methods of ablution (Table 7). When the patients were stratified according to the methods of anal cleansing after

Table 4: Prevalence of chronic diarrhoea among adult AIDS patients according to place of residence.

Place of Residence	Total number examined	No with diarrhoea	Percent prevalence
Rural	155	64	41.3
Urban	197	94	47.7
Total	352	158	44.9

When the level of education was analysed the majority of the patients (78.4%) were found to have attained a level of primary school education (Table 5). The prevalence of chronic diarrhoea was highest among patients without formal education and lowest among patients with secondary education. This difference was statistically significant (P-value < 0.05).

Table 5: Prevalence of chronic diarrhoea among adult AIDS patients according to the level of education.

Level of education	Total number examined	No with diarrhoea	Percent prevalence
None	28	18	64.3
Primary	276	124	44.9
Secondary	48	16	33.3
All	352	158	44.9

defaecation, the prevalence of chronic diarrhoea was not found to differ significantly in relation to the methods of ablution used by the patients (P-value > 0.05).

Table 7: Prevalence of chronic diarrhoea among adult AIDS patients by method of anal cleansing after defaecation.

Method of anal cleansing	Total number examined	No with chronic diarrhoea	Percent prevalence
Water	227	96	42.3
Toilet paper	116	57	49.1
Others	9	5	55.6
All methods	352	158	44.9

Others = leaves, stones, grass, maize cobs and pieces of magazines.

The clinical stage of the patients was assessed and it was found that majority of the patients (62.8%) were in clinical stage III (Table 8). When the patients were stratified according to the clinical stages of AIDS, the prevalence of chronic diarrhoea was found to differ significantly between patients who were in clinical stage III to IV and those who were in clinical stage I to II of the HIV disease (P-value < 0.05).

Table 8: Prevalence of chronic diarrhoea among adult AIDS patients according to the clinical stage of AIDS.

Clinical stage	Total number examined	No with chronic diarrhoea	Percent prevalence
I	0	0	0.0
II	104	0	0.0
III	221	135	61.1
IV	27	23	85.2
All	352	158	44.9

3.3 PARASITOLOGICAL CHARACTERISTICS

A total of 123 out of 352 (34.9%) adult AIDS patients had pathogenic intestinal parasites. The parasites were isolated as single infection in 88 out of 123 (71.5%), while the rest were mixed infection of more than one type of parasites (Table 9).

Table 9: Distribution of adult AIDS patients with pathogenic intestinal parasites according to specific parasites

Specific parasite(s)	No with parasites	Percentage
One	88	71.5
Two	30	24.4
Three	5	4.1
All	123	100.0

The parasites included Cryptosporidum spp, Trichuris trichiura, Ascaris lumbricoides, Entamoeba histolytica, hookworms, Schistosoma mansoni, Isospora belli and Strongyloides stercoralis. Ascaris lumbricoides, hookworms and Trichuris trichiura were the ones most frequently isolated in association with other parasites. A total of 8 specific parasites were isolated from the 123 positive faecal samples. The parasites were isolated in varied frequencies among the diarrhoeic and non-diarrhoeic faecal samples as shown in Table 10.

Table 10: Frequency distribution of 8 specific intestinal parasites isolated from 123 positive stool samples according to the consistency of the stool samples.

Parasite type	No(%) of P+D+	No(%) of P+D-	Total No(%)
Cryptosporidium spp	40(42.5)	0(0.0)	40(24.7)
Isospora belli	12(12.8)	0(0.0)	12(7.4)
Trichuris trichiura	11(11.7)	15(22.1)	26(16)
Schistosoma mansoni	8(8.5)	7(10.30	15(9.3)
Ascaris lumbricoides	8(8.5)	17(25.0)	25(40.3)
Strongyloides stercoralis	7(7.4)	0(0.0)	7(4.3)
Entamoeba histolytica	6(6.4)	16(23.5)	22(13.6)
Hookworms	2(2.1)	13(19.1)	15(9.3)
Total	94(100.0)	68(100.0)	162(100)

P+D+ = Parasites in diarrhoeic stool

P+D- = Parasites in non-diarrhoeic stool

Non-pathogenic intestinal parasites such as Entamoeba coli, Entamoeba hartmani, Endolimax nana, Iodoamoeba buetschlii, Chilomastix mesnili, Trichomonas hominis and Blastocystis hominis are also found in adult AIDS with chronic diarrhoea but are of doubtful significance (Hunter *et al.*,1992; Cegielski *et al.*,1993). Since this study was concerned with

the pathogenic intestinal parasites among adult AIDS patients with chronic diarrhoea, the non-pathogenic intestinal parasites were not reported.

Of the 123 adult AIDS patients with intestinal parasites, 77 (62.6%) had chronic diarrhoea. The prevalence of chronic diarrhoea among patients harbouring specific parasites was computed (Table 11). The prevalence of chronic diarrhoea was significantly higher among patients with pathogenic intestinal parasites (P-value < 0.05). The prevalence of chronic diarrhoea was highest among patients harbouring Cryptosporidium spp, Isospora belli, Strongyloides stercoralis, schistosoma mansoni and Trichuris trichiura.

Table 11: Prevalence of chronic diarrhoea among adult AIDS patients in relation to the presence of pathogenic intestinal parasites.

Type of parasite(s)	Total number examined	No with chronic diarrhoea	Percent prevalence
PARASITES PRESENT			
Cryptosporidium spp	32	32	100.0
Isospora belli	7	7	100.0
Trichuris trichiura	10	7	70.0
Ascaris lumbricoides	15	6	40.0
Schistosoma mansoni	5	4	80.0
Strongyloides stercoralis	3	3	100.0
Entamoeba histolytica	13	3	23.1
Hookworms	3	1	33.3
Mixed infections	35	14	40.0
NO PARASITES	229	81	35.5
All	352	158	44.9

Parasites that are known to be associated with chronic diarrhoea were isolated from 103 adult AIDS patients. Of the 103 patients with parasites that are known to cause diarrhoea, 70 (68.0%) had chronic diarrhoea. The prevalence

of chronic diarrhoea among the 103 adult AIDS patients in relation to their socio-demographic and clinical characteristics was subsequently computed.

In relation to age (Table 12), the prevalence of diarrhoea among patients with parasites was not found to differ significantly among the different age groups (P-value >0.05).

Table 12: Prevalence of chronic diarrhoea among adult AIDS patients with parasites known to be associated with diarrhoea by age group.

Age Group	Total number with parasites	No with chronic diarrhoea	Percent prevalence
15-24	19	16	84.2
25-34	50	28	56.0
35-44	26	19	73.1
45-64	8	7	87.5
All	103	70	68.0

The prevalence of chronic diarrhoea among patients with parasites was not found to differ significantly in relation to the sex (Table 13) of the patients (P-value > 0.05).

Table 13: Prevalence of chronic diarrhoea among adult AIDS patients with parasites known to be associated with diarrhoea by sex.

Sex	Total number with parasites	No with chronic diarrhoea	Percent prevalence
Males	45	31	68.9
Females	58	39	67.2
All	103	70	68.0

In relation to residence (Table 14), the prevalence of chronic diarrhoea in association with intestinal parasites was not found to differ significantly between patients who lived in urban areas and those who lived in rural areas (P-value > 0.05).

Table 14: Prevalence of chronic diarrhoea among adult AIDS patients with parasites known to be associated with diarrhoea by place of residence.

Residence	Total number with parasites	No with chronic diarrhoea	Percent prevalence
Urban	62	46	74.2
Rural	41	24	58.5
Total	103	70	68.0

When the level of education was analysed (Table 15), the prevalence of chronic diarrhoea among patients with parasites was not found to differ significantly in relation to the level of education (P-value > 0.05).

Table 15: Prevalence of chronic diarrhoea among adult AIDS patients with parasites known to be associated with diarrhoea by level of education.

