PREVALENCE OF ENTEROPATHOGENS IN CHILDREN WITH PERSISTENT DIARRHOEA AND MALNUTRITION WITH OR WITHOUT HIV INFECTION BEFORE AND AFTER NUTRITIONAL INTERVENTION AT UTH

BY:

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DECLARATION

I hereby certify that this Study is my work and has not been previously submitted for a Degree at any University.

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LIST OF ABBREVIATIONS

AIDS : Acquired Immune Deficiency Syndrome

E.COLI : Escherichia Coli

ENT. COLI : Entamoeba Coli

HIV : Human Immune Deficiency Virus

NCHS : National Centre for Health Statistics

OPD : Outpatient Department

PD : Persistent Diarrhoea

UTH : University Teaching Hospital
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ABSTRACT

Persistent diarrhoea leads to malnutrition and the malnutrition that develops predisposes to further persistent diarrhoea. This becomes a persistent diarrhoea-malnutrition - persistent diarrhoea vicious cycle which is a complex of infection and host immune dysfunction that involve protein, calorie and micronutrient deficiency.

The most common intestinal infection on admission were Cryptosporidium parvum (26%), salmonella spp (18%) and Giardia Intestinalis (6%), the prevalence of which was not influenced by type of malnutrition or the HIV serostatus. The HIV serostatus was found to be 54% in these children under study aged 6 – 24 months.

At the end of the study, the same enteropathogens remained dominant with Cryptosporidium (13%), Salmonella spp (30.7%) and Giardia Intestinalis (7.8%) the commonest. HIV serostatus and absence of diarrhoea didn’t influence their prevalence. Pathogens appear to have been tolerated as commensals after nutritional rehabilitation.

78% of marasmic children were HIV seropositive as compared to 37% in those with kwashiorkor.

Of the 39 children who died, 35 were tested for presence of HIV antibodies, 25 of which were found to be HIV seropositive and 10 HIV seronegative.

Cryptosporidium, marasmic-kwashiorkor, marasmus and HIV-seropositivity were associated with high mortality.
CHAPTER ONE

1.0 INTRODUCTION

1. BACKGROUND INFORMATION

Diarrhoea is defined as the passage of three or more loose or watery stools in a 24 hour period, a loose stool defined as one that takes the shape of the receiving container \(^{(1, 2, 3, 5, 6)}\). Diarrhoea continues to be a public health problem in developing countries and is responsible for an estimated 1.3 thousand million episodes and 4 million deaths each year in children under the age of five years \(^{(1, 4, 6)}\). An average of 3.3 episodes each year is experienced by these children and in areas where episodes are frequent, young children may spend more than 15% of their days with diarrhoea each year \(^{(3, 6)}\). The main cause of death from diarrhoea is dehydration and electrolyte imbalance which result from the loss of fluids and electrolytes in stool. About 80% of deaths occur in the first two years of life \(^{(9, 6)}\). However, since the introduction of oral rehydration therapy, there has been a reduction in mortality due to acute diarrhoea \(^{(3, 5)}\).

Despite this improvement in the management of acute diarrhoeal disease with oral rehydration therapy, it is estimated that diarrhoeal diseases still account for up to 30% of all hospital admission in developing countries \(^{(4, 6)}\). In fact, a review of the status of diarrhoea indicates that although there has been a general reduction in mortality due to acute dehydration, there has been little decrease in the incidence of diarrhoeal disorders \(^{(2, 4)}\).

The reduction in mortality due to acute diarrhoea has led to emergence of another serious problem of persistent diarrhoea (PD) because oral rehydration improves the dehydration but does not remove the cause of the diarrhoea episode \(^{(3, 4, 4-6)}\).

Persistent diarrhoea is defined as a diarrhoeal episode of presumed infectious etiology that begin acutely and last for 14 days or more \(^{(3, 6)}\). This excludes chronic or recurrent diarrhoeal disorders such as Tropical Sprue, Gluten sensitivity or Blind Loop Syndrome.
Using the above definition, studies in several developing countries have shown that 3–30% of acute diarrhoeal episodes in children under five years of age become persistent (3, 5). In several large community based studies of diarrhoea, it has been shown that PD is responsible for between 36% and 54% of all diarrhoea related deaths (7–12). The major cause of death is malnutrition and its associated complications. In Bangladesh, Fanveau et al demonstrated that 49% of all deaths in children with PD were related to malnutrition (4, 9). When patients have diarrhoea not only do they lose appetite and eat less. Their ability to absorb nutrients is reduced and as a result they develop malnutrition. This is evident in developing countries where prolonged and recurrent episodes of diarrhoea frequently lead to stunting and growth failure in early childhood (3, 4, 13). The consequent malnutrition further predisposes to recurrent episodes of persistent diarrhoea, and a vicious cycle of diarrhoea-malnutrition-diarrhoea follows (4, 5). The negative effect of persistent diarrhoea on nutrition is compounded by ineffective weaning practices and food withdrawal by caretakers (4, 14). Due to its effect on the nutrition of the patient, PD has been labeled as a nutritional disorder and as such optimal nutritional therapy is generally considered a cornerstone of its management (15).