Level of education	Total number with parasites	No with chronic	Percent prevalence
None	14	8	57.1
Primary	80	56	70.0
Secondary	9	6	66.7
All levels	103	70	68.0

Regarding occupation (Table 16), the prevalence of chronic diarrhoea among patients with parasites was not found to differ significantly among the different occupation groups (P-value > 0.05).

Table 16: Prevalence of chronic diarrhoea among adult AIDS patients with parasites known to be associated with diarrhoea by occupational status.

Occupational status	Total number with parasites	No with chronic diarrhoea	Percent prevalence
Peasant	43	43	65.1
Petty business	19	11	57.9
Civil servant	17	10	58.8
Business employee	12	11	91.1
Own business	8	7	87.5
Unemployed	4	3	75.0
All occupations	103	70	68.0

When defaecation habits of the patients were analysed (Table 17) the prevalence of chronic diarrhoea among patients with parasites was not found to differ significantly in relation to the methods of ablution used.

Table 17: Prevalence of chronic diarrhoea among adult AIDS patients with parasites known to be associated with diarrhoea by methods of anal cleansing after defaecation.

Method of anal cleansing	Total number with parasites	No with chronic diarrhoea	Percent prevalence
Water	69	50	72.5
Toilet paper	27	17	63.0
Others	7	3	42.9
All methods	103	70	68.0

Others = leaves, stones, grass maize cobs and pieces of magazine.

Regarding the clinical stage of AIDS among the patients (Table 18), it was found that the prevalence of chronic diarrhoea among patients with parasites was significantly higher among patients in stage III-IV of AIDS than patients in stage I-II.

Table 18: Prevalence of chronic diarrhoea among adult AIDS patients with parasites known to be associated with diarrhoea according to the clinical stage of AIDS.

Clinical stage	Total number with parasites	No with chronic diarrhoea	Percent prevalence
I	0	0	0.0
II	18	0	0.0
III	71	58	81.7
IV	14	12	85.7
All stages	103	70	68.0

Assessment was made to see if drug(s) intake in the course of the diarrhoeal illness affected isolation rates of parasites that are known to be associated with diarrhoea among the adult AIDS patients with chronic diarrhoea (Table 19). The association between drug(s) intake and having intestinal parasites that are known to cause diarrhoea was probably of borderline significance because of the small number of adult AIDS patients with chronic diarrhoea studied (Odds ratio = 2.7, 95% CL : 1.0 , 8.9).

Table 19: Distribution of adult AIDS patients with chronic diarrhoea harbouring parasites that are known to be associated with diarrhoea in relation to drug intake.

Drug	Parasites present	Parasites absent	Total
Exposed	64	70	134
Not exposed	6	18	24
Total	70	88	158

Odds ratio =2.7, 95% confidence limits: (1.0 , 8.9)

The frequency of bowel movements per day was assessed among adult AIDS patients with chronic diarrhoea with or without parasites that are known to be associated with diarrhoea to see if the frequency of bowel movements and therefore severity of the diarrhoea was related to the presence of such parasites (Table 20). There was no significant difference between the number of bowel motions per day among patients with chronic diarrhoea who had parasites and those who did not have parasites (P-value > 0.05).

Table 20: Distribution of adult AIDS patients with chronic diarrhoea harbouring intestinal parasites that are known to be associated with diarrhoea in relation to the frequency of bowel movements per day.

Parasites	Frequency of bowel movements per day. No (%)			Total No
	3-5	6-9	>= 10	
Present	13(35.1)	26(46.4)	31(47.7)	70
Absent	24(64.9)	30(53.6)	34(52.3)	88
Total	37(100.0)	56(100.0)	65(100.0)	158

Abdominal cramps were reported by 126 while pathogenic intestinal parasites were found in 77 adult AIDS patients with chronic diarrhoea (Table 21). However, the association between presence of pathogenic intestinal parasites and abdominal cramps was not statistically significant (Odds ratio = 2.1, 95% CL = 0.9 , 5.1).

Table 21: Distribution of adult AIDS patients with chronic diarrhoea harbouring pathogenic intestinal parasites in relation to the presence of abdominal cramps .

Cramps	Parasites		Total
	Present	Absent	
Present	66	60	126
Absent	11	21	32
Total	77	81	158

Odds ratio = 2.1, 95% confidence limits : (0.9 , 5.1)

3.4 ASSOCIATIONS

The associations between intestinal parasites and chronic diarrhoea among adult AIDS patients were determined by comparing AIDS patients having chronic diarrhoea with regard to exposure to intestinal parasites. The associations were computed for all pathogenic intestinal parasites first, followed by all parasites known to cause diarrhoea and then for all non-diarrhoea causing parasites. Finally associations were computed for each specific intestinal parasite separately excluding all other parasites. The results are shown in Table 22.

Table 22: The association between all, specific and mixed intestinal parasites with chronic diarrhoea among adult AIDS patients

Parasite Name	D^+P^+	D^+P^-	D^-P^+	D^-P^-	OR with 95% CL
All parasites known to cause diarrhoea	70	88	33	161	3.9 (2.3, 6.5)
All pathogenic intestinal parasites	77	81	46	148	3.1 (1.9, 4.9)
All non diarrhoea causing parasites	10	91	28	176	0.7 (0.3, 1.6)
Specific parasites excluding other parasites					
Schistosoma mansoni	4	85	1	149	7.0 (0.7, 347.6)
Mixed diarrhoea causing parasites	11	92	4	152	4.5 (1.3, 20.0)
Trichuris trichiura	7	88	3	151	4.0 (0.9, 24.5)
Ascaris lumbricoides	6	87	9	157	1.2 (0.3, 3.9)
Hookworms	1	82	2	150	0.9 (0.0, 17.8)
Entamoeba histolytica	3	84	10	158	0.6 (0.1, 2.3)
Non-diarrhoea causing parasites mixed with diarrhoea causing parasites	3	84	15	163	0.4 (0.1, 1.4)

D+P+ = Number with chronic diarrhoea and intestinal parasites.

D+p- = Number with chronic diarrhoea but no intestinal parasites.

D-P+ = Number without chronic diarrhoea but having intestinal parasites.

D-P- = Number without chronic diarrhoea and without intestinal parasites.

OR = Odds ratio, CL = Confidence limits.

The measure of effect (odds ratio) was obtained by dividing the odds of exposure to intestinal parasites among adult AIDS patients with chronic diarrhoea to the odds of exposure among adult AIDS patients without chronic diarrhoea. The findings indicate that when all pathogenic intestinal parasites were considered together, the odds ratio was 3.1 and the 95% confidence limits were (1.9, 4.9). This means that pathogenic intestinal parasites were highly associated with chronic diarrhoea among AIDS patients. Considering all parasites known to cause diarrhoea together, the odds ratio was 3.9 and the 95% confidence limits were (2.3, 6.5). Likewise, the association between chronic diarrhoea and parasites known to cause diarrhoea among adult AIDS patients was statistically significant. However when all parasites that are not known to cause diarrhoea were considered together the odds ratio was

0.7 and the 95% confidence limits were (0.3, 1.6) indicating that parasites not known to cause diarrhoea are not associated with chronic diarrhoea among adult AIDS patients.

When each specific parasite was considered alone excluding all other parasites, the odds ratios varied as shown in Table 22. None of the associations were significant probably due to small numbers as indicated by the wide confidence intervals.

Cryptosporidium spp, Isospora belli and Strongyloides stercoralis are parasites known to cause diarrhoea and were only isolated from adult AIDS patients with chronic diarrhoea indicating that they were the most likely cause of the diarrhoea.

Subsequent analysis involved a combination of all parasites known to cause diarrhoea. Since the crude measure of effect did not take into consideration potential confounding variables, a stratified analysis was conducted in order to control for the potential confounders. Stratum specific odds ratios were then computed with their 95% confidence limits. A Mantel-Haenszel weighted odds ratio (which pools the evidence of association from the individual strata while taking into account confounding variables) and the 95%

confidence limits were obtained for each potential confounding variable for comparison with the crude odds ratio and stratum specific odds ratio. Table 23 shows how the odds ratios and the 95% confidence limits were obtained in the master table, 24.

Table 23: Relationship between diarrhoea causing intestinal parasites and chronic diarrhoea among adult AIDS patients by age.

FIRST STRATUM

Age 15 - 34	Diarrhoea present	No diarrhoea	Total
Parasite present	44	25	69
No parasites	47	114	89
Total	91	139	158

Odds ratio = 4.3, 95% confidence limits (2.3 , 8.1)

SECOND STRATUM

Age 35 - 64	Diarrhoea present	No diarrhoea	Total
Parasite present	26	8	35
No parasites	41	47	159
Total	67	55	194

Odds ratio = 3.7, 95% confidence limits (1.4 , 10.1).

Crude odds ratio for all strata = 3.9

Mantel-Haenszel weighted odds ratio = 4.0, 95% confidence limits (2.4, 7.0)

When the data were divided into two strata according to the various potential confounding variables the crude odd ratios, the stratum specific odds ratios and summary odds ratios did not differ markedly as summarised in Table 24. The summary of the adjusted odds ratios were still statistically significant indicating that intestinal parasites that are known to cause diarrhoea were highly associated with chronic diarrhoea among the patients.

Table 24: Stratified analysis showing relationship between diarrhoea causing intestinal parasites and chronic diarrhoea among adult AIDS patients by their socio-demographic factors excluding age.

Stratifying variable	D+P+	D+P-	D-P+	D-P-	ORss	95%CLss	OR_{M-H}	$95\%CL_{M-H}$
Sex								
Males	31	39	14	74	4.2	(1.9, 9.5)		
Females	39	49	19	87	3.6	(1.8, 7.4)	3.9	(2.3, 6.5)
Residence								
Urban	46	48	16	87	5.2	(2.5, 10.8)		
Rural	24	40	17	74	2.6	(1.2, 5.8)	3.8	(2.3, 6.5)
Education								
Low	64	78	30	132	3.6	(2.1, 6.3)		
High	6	10	3	29	5.8	(1.0, 40.9)	3.8	(2.3, 6.4)
Occupation								
Low	41	36	23	79	3.9	(2.0, 7.9)		
High	29	52	10	82	4.6	(1.9, 11.0)	4.2	(2.5, 7.1)
Ablution methods								
Water	50	46	19	112	6.4	(3.3, 12.7)		
Toilet	20	42	14	49	1.7	(0.7, 4.0)	3.9	(2.4, 6.7)

D+P+ = Number with chronic diarrhoea and intestinal parasites

D+P- = Number with chronic diarrhoea but no intestinal parasites

D-P+ = Number without chronic diarrhoea but having intestinal parasites

D-P- = Number without chronic diarrhoea and without intestinal parasites

OR_{ss} = Stratum specific odds ratio

CL = Confidence limits

$95\%CL_{se}$ = 95%CL for stratum specific odds ratio

OR_{M-H} = Mantel-Haenszel odds ratio

$95\%CL_{M-H}$ = 95%CL for the Mantel-Haenszel odds ratio

CHAPTER 4

4.0 DISCUSSION

4.1 CHRONIC DIARRHOEA IN AIDS PATIENTS

Chronic diarrhoea is one of the major complaints of AIDS patients, occurring in about 40% of cases. At least 60% of AIDS patients report diarrhoea episodes during their illness (WHO, 1992). The present study shows that 45% (158 out of 352) of the adult AIDS patients studied had chronic diarrhoea at interview while an additional 29.8% (105 out of 352) have had diarrhoea for some time in the preceeding three months. Thus about 75% of the patients studied reported diarrhoea episodes during the course of their illness. Similar observations were made in a major referral hospital (KCMC) in the North-eastern zone of Tanzania where 75% of patients with AIDS presented with chronic diarrhoea (Howlett *et al.*,1989). However in Mwanza, chronic diarrhoea was not found to be common among factory workers who were HIV positive (N=56) (Temu *et al.*,1994). Such findings were probably due to the small number of HIV positive individuals studied.

The findings of the present study are consistent with findings from other African countries. In Kinshasa, Zaire; persistent diarrhoea was found to be a common manifestation among patients with AIDS and this was thought to be due to

poor sanitation with frequent faecal contamination of food and water (Colebunders *et al.*,1987). Persistent diarrhoea was also common among patients with AIDS in Kampala, Uganda and Lusaka, Zambia (Sewankambo *et al.*,1987; Conlon *et al.*,1990). Chronic diarrhoea has been so common among patients with AIDS that its presence is highly predictive of AIDS.

The severity of diarrhoea depends on the frequency of bowel motions and the volume of liquid stools passed in each motion. In this study it was only possible to assess the frequency of bowel motions per day so as to estimate the severity. Thus 41.8% (66 out of 158) of the adult AIDS patients with chronic diarrhoea reported the highest number of bowel motions (more than ten) per day. Diarrhoea in AIDS patients can be very severe and life threatening because such patients may loose several litres of fluids in liquid stools in a day leading to severe dehydration and electrolytes imbalance with subsequent death.

Since the prevalence of HIV/AIDS is on the increase in many parts of Tanzania (Mhalu *et al.*,1987; Killewo *et al.*,1990; Shao *et al.*,1994; MOH, 1994), the prevalence of diarrhoea among AIDS patients will also be expected to increase. This will conceivably put an extra burden on the

existing primary health care programmes particularly those related to the control of diarrhoeal diseases.

Diarrhoeal diseases independent of HIV status, are generally related to poor socio-economic status of the affected population such as low a level of education, low income, inadequate and unsafe water supply as well as poor sanitation. Diarrhoeal diseases are more common in rural areas than in urban areas because of the poorer personal hygiene and sanitation in the rural areas. In the present study several socio-demographic factors pertaining to the adult AIDS patients including those mentioned above were assessed in relation to the presence of chronic diarrhoea.

Chronic diarrhoea was found to be associated with age and level of education of the patients. However chronic diarrhoea was not found to be associated with sex, marital status, residence, occupation or the defaecation habits of the adult AIDS patients.

Diarrhoeal diseases are known to be related to the defaecation habits of the patients, in particular using water for ablution. Using water for ablution increases the risk of

existing primary health care programmes particularly those related to the control of diarrhoeal diseases.

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Diarrhoeal diseases are known to be related to the defaecation habits of the patients, in particular using water for ablution. Using water for ablution increases the risk of exposure to faeco-oral diarrhoeal diseases when there is poor

and unsafe water supply.

During the survey, there was water rationing in the Moshi and Arusha municipalities thus limiting the quantity available for general use. Poor water supply leads to unhygienic methods of defaecation, eating and handling of consumable items. In road-side settlements and villages, the investigator observed people drawing water for domestic use from shallow wells, streams, canals and rivers. These open water sources are potentially contaminated and therefore unsafe. Using water from such sources increases the chances of acquiring faeco-oral diarrhoeal diseases. Despite of all these factors, having chronic diarrhoea was not found to be associated with the methods of ablution used. Perhaps, because of low level of education and in particular lack of formal education, patients in this category may not have been informed of the faeco-oral nature of diarrhoeal diseases. Although poverty could have rendered the patients not to have access to hygienic sanitary facilities, safe and adequate water supply, having chronic diarrhoea was not found to be associated with occupation.

Chronic diarrhoea was found to be strongly associated with the clinical stage of AIDS in the patients studied. This

might have been expected because persistent diarrhoea is highly predictive of the acquired immunodeficiency syndrome.

4.2 INTESTINAL PARASITES AND CHRONIC DIARRHOEA IN ADULT AIDS PATIENTS

The aetiology of chronic diarrhoea in adult AIDS patients has remained controversial and is still debatable. Chronic diarrhoea is often thought to result from multiple factors operating simultaneously or sequentially. Different aetiological agents including various bacterial and viral agents, protozoa and helminths may be responsible for the diarrhoea in adult AIDS patients. In about 15 to 50% of the patients no specific aetiologic agents are found, suggesting that other pathological processes primarily due to the HIV itself are responsible for the diarrhoea (Smith *et al.*,1988; Kotler *et al.*,1990).