However, in some cases one cannot distinguish cause from effect as has been shown by some studies of malnourished children being more prone to developing persistent diarrhoea (14). In Bangladesh, malnourished children had a 68 fold increased risk of death from persistent diarrhoea than better nourished children in the same community (9). Hence management of patients with persistent diarrhoea can be very frustrating to family members as well as health professionals. Sometimes, patients are often treated with expensive intravenous fluids and ineffective drugs (6). As a result, this leads to an economic burden for developing countries, where resources are scarce (41).
2. STATEMENT OF THE PROBLEM

Diarrhoea and malnutrition are significant contributors to morbidity and mortality in Zambia. According to the last Zambia Demographic and Health Survey (ZDHS) report of 1996, children under (five) 5 years of age comprise 20% of the population (population of Zambia - 9.5 million). Under-five mortality is 197 deaths/1000 births. Forty-two (42%) percent of children under five years are stunted compared to an international reference (National Center for Health Statistics NCHS) indicating chronic malnutrition (ZDHS 1996).

In 1987 a National Baseline Survey report showed the diarrhoea incidence rate for under-five children of 27% for urban and 22% for rural areas\(^{(16)}\). The majority of those suffering were between 1 – 5 years (69%) while 31% were under age of one year. 95% of cases had diarrhoea that lasted more than 21 days\(^{(16)}\). The problem has become worse with the current pandemic of Acquired Immune Deficiency Syndrome (AIDS).

Diarrhoeal diseases appear to be common in young children with HIV/AIDS\(^{(18)}\). In a study of Zambian children with diarrhoea and malnutrition, 192 of 198 (97%) of the HIV positive children aged between 1 and 2 years had diarrhoeal illness at presentation, severe malnutrition was seen in 172 (87%)\(^{(18)}\).

Management of diarrhoea in Zambia, as in many other parts of the world, is based on the World Health organization (WHO) recommendation. The emphasis is on the use of oral rehydration solution for acute diarrhoea and continued feeding. Antibiotics are used when a specific bacteria is isolated for example *Shigella Spp.*

Few studies have been done to find the cause of PD in children with PD and malnutrition. Therefore, the objective of this study was to find the prevalence of enteropathogens in children with persistent diarrhoea and malnutrition with or without HIV infection before and after nutritional intervention at UTH in Lusaka.
JUSTIFICATION OF THE STUDY

It is important to know the common enteropathogens in children with persistent diarrhoea and malnutrition in order to formulate guidelines for the management of the patients.
CHAPTER TWO

LITERATURE REVIEW

With the increase in morbidity and mortality due to PD, a number of studies have been done to try and gain more insight into this problem and hence come up with management strategies. The studies done in other parts of the world have tried to look at the organisms that cause PD and the risk factors. The likely risk factors are age of the patient, nutritional status and immune status. Recognition of factors predisposing to prolongation of diarrhoea may allow interventions aimed at prevention of PD as well as institution of early and effective therapy (4).

AGE OF CHILD

The younger infant is at greatest risk of developing PD(3,4). In Northern India, the incidence of PD in infancy was five to six-fold higher than in the subsequent 2 years (1 - 9). The risk was greatest in 6 – 11 months age group when weaning often occurs (1 - 9). Studies from rural Bangladesh and former Zaire (Democratic Republic of Congo) on age distribution have reported similar data (20, 22).

However, in a study of 677 children under 3 years of age in a Peri Urban community near Lima, Peru, Lanat et al found that the proportion was highest in the children aged 6 – 35 months (22). Houselham et al in South Africa observed that the likelihood of PD was highest in the very young infant (<3 months of age), especially in association with malnutrition (23). This pattern reflects the combined effects of declining levels of maternally acquired antibodies, the lack of active immunity in the infant, the introduction of food that may be contaminated with faecal bacteria, and direct contact with human or animal faeces when the infant starts to crawl (6).
MALNUTRITION

Malnutrition is also a risk factor for PD (3). In Brazil, Schorling et al observed an increased frequency of diarrhoea with an almost two fold increase in diarrhoea burden in malnourished children (24, 25). Malnutrition defined either as weight for age <75% of the NCHS mean or length for age <85% (24, 25). In Bangladesh, Fauvea et al found that of the 1,934 diarrhoeal associated deaths, 49% were in malnourished children with PD (24). Data from a study done in Vietnam also showed that children with persistent diarrhoea were often severely malnourished (13).

IMMUNITY

Although the association between malnutrition and persistent diarrhoea is multifactorial, immunity predisposing to increased risk of infection has been a major focus of attention. The risk of developing PD can be predicted from the capacity of children to produce delayed type hypersensitivity reactions to standard skin test antigen (3). This suggests that intact cell mediated immunity contributes to the ability to terminate enteric infection (3, 5). In Peru and Bangladesh studies have shown that children with impaired skin test responses were likely to develop PD than were children with normal responses on follow up (3, 27, 28).

Additional evidence linking nutritional status and immunity to increased risk of persistent diarrhoea has emerged from studies of HIV infected infants. Thea et al (21) identified significant growth faltering 6 – 8 weeks prior to the onset of PD in HIV infants in a Cohort of 429 Zairean (Congoles) infants (53 with HIV infection, 139 uninfected offspring of infected mothers and 191 born to uninfected mothers). Similar data from Baltimore has also highlighted an increased risk of repeated episodes of diarrhoea and development of PD in HIV infection (29). However, impaired immune function, poverty, lack of breast feeding, inadequate or inappropriate foods, and increased pathogen transmission may play a role in this association (4). The association of persistent diarrhoea with alteration in nutritional status, immunity, food and water contamination strongly suggest an infective basis. A number of light microscopic and ultrastructural studies of the bowel mucosa in children with persistent diarrhoea are also indicative of an enteropathogenic pathogenesis (30, 31).
ENTEROPATHOGENS / INFECTIONS

Most of the enteropathogens that cause acute diarrhoea have also been associated with persistent diarrhoea. As such the enteropathogens identified in patients with persistent diarrhoea are divided into two broad group: (1) those that are isolated with about equal frequency from episodes of acute and persistent diarrhoea like *Shigella* Spp, non typhoid *Salmonella*, enterotoxigenic *Escherichia Coli (E.Coli)*, *Campylobacter Jejuni*, (2) those that are isolated with greater frequency from episodes of persistent diarrhoea. These are Enteroadherent E.Coli (EAEC), Enteropathogenic E.Coli (EPEC) and *Cryptosporidium*.