The present study was concerned with known pathogenic intestinal parasitic agents (protozoa and helminths) that were found in the adult AIDS patients with chronic diarrhoea. In this study parasitic agents including protozoa and helminths that are known to be pathogenic were found in 77 out of 158 (48.7%) adult AIDS patients with chronic diarrhoea. Previous studies in Kinshasa, Zaire; Lusaka,

Zambia and Kampala, Uganda have shown that intestinal parasites are found among AIDS patients with chronic diarrhoea in varied proportions (Henry *et al.*,1986; Colebunders *et al.*,1987; Conlon *et al.*,1990). In the three studies above the pathogenic intestinal parasites that were commonly isolated included Cryptosporidium spp, Isospora belli, Strongyloides stercoralis, Trichuris trichiura, Ascaris lumbricoides, hookworms, Schistosoma mansoni and Entamoeba histolytica (cysts and trophozoites). Cryptosporidium spp and Isospora belli were only isolated from patients with chronic diarrhoea in the three studies.

The findings of the present study re-affirm the findings of the studies done in the three African countries. In the present study the same spectrum of parasites were isolated from the adult AIDS patients with chronic diarrhoea. This indicates that the profile of intestinal parasitic infections among African patients with enteropathic AIDS is almost similar.

Although Giardia lamblia is commonly isolated from patients with diarrhoea in parts of the study area, it was not found among the studied patients. Repeated examinations (including samples of mucus when present) could neither identify

trophozoites nor cysts. This was perhaps due to the fact that giardia trophozoites are not so common in diarrhoeic stools. Cysts, the form commonly found in faeces, are irregularly excreted and therefore could be missed. Similarly it was not possible to detect amoebic trophozoites in the diarrhoeic stool specimens. Repeated examinations of the diarrhoeic specimens (by warming the slides and taking samples of mucus with blood when present) could not detect trophozoites. Similar observations were made in Lusaka, Zambia (Hunter *et al.*,1992; Khumalo-Ngwenya *et al.*,1994).

Other studies on Tanzanian patients with AIDS and chronic diarrhoea have been performed in Dar es Salaam and Mwanza. In the Dar es Salaam study on paediatric patients with chronic diarrhoea and AIDS, a number of parasites including Cryptosporidium spp were isolated. However no Isospora belli was isolated from the paediatric patients with chronic diarrhoea and AIDS (Cegielski *et al.*,1993). In the Mwanza study, isolation of pathogenic intestinal parasites among patients with diarrhoea who were HIV positive (N = 56) was fairly low. Hookworms (24.7%) and Schistosoma mansoni (10.7%) were the most common agents isolated while Cryptosporidium spp was isolated in only one case (Temu *et al.*,1994). The study population sample of 56 was probably too small to make

firm conclusions about the findings.

Of the protozoan infections, Crypotosporidium spp and Isospora belli are the ones that are consistently found in association with chronic diarrhoea among AIDS patients. These parasites are found only in patients with chronic diarrhoea. In the present study, the two parasite types were isolated from 40 out of 158 (25.3%) and 12 out of 158 (7.6%) of the adult AIDS patients with chronic diarrhoea respectively. Investigations for intestinal parasitic infections in AIDS patients in Africa have largely focused on patients with chronic diarrhoea. Cryptosporidium spp and Isospora belli have been found consistently in such patients, at prevalences varying from 8 to 32% and 12 to 19%, respectively in studies of 42-46 patients with AIDS and chronic diarrhoea (Henry *et al.*,1986; Colebunders *et al.*,1987; Conlon *et al.*,1990). In nearly all of the studies, these protozoa were more common than the other parasitic protozoa and helminths.

The prevalence of the two parasite types in the present study was different from the observed prevalence in the above studies probably due to seasonal variation in parasite frequencies, the prevalence of the parasites being generally low in the study area or differences in the level of

immunosuppression among the studied patients (patients in late stages of AIDS are more likely to develop coccidiosis than patients in early stages).

These coccidian parasites are opportunistic intestinal parasitic infections that have recently been regarded as being of public health importance as a result of the occurrence of the HIV/AIDS pandemic (Soave and Johnson, 1988). They are opportunistic because in immunocompetent human hosts they cause acute self-limited diarrhoea while in immunocompromised hosts including those with the acquired immunodeficiency syndrome they cause severe and persistent diarrhoea which can be fatal.

Using advanced techniques such as endoscopy of the intestinal tract, histology and culture of materials obtained from the intestinal lining, it has consistently been shown that these coccidian parasites are the commonest pathogenic intestinal parasites present in African patients with AIDS and chronic diarrhoea. Such techniques have led to the findings that Cryptosporidium spp and Isospora belli had a combined frequency of up to 60% among African adult AIDS patients with chronic diarrhoea (Lucas *et al.*,1988; Colebunders *et al.*,1987).

To establish whether the coccidian protozoa were the cause of the chronic diarrhoea observed in AIDS patients, a study was done in the United States on patients with AIDS and chronic diarrhoea to determine the relationship between intestinal injury and the presence of parasitic infections among the patients (Kotler *et al.*,1990). In the study by Kotler *et al* it was concluded that a significant proportion of patients with enteropathic AIDS and intestinal injury had identifiable complicating infections which include Cryptosporidium spp.

The clinical features of the diarrhoeal syndrome in the patients with coccidiosis are characteristic. Typically there are from three to ten non-bloody bowel movements of variable volume, occurring at irregular intervals throughout the day. When severe the diarrhoea is associated with dehydration and electrolytes abnormalities, predominantly hypokalaemia. Thus, it is now widely accepted that coccidian infection in patients with AIDS is strongly associated with persistent diarrhoea which may be torrential with 1 to 15 litres of liquids being passed out and lost daily (Fang *et al.*,1992). The findings of the present study supports the commonly held view that the intracellular coccidian parasites, Cryptosporidium spp and Isospora belli are opportunistic infections significantly more associated with HIV infection

than the more common gut parasites (Khumalo-Ngwenya *et al.*,1994).

In the present study Entamoeba histolytica cysts were isolated individually from 1.9% of the patients with chronic diarrhoea. The low prevalence of E.histolytica observed in an otherwise endemic study area was probably due to eradication by previous anti-protozoal treatment, because there seemed to be a borderline significant difference in the infection prevalence among adult AIDS patients with chronic diarrhoea with different medication histories. The number of such patients may have been inadequate, or medication histories may have been unreliable. Nevertheless, because the zymodeme pattern of these amoebae was not determined, it cannot be concluded whether they were of pathological significance or not. The presence of cysts in the patients is of no pathological significance unless they were of a pathological strain (pathogenic zymodeme)(Manson-Bahr and Bell, 1987; Reeds *et al.*,1991).

Since cell mediated immunity is important in host defense against amoebiasis (Sepulveda *et al.*,1982), one might predict that colonized patients with AIDS would be at a high risk of invasive disease if the amoeba isolates are of pathogenic

zymodeme. However invasive amoebiasis was found to be rare in studies done in the US and the reasons for this was that the amoeba isolates were of non-pathogenic zymodeme (Reed *et al.*, 1990). The findings of the present study support the view that common parasites such as E.histolytica are not opportunistic in AIDS patients (Lucas, 1990; Guerrant and Bobak, 1991; Hunter *et al.*,1992; Khumalo-Ngwenya *et al.*,1994).

Host defense against giardiasis is related to humoral and secretory immune functions (Stevens *et al.*,1982). This may be expected to be abnormal in patients with AIDS, thus making them more susceptible to giardiasis. In this study, giardia was not found in any of the faecal samples perhaps because of the low sensitivity of single stool examinations which might not have allowed for the detection of giardia trophozoites or cysts.

The helminths isolated individually from the adult AIDS patients with chronic diarrhoea in this study included Trichuris trichiura (4.4%), Schistosoma mansoni (2.5%), Strongyloides stercoralis (1.9%) and hookworms (0.6%). With the exception of Strongyloides stercoralis, previous reports did not show any interactions between HIV disease and

helminthic diseases.

In Jamaica a strong association was shown to exist between HIV infection and parasitologically proved Strongyloides stercoralis (Robinson *et al.*,1990). The presence of HIV infection in areas which are endemic for Strongyloides stercoralis raises the fear of increased prevalence of strongyloidiasis and the hyperinfection syndrome characterized by massive strongyloidiasis in colonized patents with AIDS. This fatal condition is known to be precipitated by the administration of immunosuppressive drugs including steroids to patients with strongyloidiasis. This is of concern to health workers because patients with AIDS may have other conditions like bronchial asthma or severe eczema which may require administration of steroids.

Previous studies have shown that the prevalence of Strongyloides stercoralis in African patients with AIDS was the same as that in the general population (Lucas; 1988). Furthermore, cases of hyperinfection syndrome in patients with AIDS have been noted to be very rare (Conlon *et al.*,1990; Fleming, 1990) and it was suggested that probably the components of cell medicated immunity affected by the HIV disease are not important for containing infections with this

parasite. Thus the low prevalence of strongyloidiasis observed among the adult AIDS patients with chronic diarrhoea in the present study was perhaps due to similar reasons. The reason why AIDS does not predispose patients colonized with Strongyloides stercoralis to disseminated strongyloidiasis remains to be explained.

Trichuris trichiura and Schistosoma mansoni which are parasites known to be associated with chronic diarrhoea were also isolated from the patients studied. Even if they were not directly responsible for initiating the observed diarrhoea in patients studied, their presence might have caused exacerbations and therefore continuation of the diarrhoea.

Ascaris lumbricoides and hookworms which are parasites known not to be associated with chronic diarrhoea were also isolated from the adult AIDS patients studied. Their presence in AIDS patients with chronic diarrhoea might have contributed to the morbidity caused by the HIV itself or other aetiological agents thereby speeding the downward spiral in the health of the affected patients. The presence of such parasites is known to contribute to the immuno-compromisation caused by AIDS (Girdwood, 1989).

Newly recognized organisms, such as the microsporidium Enterocytozoon bieneusi, which has been reported from American AIDS patients may play an important role in Africa (Canning and Hollister, 1990; Orenstein *et al.*,1990). However the diagnostic methods used in this study did not allow for the detection of this parasite.

Since adult AIDS patients with chronic diarrhoea are in the most severe stages of the disease i.e. clinical stage III or IV, it implies that the immunological abnormalities in them are also very severe. These immunological abnormalities result from selective destruction of CD_4 cells (T_4 helper lymphocytes) by the HIV. This leads to functional defects virtually in every part of the immune system, including humoral and cellular immunity (WHO[c],1992). Generally, conditions which depress cell-mediated immunity, predominantly increase vulnerability to parasitic infections (Girdwood.,1989).

Since host immune responses are likely to have an effect on establishment of patent infection as well as an influence on parasite loads (Robinson *et al.*,1991), patients in such severe functional defects in their immune mechanisms will be expected to show abnormal host responses in terms of being

unable to contain the enteric parasitic infections. Thus susceptibility to enteric parasitic infections among AIDS patients and therefore progression to disease would greatly be increased because it is now widely accepted that host responses to parasitic infections are both cell mediated and humoral antibody mediated (Mauel 1982; Roitt 1988). Susceptibility to infections by enteric parasites would be expected to increase because exposure to the infective stages of intestinal parasites in the environment is independent of the host immunological status and therefore progression to disease once infected will entirely depend on the level of host immunity (Robinson *et al.*,1991).

Cell mediated responses are reactions to invading parasites by lymphocytes (CD_4 helper T cells) and natural killer (NK) cells. The humoral mediated responses are mainly mediated by I_gE antibody which is important in the defence of the body against parasites. The I_gE is carried as a cytophilic antibody on the surface of macrophage, platelets and eosinophils so as to effect antibody dependent cell cytotoxicity. Furthermore antigens from parasites may cross-link with I_gE antibody molecules to release pro-inflammatory molecules that help to amplify inflammatory reactions and killing responses to invading parasites (Wardle, 1992). All these important

immunological defenses which may be important for containing infective stages or mature forms of enteric parasitic infections may be expected to be critically deranged in Acquired Immunodeficiency Syndrome (AIDS).

The immunological defenses are impaired to a degree that protection against the development of clinical infection is reduced hence isolation rates of intestinal parasites known to cause diarrhoea would expectedly be higher in adult AIDS patients with chronic diarrhoea than in those without chronic diarrhoea. Thus in this study the prevalence of chronic diarrhoea among the adult AIDS patients harbouring parasites that are known to be associated with diarrhoea was highest in patients who were in clinical stage III to IV and was zero in patients who were in clinical stage I to II.

In the present study several socio-demographic factors and clinical characteristics pertaining to patients harbouring parasites that are known to be associated with chronic diarrhoea were analysed in relation to the presence of chronic diarrhoea. This analysis was meant to show whether the prevalence of parasite related chronic diarrhoea was related to the socio-demographic characteristics of the patients or their immunological status or both.

The prevalence of chronic diarrhoea among adult AIDS patients with parasites that are known to cause diarrhoea was not found to be associated with the socio-demographic characteristics of the patients. This might have been expected because infection with the parasites is through exposure to the infective stages in the environment which occurs independent of the host immunological status. Host immune-responses which might have been expected to be deranged by AIDS are likely to have been responsible for the establishment of patent infection as well as influencing the parasite loads hence development and persistence of the diarrhoea, and not the patients' socio-demographic characteristics (Robinson *et al.*,1991).

Chronic diarrhoea among adult AIDS patients with parasites was found to be strongly associated with the clinical stage of AIDS in the patients. This can be explained by the fact that patients with chronic diarrhoea are all in clinical stages III to IV of the HIV disease which are the most severe stages of AIDS. The level of immunity is expected to be relatively lower in stages III to IV of AIDS than stages I to II consequently making patients in these stages extremely vulnerable to parasite related diarrhoeal diseases. This might have been expected because once infected with parasites

that are known to cause diarrhoea, progression to diarrhoeal disease depends on the level of host immunity. The immune defense mechanisms in these stages of the HIV disease are critically deranged leading to inability to contain such infections.

In the present study it was observed that the association between drug(s) intake and having intestinal parasites that are known to be associated with chronic diarrhoea was of borderline significance probably due to the small number of adult AIDS with chronic diarrhoea studied. Drug(s) intake would have been protective (Odds ratio < 1). This suggests that the aetiology of chronic diarrhoea among the adult AIDS patients may not have been treatable causes only. Furthermore, majority of the drugs taken were not anti-parasitic and therefore would not have affected the parasite status of the patients. With the exception of the antiprotozoal metronidazole which is amoebicidal, the other drugs presumed to have been taken previously are not known to affect other intestinal protozoa or helminths. Alternatively the medication histories may have been unreliable in that, although prescribed, the drug(s) may not have been taken by the patients.

It was of interest to see if the presence of parasites that are known to be associated with diarrhoea increased the severity of diarrhoea and abdominal cramps among patients with chronic diarrhoea. However, there was no significant difference in the frequency of bowel motions per day and presence of abdominal cramps observed between adult AIDS patients having chronic diarrhoea with or without intestinal parasites. This suggests that probably other aetiological agents including the HIV agent itself may contribute to the severity of the diarrhoea, abdominal cramps and other abdominal symptoms.