Several studies have shown that these organisms have an unusual capacity to cause persistent diarrhoea \(^{(32,33)}\). In the case of *cryptosporidium*, and possibly *Shigella Spp.*, this is especially true for children with pre-existing malnutrition \(^{(3,4)}\). In Thai orphanages, *cryptosporidium* infection was noted in children with significantly lower weight for height measurements than infected controls \(^{(32,33)}\). Similar data was obtained from Peru, Israel and Jamaica where *cryptosporidium* infected patients were more likely to be malnourished \(^{(32,33,34)}\). However, it is important to note that *cryptosporidium* can cause diarrhoea in children with good nutritional status. This is usually self limiting in this group of patients but in malnourished and those with AIDS the diarrhoea can be prolonged and fatal \(^{(45)}\). Some Researchers in the developing countries have shown that *cryptosporidium* infection was common in the first year of life \(^{(6)}\). In Guinea Bissau, West Africa, *cryptosporidium* was common in younger children (median age 12 months) \(^{(25)}\). In cases of persistent diarrhoea prevalence of *cryptosporidium* was 15% and mortality of 2.9% in the population under study \(^{(25)}\).

Another group of enteropathogens that are of considerable interest is enteroadherent E.Coli which has been found in children with persistent diarrhoea from a number of different locations. The enteroadherent E.Coli strains, some of which are also enteropathogenic E.Coli, are characterised by their capacity to adhere to the intestinal mucosal brush border. At least three patterns of adhesion are recognized, localized adhesion (LA), diffuse adhesion (DA), and aggregative adhesion (AA). LA E.Coli have been associated with PD during which they colonise the small bowel and produce characteristic mucosal changes (brush border effacement and pedestal formation) \(^{(3)}\).
However, in India (1–9), Bangladeshi (36), and Mexico (37) AA E.Coli were found to be more frequent in the acute phase of the PD. A study done in Democratic Republic of Congo (former Zaire) traditional EPEC strains were found in 11% (5/46) of HIV-positive Congolese infants with acute diarrhoea versus 12% (26/223) of HIV negative infants with diarrhoea in a neighbouring country (20). The same study showed that 41% of HIV positive infants with diarrhoea and 30% of HIV negative infants with diarrhoea had E.Coli that hybridized with a DNA probe that detects some aggregatively adherent strains.

In Zambia, a study done at UTH, adherent E.Coli were isolated from 69% (55/80) of HIV positive adults with diarrhoea compared with 25% (3/12) of HIV negative patients (47). The cell adherence patterns of the adherent E.Coli in the HIV positive were nearly equally distributed: Aggregative (20/55, 33%), Diffuse (20/55, 36%) and Localised (15/55, 26%) (47). None of the Adherent E.Coli from these patients hybridized with EPEC enteroadherence factor (EAF) probe, indicating that these were not traditional EPEC strains (47). Other research findings in other parts of the world have included description of the bundle forming pilus (BFP) gene which encodes the putative adhesion mediating initial adherence of EPEC to mucosal surface, and the E.Coli attaching and effacing (eae) gene, required for intimate attachment of EPEC to epithelial cells (48).

In other studies for example, a community based study in Chile, Santiago, in patients with persistent diarrhoea, the following organisms were isolated (41). See table A below.

**TABLE A:**

<table>
<thead>
<tr>
<th>BACTERIOLOGICAL STUDIES</th>
<th>NUMBER: 56/58</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPEC</td>
<td>12</td>
<td>21.5%</td>
</tr>
<tr>
<td><em>Shigella Spp.</em></td>
<td>4</td>
<td>7.2%</td>
</tr>
<tr>
<td><em>Campylobacter Jejuni</em></td>
<td>4</td>
<td>7.2%</td>
</tr>
<tr>
<td><em>Salmonella Enteridis</em></td>
<td>1</td>
<td>1.8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PARASTOLOGICAL STUDIES</th>
<th>NUMBER: 57/58</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Giardia Intestinalis</em></td>
<td>8</td>
<td>14%</td>
</tr>
<tr>
<td><em>E. Histolytica</em></td>
<td>6</td>
<td>10%</td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td>2</td>
<td>3.5%</td>
</tr>
</tbody>
</table>
The bacteria isolated with highest frequency was EPEC and the parasite is Giardia intestinalis. Campylobacter Jejuni and Shigella Spp. were equally frequent in terms of isolation among the bacteria. In Ethiopia, Gedilu et al isolated Campylobacter Jejuni among children aged 1 – 5 years presenting with persistent diarrhoea\(^{(44)}\).

In Zambia, few studies have been done to look at the aetiology of persistent diarrhoea in children with malnutrition. Some studies that have been done are on acute diarrhoea at UTH. From March 1974 to June 1975, Chintu et al looked at 800 children with diarrhoea, 500 outpatients and 300 inpatients\(^{(50)}\).

In that study, bacterial pathogens commonly isolated from stool were Enteropathogenic Escherichia Coli (18%), Shigella Spp. (14%) and Salmonella Spp. (3.5%).

In another study done at UTH between 1992 – 1994, Matsubayashi et al looked at the aetiology of acute diarrhoea in children below 5 years of age, finding the following, Table B.

**TABLE B:**

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>0 – 2 YEARS</th>
<th>2 – 5 YEARS</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EPEC</strong></td>
<td>80 (14.9%)</td>
<td>13 (12.7%)</td>
<td>92 (14.6%)</td>
</tr>
<tr>
<td><strong>Shigella Dysenteria</strong></td>
<td>32 (6.0%)</td>
<td>12 (11.8%)</td>
<td>44 (6.9%)</td>
</tr>
<tr>
<td><strong>Shigella Boydii</strong></td>
<td>8 (1.5%)</td>
<td>3 (29%)</td>
<td>11 (1.7%)</td>
</tr>
<tr>
<td><strong>Shigella Flexenli</strong></td>
<td>8 (1.5%)</td>
<td>2 (2.0%)</td>
<td>10 (1.6%)</td>
</tr>
<tr>
<td><strong>Salmonella Spp.</strong></td>
<td>8 (1.5%)</td>
<td>1 (1.0%)</td>
<td>9 (1.4%)</td>
</tr>
<tr>
<td><strong>Yersinia Enterocolitica</strong></td>
<td>8 (1.5%)</td>
<td>0 (0.0%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td><strong>Vibrio Cholerae</strong></td>
<td>10 (1.9%)</td>
<td>9 (8.8%)</td>
<td>19 (3.0%)</td>
</tr>
<tr>
<td><strong>TOTAL:</strong></td>
<td>537 ((100%))</td>
<td>102 (100%)</td>
<td>639 (100%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PARASITE</th>
<th>N-548 0-2 years</th>
<th>N-102 2-5 years</th>
<th>N-650 TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cryptosporidium</strong></td>
<td>22 (4.0%)</td>
<td>0 (0.0%)</td>
<td>22 (4.0%)</td>
</tr>
<tr>
<td><strong>Giardia Intestinal</strong></td>
<td>3 (0.5%)</td>
<td>2 (2%)</td>
<td>5 (0.5%)</td>
</tr>
<tr>
<td><strong>Ascaris Lumbricoids</strong></td>
<td>6 (1.1%)</td>
<td>7 (6.9%)</td>
<td>12 (0.2%)</td>
</tr>
<tr>
<td><strong>Hymenolepis Nama</strong></td>
<td>0 (0.0%)</td>
<td>1 (1.0%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td><strong>SUB TOTAL:</strong></td>
<td>31 (5.6%)</td>
<td>10 (9.9%)</td>
<td>40 (5.6%)</td>
</tr>
</tbody>
</table>
EPEC was the bacteria isolated the most while cryptosporidium was the most frequently isolated parasite, both organisms were common in the age group 0 - 2 years.

Chintu et al conducted another study which looked at intestinal parasites in HIV seropositive Zambian children with diarrhoea between the ages 15 months and 5 years (45). In that study, the most frequently isolated parasite was cryptosporidium Spp 14% from HIV positive children, and 6% from HIV negative children (45). They also reported no significant interactions between HIV infection and other gut parasitic infection (45).
CHAPTER THREE

OBJECTIVES

GENERAL

1. To define the prevalence of protozoa and bacteria in children with persistent diarrhoea and malnutrition with or without HIV infection in Lusaka.

SPECIFIC

1. To investigate any difference in prevalence of protozoa and bacteria before and after treatment of malnutrition and persistent diarrhoea.

2. To investigate any association between the type of malnutrition and the HIV status of the patient (HIV positive and HIV negative.)

3. To define enteropathogens according to the following subset criteria:
   (a) Kwashiorkor, Marasmus-Kwashiorkor and Underweight.
   (b) HIV positive / HIV negative.
CHAPTER FOUR

METHODOLOGY

STUDY DESIGN

This was a descriptive study of children aged 6 to 24 months admitted with a diagnosis of persistent diarrhoea and malnutrition between March 1998 and May 2000.

STUDY SITE

The study was conducted at the University Teaching Hospital, Department of Paediatrics on the Nutrition Ward (A07). The UTH is the biggest hospital in Lusaka and it is the major site of ambulatory care for a large proportion of the City. It is also the only teaching hospital and tertiary referral centre for the whole country.

UTH Paediatrics Wing consists of an Outpatient (OPD), Admission Ward, Paediatrics Intensive Care Unit (PICU), Paediatrics Review Clinic, Nutrition Ward and General Wards, with a bed capacity of 500. Patients who come to the hospital are screened by Clinical Officers and Medical Officers in the OPD. They are then admitted to the Admission Ward, a provisional diagnosis and preliminary investigations are done. However, patients with Protein Energy Malnutrition, after being seen, are admitted straight to the Nutrition Ward without passing through Admission Ward. In Admission Ward, patients are reviewed by a Consultant, then transferred to general wards. Patients requiring critical care are admitted to Paediatrics Intensive Care Unit.

The Nutrition Ward has a capacity of 61 cots and beds. It is a busy ward with more than 100% bed occupancy especially during the peak period (September to December). This ward is divided into 4 bays (1 – 4) for purposes of effective management and ease monitoring. All children admitted to this Nutrition Ward undergo a period of treatment and nutritional rehabilitation of 2 – 4 weeks.