It is important to note that in this study 77 out 158 (48.7%) of the adult AIDS patients with chronic diarrhoea had pathogenic intestinal parasites that could be diagnosed and treated in most developing countries. Since chronic diarrhoea often results from multiple factors operating simultaneously or sequentially, specific diagnosis of and treatment for the specific pathogens that may contribute to the initiation or continuation of the diarrhoea could reduce or stop the diarrhoea at least for a while. This is supported by the findings of Henry *et al* (1986) and Smith *et al* (1988). In the study by Henry *et al*, it was observed that simple microscopy of unconcentrated stool samples could detect enteric

parasites in 41% of adult AIDS patients with chronic diarrhoea in Zaire. In the study by Smith *et al*, patients treated specifically for their enteric pathogens showed improvement of diarrhoea hence emphasizing the importance of specific diagnosis and therapy of such infections.

4.3 ASSOCIATION OF INTESTINAL PARASITES WITH CHRONIC DIARRHOEA IN ADULT AIDS PATIENTS

To study disease association, cross-sectional surveys are most appropriate for many of the common parasitic diseases like intestinal protozoa and helminths whose prevalence are relatively high (Smith *et al*.,1988). However, cause and effect relationships are difficult to sort out because both occur at the same time. In studying disease associations through the conduct of a cross-sectional survey, the prevalence of both the disease and the factor are assessed among the same individuals in the population.

In the present study, the prevalence of chronic diarrhoea as well as that of parasites known to cause diarrhoea among the selected adult AIDS patients were determined. The design adopted was that of a case-control study and hence the measure of effect was the odds ratio. Infection status with intestinal parasites among cases and controls were determined

and odds ratios techniques used to compare cases and controls. Association was considered statistically significant when the 95% confidence limits on the odds ratio did not include 1.0. In this study when all parasites known to cause diarrhoea were considered together, the odds ratio was found to be 3.9 and the 95% confidence limits were (2.32 , 6.52). This means that parasites known to cause diarrhoea are strongly associated with chronic diarrhoea among adult AIDS patients.

Further appraisal concerned the assessment of the influence of bias, modifying and confounding factors on the association and causality (Abramson, 1990). The influence of bias was taken care of during study design and execution of the study in the following way: Recall bias for the duration of diarrhoea by interviewees was minimized by shortening the recall period and limiting it to events of the last two or more weeks as opposed to events of more than one month according to WHO. The limitation of this recall period of events of more than one month would have been that a number of patients would report diarrhoea of less than one month therefore be misclassified as not having chronic diarrhoea.

Selection bias by the investigator was reduced or minimized by blinding the investigator about the identity of the specimens to be examined. The investigator did not know whether the prepared smears were from diarrhoeic or non-diarrhoeic specimens hence reducing chances of differential search for parasites in the smears.

The influence of confounding variables was taken care of during data analysis by stratifying according to the potential confounding variables. The Mantel-Haenszel weighted odds ratios and the 95% confidence limits were obtained for each potential confounding variable. Even After adjusting for such variables the adjusted odds ratios remained highly statistically significant indicating a strong association of intestinal parasites with chronic diarrhoea among adult AIDS patients.

When the specific parasites were considered individually by excluding all other parasites, Schistosoma mansoni, Trichuris trichiura and Ascaris lumbricoides consistently showed odds ratios greater than 1.0. However these were not statistically significant because the 95% confidence limits included 1.0.

Although Schistosoma mansoni and Trichuris trichiura are parasites that are known to cause diarrhoea, the absence of statistical significance in this study was probably due to the small numbers of subjects from whom these parasites were isolated. Absence of statistical significance for hookworms and Ascaris lumbricoides may also be explained by the fact that these parasite are not known to cause diarrhoea.

Although Entamoeba histolytica is a parasite known to cause diarrhoea, this parasite had an odds ratio of 0.6 which was not statistically significant. This is consistent with the findings of another study in which it was concluded that invasive amoebiasis was rare and this was explained by the fact that the amoebae isolates were of non-pathogenic zymodeme (Reeds *et al.*,1991). However in this study the zymodeme patterns of the amoebae isolates were not determined.

When parasites that are not known to cause diarrhoea occurred in multiple infections with parasites known to cause diarrhoea, in the analysis an odds ratio of 0.4 was obtained and was not statistically significant indicating that the component of diarrhoea-causing parasites responsible for the association was diluted by the combination. For multiple

infections with parasites known to cause diarrhoea, in the analysis an odds ratio of 4.5 was obtained and this was statistically significant indicating that they were associated with the diarrhoea.

It important to note that Cryptosporidium spp, Isospora belli and Strongyloides stercoralis were parasites known to cause diarrhoea that were only isolated from patients with chronic diarrhoea. Cryptosporidium spp and Isospora belli have been described as being causally associated with chronic diarrhoea in patients with the acquired immunodeficiency syndrome (Current,1989; Fleming,1990).

Although Strongyloides stercoralis is rarely isolated from patients with AIDS (Lucas,1988), the fact that this parasite was only isolated from patients with chronic diarrhoea who are in clinical stage III or IV of the HIV disease suggests that in late stages of AIDS, patients become more susceptible to strongyloidiasis. Perhaps this is one of the reasons why in Jamaica, a strong association was shown to exist between HIV infection and parasitologically proved Strongyloides stercoralis (Robinson *et al.*,1990).

The causal mechanisms of intestinal parasites in chronic diarrhoea have been described by several authors. Cryptosporidium spp and Isospora belli are postulated to cause atrophy and hyperplasia in the intestinal villi and crypts respectively consequently leading to malabsorption and impaired digestion in the small bowel, while a toxin - mediated hypersecretion into the gut leads to secretory diarrhoea (Current, 1989; Kotler *et al.*,1990). Trichuris trichiura on the other hand is known to cause invasive diarrhoea through damage of the mucosa of the large intestine leading to bloody diarrhoea or mucopurulent diarrhoea. The ulcers formed by mucosal damage provide a suitable site for tissue invasion by Entamoeba histolytica which also causes invasive diarrhoea (Manson - Bar and Bell, 1987).

Schistosoma Mansoni is known to cause oedema, erythema, haemorrhages and ulcerations in the large intestine leading to bloody diarrhoea (Prata, 1982). Strongyloides stercoralis is known to cause superficial damage of the small intestinal mucosa often with excessive secretion of mucus. In severe infection, the mucosa is flattened and becomes atrophic and the pathological picture resembles that of tropical sprue (Muller, 1975).

4.4 LIMITATIONS OF THE STUDY

Causality:

The use of prevalent data restricts the investigator from making firm conclusions on causality because under such study circumstances it cannot be proven that chronic diarrhoea and intestinal parasites are causally related. This is because it is not possible in a cross-sectional study to show whether the patients acquired intestinal parasites first and then developed diarrhoea or they developed diarrhoea first and subsequently acquired the intestinal parasites.

Level of immunity:

A clinical staging was used to reflect the level of host immunity which would in turn be related to the degree of protection against the development of clinical enteric parasitic infections. The clinical staging needed to be supported by CD4+ lymphocytes counts or total lymphocytes counts. However CD4+ or total lymphocytes counts was not done. Cell count is more objective in ascertaining the level of host immunity than clinical evaluation which is somehow subjective. A combined clinical and laboratory classification provides a useful framework of reference for comparing results of investigations in different parts of the world.

Inability to do multiple stool examinations:

Multiple stool examinations were needed for the detection of

Cryptosporidium spp because the parasites are usually shed intermittently. Because of time constraints single stool examinations were done hence the isolation rate of this protozoa might have been slightly low. Giardia lamblia could have been missed because of similar reasons. Generally single stool examinations have low sensitivity.

CHAPTER 5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

This study has re-affirmed that chronic diarrhoea is a major complaint of adult AIDS patients in North-eastern Tanzania. Diarrhoea occurs in about 45% of the cases and at least 70% of the patients would report diarrhoeal episodes during their illness. Thus diarrhoea is one of the commonest causes of morbidity and possibly mortality among adult AIDS patients in Moshi and Arusha areas. Intestinal parasites that are known to cause diarrhoea can be detected in about 44% of adult AIDS patients with chronic diarrhoea.