On admission, patients are usually very ill and are put in Bay 1 where the initial phase of treatment starts.
Initial phase of treatment is the resuscitation period when patients are put on cow’s milk based diet, antibiotics and micronutrients. The cow’s milk is fortified with sugar and cooking oil to increase the caloric value to 100 calories per 100 ml. Most of the patients are fed by Nasogastric tubes.

When they start improving and gain their appetite, these patients are moved to Bay 2 where Soya porridge is introduced in addition to the cow’s milk. At this time they are usually able to take by mouth. This period begins the rehabilitation phase. This phase will go on until patients reach Bay 3 – 4 when preparations for discharge start. Before patients are discharged, Health Education is given to parents on the importance of nutrition, risk factors, personal hygiene and Family Planning. While on the ward patients are weighed once every week and all those that are not up to date with immunization are immunized. After discharge, patients are followed up in the Nutrition Clinic where they are reviewed regularly by the Nutritionist and Medical Officers for growth monitoring and continued Health Education.

STUDY POPULATION

All eligible children aged 6 – 24 months with persistent diarrhoea and malnutrition admitted to Nutrition Ward during the study period were recruited.

SELECTION OF STUDY SUBJECTS

INCLUSION CRITERIA

1. Age 6 to 24 months.
2. Diarrhoea for more than 14 days with malnutrition.

EXCLUSION CRITERIA

1. Outside age range.
2. Seriously ill – with failure to improve after resuscitation.
3. Patients who required isolation, for example measles and chicken pox patients.
5. Those who could not be followed up for various reasons, for example living outside Lusaka.

**DEFINITION OF PERSISTENT OF DIARRHOEA AND MALNUTRITION**

In this study, diarrhoea was defined as the passage of three or more liquid motions taking the shape of the receiving container within 24 hours period and persistent diarrhoea was defined as diarrhoeal episode of presumed infectious etiology that last more than 14 days. Malnutrition was defined according to the Wellcome classification.

**TABLE C: WELLCOME CLASSIFICATION**

<table>
<thead>
<tr>
<th>MALNUTRITION</th>
<th>BODY WEIGHT % OF STANDARD</th>
<th>OEDEMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>80 – 60</td>
<td>Absent</td>
</tr>
<tr>
<td>Marasmus</td>
<td>&lt; 60</td>
<td>Absent</td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>80 – 60</td>
<td>Present</td>
</tr>
<tr>
<td>Marasmic-Kwashiorkor</td>
<td>&lt; 60</td>
<td>Present</td>
</tr>
</tbody>
</table>

**SAMPLING**

All children who fulfilled the criteria were recruited to the study after parents / guardians gave consent. This was conducted in the Nutrition Ward during week days from Monday to Friday, 08.00 to 15.00 hours from March 1998 to May 2000. The first case was selected at random and there after patients were recruited consecutively if their parents gave consent.
SAMPLE SIZE

Sample size was calculated with the following assumption based on studies in the Department of Paediatrics. For population size of 30,000; for:

1. **Parasites**: Expected frequency was 12% and worst acceptable is 17%.
2. **Bacteria**: Expected frequency was 25.50%, worst acceptable is 32.00%.

The following estimates were calculated at 95% confidence level and a power of 80%.

<table>
<thead>
<tr>
<th>CONFIDENCE LEVEL</th>
<th>SAMPLE SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Parasites</td>
<td>95%</td>
</tr>
<tr>
<td>2. Bacteria</td>
<td>95%</td>
</tr>
</tbody>
</table>

Hence for this study, the sample size was calculated to be 172.

SUBJECT MANAGEMENT

1. **DATA COLLECTION**
   Information on recruitment was collected using a standard Questionnaire as shown below: (See Appendix)
   (a) History of current illness
   (b) Past medical history
   (c) Nutritional history

2. **FOLLOW UP OF STUDY SUBJECTS**
   Children were followed up for up to a maximum of 4 weeks

3. **COLLECTION OF SAMPLES**
   All eligible subjects had 3 samples of stool collected on 3 consecutive days on admission and just before discharge from hospital 4 weeks later for bacteria and parasite analysis at University Teaching Hospital Laboratory. The samples were collected in a plastic container with a tight fitting leak proof lid between 08.00 – 15.00 hours.
Anonymous and retrospective HIV testing was carried out on coded blood samples, but in case of clinical need, HIV testing was carried out after fully informed consent with pre- and post test counselling.

LABORATORY PROCEDURES

The stool samples reached the laboratory within 1 hour of collection where they were plated on McConkey Agar, Sorbital MacConkey and Xylose Lysine Desoxycholate Agar. After culture, standard biochemical tests were used to identify the various bacteria isolated. However, for E.Coli, five colonies were picked and grown overnight in a liquid medium and subsequently frozen at minus 20 celsius (-20 celsius) in 50% glycerol. These samples were packed in dry ice and then transported by air to London where they were tested for HEP-2 cell adherence assay.

Immediately after plating the stool, further examination of stool under the microscope was done to identify trophozoites, ova, cysts and parasites present. This was done by using 2 main methods:

1. As saline wet mounts using 2 types.
   (a) The first type involved placing a drop of normal saline on one end of a slide, then using a tooth pick, a small portion of stool was picked and emulsified on the drop of saline, then the preparation was covered with a cover slip.
   (b) The second type was the formal-saline concentration technique. This involved shaking approximately 2g of stool specimen in 7 ml of formal-saline with 3 ml of Ether before centrifugation at 3000 rpm for 5 minutes. The deposit was mixed well then the preparation was covered with a cover slip after putting on a slide.

2. Air-dried thin smears, which were stained by a modified Ziehl-Neelsen technique for Cryptosporidium and Isospora Oocyte. This involved fixing the preparation in Methanol for 3 minutes, staining with Carbol Fuchsin for 10 minutes, descolourizing with 3% acid alcohol for 1 minute, then washing in running tap water, counter staining with 0.25% Malachite green for 1 minute, washing off the stain with clean water and air drying.
3. HIV serological testing was performed using duplicate tests with the capillus (Trinity Biotect, Dublin) and ELISA (Bionor, Norway). As HIV antibody status may not reflect the true absence or presence of HIV infection in children below the age of 15 months, the analysis included stratification according to age below or above this age.

DATA ANALYSIS

Data collected was analysed using EPI INFO. Descriptive data is given using frequencies. Assessment of the relationship between infection and HIV status was performed using the Odds Ratio with stratification for age below/above 15 months. Hypothesis testing used either Chi-Square (with Yates correction) or Fischer's exact tests.

ETHICAL CONSIDERATION

The study was approved by the Research Ethics Committee of the University of Zambia, School of Medicine.
CHAPTER FIVE

RESULTS

A total of 200 children with persistent diarrhoea and malnutrition were enrolled in the study over the period March 1998 to May 2000. There were 94 boys (47%) and 106 girls (53%). The age range was 6 – 24 months (mean age 15 months). There was no difference in the demographic and clinical characteristics between boys and girls except for HIV serostatus which did differ. In 196/200 children, HIV test was performed out of which 106 were HIV positive (54%) while 90 children were HIV negative (46%). Four patients died before blood samples for HIV test were obtained. In the 106 HIV positive group, girls were more likely to be HIV positive (67 girls) than boys (39) (OR 2.7, 95% CI, 1.4 – 5.1; p-value 0.001); Table I. HIV antibodies were detected more frequently in children above 15 months: 79 out of 140 children (65%) over 15 months of age compare to those below 15 months of age 27 out of 56 (48%) – (p-value 0.38). However, this was not statistically significant.

<table>
<thead>
<tr>
<th></th>
<th>MALE: N=94</th>
<th>FEMALE: N=106</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months, mean, IQR)</td>
<td>17.5 (14 – 20)</td>
<td>18 (14 - 22)</td>
<td>0.26</td>
</tr>
<tr>
<td>HIV Serology</td>
<td>39 / 94</td>
<td>67/102</td>
<td>0.001</td>
</tr>
<tr>
<td>WELLCOM CLASSIFICATION:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>11</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Marasmus</td>
<td>17</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>46</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Marasmic-Kwashiorkor</td>
<td>20</td>
<td>23</td>
<td>0.44</td>
</tr>
<tr>
<td>Fever</td>
<td>23</td>
<td>35</td>
<td>0.22</td>
</tr>
</tbody>
</table>
MALNUTRITION

The commonest type of malnutrition in these children was Kwashiorkor 93/200 (47%) followed by Marasmus 45/200 (23%), then Marasmic-Kwashiorkor 43/200 (22%) and underweight 19/200 (10%). There was a significant association between the type of malnutrition and HIV serological status, the marasmus and marasmic-kwashiorkor patients being more likely to be HIV positive. Out of the 45 patients with marasmus, 35 were HIV positive and 10 were HIV negative while among the 41 with marasmic-kwashiorkor, 28 were HIV positive and 13 were HIV negative (p-value 0.00).

**TABLE 2: TYPE OF MALNUTRITION AND HIV SEROSTATUS**

<table>
<thead>
<tr>
<th>TYPE OF MALNUTRITION</th>
<th>HIV SEROLOGICAL STATUS</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POSITIVE</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>Marasmus</td>
<td>35 (78%)</td>
<td>10 (22%)</td>
</tr>
<tr>
<td>Marasmic-Kwashiorkor</td>
<td>28 (68%)</td>
<td>13 (32%)</td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>34 (37%)</td>
<td>57 (63%)</td>
</tr>
<tr>
<td>Underweight</td>
<td>9 (47%)</td>
<td>10 (53%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>106 (54%)</strong></td>
<td><strong>90 (46%)</strong></td>
</tr>
</tbody>
</table>

As HIV antibodies in children below 15 months may not show a true reflection of HIV infection, an analysis was done for children below 15 months and above 15 months in relation to the various types of malnutrition. The number of HIV seropositive patients was high in marasmus and marasmic-kwashiorkor whether above or below 15 months. Similary in kwashiorkor group the seronegative were more in both age groups (below 15 and over 15 months).
The only difference was in underweight patients where in those over 15 months, the HIV seropositive were more compared to those below 15 months (shown in table 3).