Cryptosporidium spp. and Isospora belli are opportunistic infections most commonly found in AIDS patients with chronic diarrhoea in Moshi and Arusha. Cryptosporidium spp, Isospora belli and Strongyloides stercoralis are important parasites known to cause diarrhoea that are the most likely cause of

diarrhoea in patients with AIDS. Intestinal parasites known to cause diarrhoea considered together, are strongly associated with chronic diarrhoea among adult AIDS patients. While selected parasitic agents may be of importance in HIV-seropositive adult AIDS patients with chronic diarrhoea, the high proportion of adult AIDS patients who had chronic diarrhoea in the absence of identified parasitic infections indicates the existence of other infectious agents, or that alternative mechanisms other than infections are responsible for the diarrhoea. The findings of the present study and those published previously in Africa indicate that intestinal parasites, appear to explain only a fraction of the diarrhoea in HIV-seropositive adults.

The risk of developing parasite related diarrhoeal diseases after acquiring intestinal parasitic infections from the environment depend on the immunological status of the affected patients (in this case lowered by the HIV) and not other socio-economic and demographic factors. It is not possible to establish that parasites known to be associated with diarrhoea were causal to the reported diarrhoea among the patients because in a cross-sectional study the temporal relationships events cannot be established. Even if the parasites did not initiate the diarrhoea, their presence

would have caused exacerbation and continuation of the diarrhoea. This might have been expected because parasitic infections independent of HIV are known to cause lowered body resistance, hence in the presence of AIDS, the immunological defenses are impaired further to the extent that protection against the development of parasite related diarrhoea is further reduced.

5.2 RECOMMENDATIONS

Since diarrhoea is so common among AIDS patients who are in turn increasingly requiring care, there is a need to strengthen existing primary health care programmes, especially the diarrhoeal diseases control programme so as to cope with the HIV associated chronic diarrhoea in patients with HIV disease. There is therefore a need to strengthen counselling services and home based care with the aim of providing supportive treatments for the patients with chronic diarrhoea.

There is a need to strengthen laboratory facilities at all levels of health care delivery centres so as to be able to diagnose specific diarrhoea causing parasites so that specific therapies when available can be administered. Since treatment of opportunistic infections including those which

cause chronic diarrhoea has a considerable palliative effect which improves the quality of life, there is a need to do further studies to determine which drugs are suitable for such type of treatment in our set up. Thus clinical trials should be done on drugs such as co-trimoxazole, spiramycin and diclazuril for the purpose of finding effective therapy for isosporiasis and cryptosporidiosis which are life threatening infections in patients with AIDS. Together with clinical trials, carefully controlled studies are needed for the experimental and clinical evaluation of any cellular, humoral or immunomodulatory agents. There is a need for a concerted effort towards control of the endemic intestinal parasitic infections so as to break the vicious cycle of immunocompromisation and parasitic infections which is expected to be worsened by the occurrence of AIDS.

A comprehensive study on the association of microbial, viral and parasitic agents with chronic diarrhoea is suggested. Such a study might provide more information about the aetiology of diarrhoea in AIDS patients particularly by establishing cause and effect relationships. The best design to use in view of the nature of the problem is a case-control study so as to get an urgent answer to the care of adult AIDS patients with chronic diarrhoea. For such a study, newly

diagnosed HIV sero-positive adult patients with chronic diarrhoea will be recruited as cases while newly diagnosed HIV sero-positive adult patients without chronic diarrhoea will be recruited as controls. Various exposures to microbial, viral and parasitic agents will be investigated and compared between those with chronic diarrhoea and those without, to see if the diarrhoea is associated with any infectious agents. Infection status of HIV-seronegative adults with and without chronic diarrhoea will be determined so as to get the distribution of the infections in the general population and whether such infections are associated with diarrhoea or not. A prospective cohort study will not be appropriate because it will not be ethical to observe and follow up HIV individuals who are infected with the various agents to be investigated. Since in such a cohort study AIDS patients with and without microbial, viral or intestinal parasitic agents will be followed up for development of chronic diarrhoea, it will be unethical to follow up such patients without treatment.

REFERENCES

Abramson JH. Making sense of associations. In: Survey Methods in Community Medicine. Fourth edition. Churchill Livingstone, Edinburgh, London, Melbourne and New York; 1990: 251-63.

Canning EU and Hollister WS. Enterocytozoon bieneusi (Microspora): Prevalence and pathogenicity in AIDS patients. Trans R Soc Trop Med Hyg 1990; **84:** 181-6.

Cegielski JP, Msengi AE, Dukes CS, Mbise RL, Redding-Lallinger R, Minjas JN, Wilson ML, Shao J and Durack DT. Intestinal parasites and HIV infection in Tanzanian children with chronic diarrhoea. AIDS 1993; **7:** 213-21.

Cheesbrough M. Techniques used to identify parasites. In: Medical Laboratory Manual for Tropical Countries. Volume I, 2nd Ed. Harley Court, Jordan Hill, Oxford: ELBS/Tropical Health Technology/Butterworth-Heinemann, 1987: 178-220.

Colebunders R, Francis H, Mann JM, Bila KM, Izaley L, Kimputu L, Behets F, Van der Groen G, Quinn TC, Curran JW and Piot P. Persistent diarrhoea strongly associated with HIV infection in Kinshasa, Zaire. Am J Gastroenterol 1987; **82:** 859-64.

Conlon CP, Pinching AJ, Perera CU, Moody A, Luo, NP and Lucas SB . HIV related enteropathy in Zambia: a clinical microbiological and histological study. Amer J Trop Med Hyg 1990; **42**(1): 83-8.

Cook GC . Small intestinal coccidiosis: an emergent clinical problem. J infect 1988; **16**: 213-9

Current WL. Cryptosporidiosis: In: New Strategies in Parasitology. Churchill Livingstone; Edinburgh, London, Melbourne and New York, 1989: 257-71

Dallabetta GA and Miotti PG. Chronic diarrhoea in AIDS patients in the tropics: a review. Trop Doct 1992; **22**: 3-9

DeHovitz JA, Pape JW, Boncy M and Johnson WD. Clinical manifestations and therapy of Isospora belli infections in patients with the acquired immunodeficiency syndrome (AIDS). N Engl J Med 1986; **315**(2): 87-90

Fang G-D, Zu S-X, Fayer R, and Guerrant RL. Cryptosporidiosis: pathogenesis and immunology. Parasitology Today 1992; **8**: 24-7

Fleming AF. Opportunistic infections in AIDS in developed and developing countries.
Trans R Soc Trop Med Hyg 1990; **84** Suppl 1: 1-6

Gilks CF and Ojoo SA. A practical approach to the clinical problems of the HIV - infected adults in the tropics.
Trop Doct 1991; **21**: 90-7

Girdwood RWA. Protozoan infections in the immunocompromised patient - the parasites and their diagnosis.
J Med Microbiol 1989; **30**: 3-16

Glatt AE, Chirgwin K and Landesman SH. Current concepts: treatment of infections associated with human immunodeficiency syndrome. N Engl J Med 1988; **318**(22): 1439-48

Guerrant RL and Bobak DA. Review article: Medical Progress: Bacterial and protozoal gastroenteritis.
N Engl J Med 1991; **325**: 327

Henry MC, De Clercq D, Lokombe B, Kayembe K, Kapita B, Mamba K, Mbendi N and Mazebo P. Parasitological observations of chronic diarrhoea in suspected AIDS adult patients in Kinshasa (Zaire). Trans R Soc Trop Med Hyg 1986; **8**: 309-10

Howlett WP, Nkya WM, Mmuni KA and Missalek WR. Neurological disorders in AIDS and HIV disease in the Northern Zone of Tanzania. AIDS 1989; 3: 289-96

Hunter G, Bagshawe AF, Baboo KS, Luke R and Prociv P. Intestinal parasites in Zambian patients with AIDS. Trans R Soc Trop Med Hyg 1992; 86: 543-5

Khumalo-Ngwenya B, Luo NP, Sukuntu R, Sakala-Kazembe F, Baboo KS, Mathewson J and Zumla A. Gut parasites in HIV-seropositive Zambian adults with diarrhoea. East Afr Med J 1994; 71(6): 379-83

Killewo J, Nyamuryekunge K, Sandstrom A, Bredberg-Raden U, Wall S, Mhalu FS and Biberfeld G. Prevalence of HIV-1 infection in the Kagera region of Tanzania: A population based study. AIDS 1990; 4: 1081-5

Klepp K-I, Lie GT, Lugoe LW and Ngomuo ET. AIDS and its consequences for families, health care and education in Arusha and Kilimanjaro, Tanzania. Forum for Development Studies 1993; 1: 63-5

Kotler DP, Francisco A, Clayton F, Scholes JV and Orenstein JM. Small intestinal injury and parasitic diseases in AIDS. Ann Intern Med 1990; **113**(6): 444-9

Lucas SB. AIDS in Africa - clinicopathological aspects. Trans R Soc Trop Med Hyg 1988; **82**: 801-2

Lucas SB. Missing infections in AIDS. Trans R Soc Trop Med Hyg 1990; **84** Suppl 1: 34-8

Lutz W, Chalmers J, Hepburn W and Lockerbie J. Questionnaire Design. In: Health and Community Surveys. Volume II, First edition. Macmillan Press Limited, London and Basingstoke; 1992: 1-87

Manson - Bahr PEC and Bell DR. Diarrhoea caused by protozoa. In: Manson's Tropical Diseases. Nineteenth edition. ELBS/Baillie Tindal 24-28 Oval Road, London NW1 7DX; 1987: 301-13

Mauel J. Effector and Escape Mechanisms in Host-Parasite Relationships. Progress in Allergy 1982; **31**: 22-5

Mhalu FS, Bredberg-Raden U, Mbena E, Pallangyo K, Kingo J, Mbise RL, Nyamuryekunge K and Biberfeld G. Prevalence of HIV infection in healthy subjects and group of patients in Tanzania. AIDS 1987; 1: 217-21

Ministry of Health Tanzania. HIV/AIDS/STD Surveillance Report. In: National AIDS Control Programme. Ministry of Health, Dar es Salaam Report No.8; 1994: 3-37

Ministry of Health Tanzania. Introduction and statement of the problem. In: National Deworming Propgramme 1993-1995. Ministry of Health, Dar es Salaam; 1992: 1-2

Mnyika KS, KLepp KI, Kissila PE and Ole-King'ori N, editors. Population based KABP study and HIV screening in Arusha, Tanzania. Proceedings of The IX International Conference on AIDS/STD World Congress, Berlin; 1993

Mnyika KS, Klepp K-I, Nilsen S, Kissila PE and Ole-King'ori N. Prevalence of HIV-1 infection in urban, semi-urban and rural areas in Arusha region, Tanzania. AIDS 1994; **8:** 1477-81

Muller R. Strongyloides stercoralis. In: Worms and disease - A manual of medical helminthology. First edition. William Heinemann Medical Books Limited, London, 1975: 68-71

Orenstein JM, Chiang J, Steinberg W, Smith PD, Rotterdam H and Kotler DP. Intestinal microsporidiosis as a cause of diarrhoea in human immunodeficiency virus infected patients. Hum Pathol 1990; **21**: 475-81

Pinching AJ and Jeffries DJ. Therapy for HIV infection and AIDS. Brit Med Bull 1988; **41**(1): 115-29

Prata A. Infection with Schistosoma mansoni. In: Jordan and Webbe, editors. Schistosomiasis-epidemiology, treatment and control. Heinemann Medical books, London; 1982: 105-9

Reed SL, Wessel DW, and Davis CE. Entamoeba histolytica infections and AIDS. Am J Med 1991; **90**: 269-71

Roitt I. Immunity to parasitic infections. In: Essential Immunology. Seventh edition. Oxford Blackwell Scientific Publications, 1991: 164-7

Robinson RD, Lindo JF and Neva FA. Interaction between HTLV-1 and Strongyloides stercoralis infection resulting in lowered IgE levels in Jamaica. West Indian Med J 1990; 39 Suppl 1: 35-7

Robinson RD, Murphy EL, Wilks RJ, Neva FA, Terry SI, Hanchard B, Figueroa JP and Blattner WA. Gastro-intestinal parasitic infections in healthy Jamaican carriers of HTLV-1 J Trop Med Hyg 1991; **94**(6): 411-5

Sepulveda B and Martinez-Palomo A. Immunology of amoebiasis by Entamoeba histolytica. In: Cohen S, Warren KS, editors. Immunology of Parasitic Infections. Oxford, Blackwell; 1982: 170-91

Sewankambo N, Mugerwa RD, Goodgame R, Carswell JW, Moody A, Lloyd G and Lucas SB. Enteropathic AIDS in Uganda. An endoscopic, histological and microbiological study. AIDS 1987; 1(1): 9-13

Shao J, Brubar G, Levin A, Kibauri A, Masesa E, Siso Z, Konings E, Clayton Y, Kumby D and Allexander S. Population-based study of HIV-1 infection in 4086 subjects in Northwest Tanzania. J Acquir Immun Defic Syndr 1994; 7: 397-402

Smith PD, Lane HC, Gill VJ, Manischewitz JF, Gerald MS, Quinnan V, Fauci AS and Maswe H. Intestinal infections in patients with the acquired immunodeficiency syndrome (AIDS) Ann Intern Med 1988; **108**(3): 328-33

Soave R and Johnson WD. Cryptosporidium and Isospora belli infections. J Infect Dis 1988; **157**(2): 225-29

Stevens DP. Giardiasis : immunity ,immunopathology and immunodiagnosis. In: Cohen S, Warren KS, editors. Immunology of Parasitic Infections. Oxford, Blackwell; 1982: 192-203

Temu MM, Barongo L, Senkoro K, Klokke AH and Boerma JT. Diarrhoea and HIV infection among urban factory workers in Mwanza, Tanzania. Proceedings of the 12th Annual Joint Scientific Conference, National Institute for Medical Research, 1994. Preliminary report, Programme and Abstracts; A45: pp 32

Wardle EN. Schistosomiasis: an exercise in immunobiology. Medicine Digest 1992; 18(12): 3-4

Weller IVD. Gastrointestinal and hepatic manifestations of AIDS. In: Adler MW, editor. ABC of AIDS. Articles published in the British Medical Journal. Tavistock Square, London WC1H 9JR, 1st Edition(4th impression) 1988; 27-9

WHO. Interrelations of tropical diseases and HIV infection. In: Report of an informal consultation held at the Kenya Medical Research Institute, Nairobi, December 1-4, TDR/GPA/TD-HIV/87.3, 1987: 45-47

WHO. Epidemiological study designs appropriate for investigating interaction between HIV infection and tropical diseases. AIDS Tech Bull 1989; 2: 17-8

WHO[a]. Clinical spectrum of HIV infection. In: Piot P, Kapita BM, Ngugi EN, Mann JM, Colebundars R and Wabitsch R, editors. AIDS in Africa: a manual for physicians. World Health Organization, Geneva 1992: 21-42

WHO[b]. HIV infection in children. In: Piot P, Kapita BM, Ngugi EN, Mann JM, Colebunders R and Wabitsch R, editors. AIDS in Africa: a manual for physicians. World Health Organisation, Geneva 1992: 63-71

WHO[c]. Aetiology and pathogenesis of HIV. In: Piot P, Kapita BM, Ngugi EN, Mann JM, Colebunders R and Wabitsch R, editors. AIDS in Africa: a manual for physicians. WOrld Health Organisation, Geneva 1992: 4-12