**TABLE 3: CLINICAL FEATURES OF MALNUTRITION IN RELATION TO HIV SEROLOGICAL STATUS AND AGE**

<table>
<thead>
<tr>
<th>WELLCOME CLASSIFICATION</th>
<th>OVER 15 MONTHS</th>
<th>UNDER 15 MONTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV Positive</td>
<td>HIV Negative</td>
</tr>
<tr>
<td>Underweight</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Marasmus</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Kwashiokor</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Marasmus-Kwashiorkor</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.0004</td>
<td></td>
</tr>
</tbody>
</table>

**PARASITE AND BACTERIA DETECTION RATE**

The commonest parasites isolated were *Cryptosporidium parvum* 51/200 (26%), *Giardia intestinalis* 11/200 (6%) and *Ascaris lumbricoides* 10/200 (5%). Yeast were also isolated in high numbers but this is probably a commensal 76/200 (38%). In the bacteria group, *Salmonella* spp were the commonest 36/200 (18%). During the rainy season of 1998 to 1999, there was an outbreak of cholera. As a result, 6/200 cases (3%) of cholera were recorded (table 4).
### TABLE 4: PARASITES AND BACTERIA DETECTION RATE

#### 4A: PARASITES

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>NO: 200 (FREQ)</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium</td>
<td>51</td>
<td>26%</td>
</tr>
<tr>
<td>Giardia</td>
<td>11</td>
<td>6%</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>10</td>
<td>5%</td>
</tr>
<tr>
<td>Yeast</td>
<td>76</td>
<td>38%</td>
</tr>
<tr>
<td>Entamoeba Coli</td>
<td>4</td>
<td>2%</td>
</tr>
<tr>
<td>Isospora belli</td>
<td>4</td>
<td>2%</td>
</tr>
<tr>
<td>Blastocyst Hominis</td>
<td>4</td>
<td>2%</td>
</tr>
<tr>
<td>Hook Worm</td>
<td>3</td>
<td>1.5%</td>
</tr>
<tr>
<td>Endolimax nana</td>
<td>2</td>
<td>1%</td>
</tr>
<tr>
<td>Microsporidia</td>
<td>1</td>
<td>0.5%</td>
</tr>
<tr>
<td>None</td>
<td>21</td>
<td>11%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### TABLE 4B: BACTERIA

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>NO: 200 (FREQ)</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella SPP</td>
<td>36</td>
<td>18%</td>
</tr>
<tr>
<td>Shigella Spp</td>
<td>4</td>
<td>2.0%</td>
</tr>
<tr>
<td>Cholera</td>
<td>6</td>
<td>3%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
HIV STATUS AND INTESTINAL INFECTION

There was no significant association between the type of organism isolated and the HIV serostatus of the patients. Even the commonest intestinal infection *cryptosporidium* and *salmonella* spp did not show any difference between the two groups HIV negative and HIV positive (Table 5).

### TABLE 5: HIV STATUS AND INTESTINAL INFECTION

<table>
<thead>
<tr>
<th>PARASITE</th>
<th>NO: 90 HIV NEGATIVE</th>
<th>NO: 106 HIV POSITIVE</th>
<th>ODDS RATIO</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptosporidium Parvum</em></td>
<td>17 (19%)</td>
<td>30 (28%)</td>
<td>1.72</td>
<td>0.82 – 3.60</td>
</tr>
<tr>
<td><em>Giardia Intestinalis</em></td>
<td>7 (8%)</td>
<td>4 (4%)</td>
<td>0.47</td>
<td>0.11 – 1.89</td>
</tr>
<tr>
<td><em>Yeast Cells</em></td>
<td>30 (33%)</td>
<td>45 (45%)</td>
<td>1.50</td>
<td>0.80 – 2.83</td>
</tr>
<tr>
<td><em>Ascaris Lumbricoides</em></td>
<td>5 (6%)</td>
<td>5 (4.7%)</td>
<td>0.85</td>
<td>0.08 – 3.57</td>
</tr>
<tr>
<td><em>Isospora belli</em></td>
<td>2 (2.2%)</td>
<td>2 (1.9%)</td>
<td>0.85</td>
<td>0.08 – 8.82</td>
</tr>
<tr>
<td><em>Entamoeba Coli</em></td>
<td>2 (2.2%)</td>
<td>2 (1.9%)</td>
<td>0.85</td>
<td>0.08 – 8.82</td>
</tr>
<tr>
<td><em>Hook Worm</em></td>
<td>2 (2.2%)</td>
<td>1 (0.9%)</td>
<td>0.43</td>
<td>0.01 – 6.17</td>
</tr>
<tr>
<td><em>Blastocyst Hominis</em></td>
<td>2 (2.2%)</td>
<td>2 (1.9%)</td>
<td>0.85</td>
<td>0.08 – 8.82</td>
</tr>
<tr>
<td><em>Endolimax Nana</em></td>
<td>0</td>
<td>2 (1.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Microsporidia</em></td>
<td>1 (1.1%)</td>
<td>0</td>
<td>0.00</td>
<td>0.00 – 15.22</td>
</tr>
</tbody>
</table>

**BACTERIA:**

| *Salmonella Spp.*            | 15 (17%)            | 21 (20%)              | 1.25       | 0.56 – 2.79 |
| *Shigella Spp.*              | 1 (1.1%)            | 3 (2.8%)              | 2.63       | 0.23 – 67.66 |
| *Vibrio Cholera*             | 5 (6%)              | 1 (0.9%)              | 0.10       | 0.01 – 1.50 |
TYPE OF MALNUTRITION AND INTESTINAL INFECTION

Children in this study had different types of malnutrition with each child having one type based on the wellcome classification. However, the isolation rate of the various enteropathogens didn’t differ among these types of malnutrition. None of these enteropathogens were significant in the statistical analysis.

**TABLE 6: TYPE OF MALNUTRITION AND INTESTINAL INFECTION**

<table>
<thead>
<tr>
<th>PARASITES</th>
<th>NO : 19 UNDERWEIGHT</th>
<th>NO : 45 MARASMUS</th>
<th>NO : 91 KWASH</th>
<th>NO : 41 MARASMIC-KWASH</th>
<th>TOTAL</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptosporidium</em></td>
<td>6</td>
<td>14</td>
<td>22</td>
<td>9</td>
<td>51</td>
<td>0.65</td>
</tr>
<tr>
<td><em>Giardia</em></td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>11</td>
<td>0.64</td>
</tr>
<tr>
<td><em>E. histolytica</em></td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>0.58</td>
</tr>
<tr>
<td><em>T. belli</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.69</td>
</tr>
<tr>
<td><em>T. Nana</em></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>0.93</td>
</tr>
<tr>
<td><em>D. oocyst</em></td>
<td>7</td>
<td>15</td>
<td>36</td>
<td>18</td>
<td>76</td>
<td>0.83</td>
</tr>
<tr>
<td><em>E. Turns</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0.85</td>
</tr>
<tr>
<td><em>Giardia Coli</em></td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>0.58</td>
</tr>
<tr>
<td><em>E. acridium</em></td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>10</td>
<td>0.83</td>
</tr>
<tr>
<td><em>Protozoa</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.77</td>
</tr>
<tr>
<td><strong>BACTERIA:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. terrinella</em></td>
<td>4</td>
<td>9</td>
<td>14</td>
<td>9</td>
<td>36</td>
<td>0.77</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>0.49</td>
</tr>
<tr>
<td><em>S. enterica</em></td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>0.51</td>
</tr>
</tbody>
</table>
INTESTINAL INFECTION AFTER FOUR WEEKS TREATMENT IN HOSPITAL

After 4 weeks of treatment in hospital, the dominant intestinal infections remained Salmonella spp. (30.7%), Cryptosporidium (13.7%) and Giardia Intestinalis (7.8%). Other organisms isolated were Isospora belli (1.3%), Entamoeba Coli (1.3%), Blastocyst and Microsporidium (0.7%). Yeast (39.2%) was also isolated in high numbers just like the results obtained on admission (Table 7).

TABLE 7: INTESTINAL INFECTION AFTER FOUR WEEKS TREATMENT IN HOSPITAL

<table>
<thead>
<tr>
<th>ORGANISM PARASITE</th>
<th>NO : 153</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium</td>
<td>20</td>
<td>13.1%</td>
</tr>
<tr>
<td>Giardia Intestinalis</td>
<td>12</td>
<td>7.8%</td>
</tr>
<tr>
<td>Isospora belli</td>
<td>2</td>
<td>1.3%</td>
</tr>
<tr>
<td>Ent. Coli</td>
<td>2</td>
<td>1.3%</td>
</tr>
<tr>
<td>Blastocyst</td>
<td>1</td>
<td>0.7%</td>
</tr>
<tr>
<td>Yeast Cells</td>
<td>60</td>
<td>39.2%</td>
</tr>
<tr>
<td>NONE</td>
<td>14</td>
<td>9.2%</td>
</tr>
<tr>
<td>Hook Worm</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ascaris</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Microsporidium</td>
<td>1</td>
<td>0.7%</td>
</tr>
<tr>
<td>Endolimax Nana</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BACTERIA:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Spp.</td>
<td>47</td>
<td>30.7%</td>
</tr>
<tr>
<td>Shigella Spp.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
INTESTINAL INFECTION AND HIV SEROSTATUS AFTER MALNUTRITION TREATMENT

The HIV serostatus did not seem to affect the prevalence of the intestinal infection even after patients had recovered from their illness. There was no significant difference in intestinal infection between the HIV seropositive and HIV seronegative patients. *Salmonella* Spp, *Cryptosporidium* and *Giardia Intestinalis* remained the commonest isolated enteropathogens in the two groups with their detection rate not influenced by HIV serostatus, (Table 8).

In this study, certain patients had some enteropathogens isolated from stool collected on recruitment and on discharge after treatment. This is shown in Table 9 where *Salmonella* Spp, *Cryptosporidium* and *Giardia Intestinalis*, the dominant intestinal infections are listed.

**TABLE 8: HIV STATUS AND INTESTINAL INFECTION**

<table>
<thead>
<tr>
<th>ORGANISM PARASITE</th>
<th>HIV –VE</th>
<th>HIV +VE</th>
<th>P-VALUE</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium</td>
<td>7</td>
<td>13</td>
<td>0.296</td>
<td>1.89</td>
<td>0.64 – 5.6</td>
</tr>
<tr>
<td>Isospora belli</td>
<td>1</td>
<td>1</td>
<td>0.505</td>
<td>0.94</td>
<td>0.02 – 35.58</td>
</tr>
<tr>
<td>Endolimax Nana</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blastocyst</td>
<td>1</td>
<td>0</td>
<td>0.483</td>
<td>0.00</td>
<td>0.00 – 16.63</td>
</tr>
<tr>
<td>Yeast Cells</td>
<td>25</td>
<td>35</td>
<td>0.243</td>
<td>1.56</td>
<td>0.76 – 3.19</td>
</tr>
<tr>
<td>Hook Worm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Entamoeba Coli</td>
<td>2</td>
<td>0</td>
<td>0.232</td>
<td>0.00</td>
<td>0.00 – 3.89</td>
</tr>
<tr>
<td>Ascaris</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Microsporidium</td>
<td>1</td>
<td>0</td>
<td>0.483</td>
<td>0.0</td>
<td>0.0 – 16.63</td>
</tr>
<tr>
<td>NONE</td>
<td>6</td>
<td>8</td>
<td>0.877</td>
<td>1.28</td>
<td>0.37 – 4.46</td>
</tr>
</tbody>
</table>

**BACTERIA:**

<table>
<thead>
<tr>
<th></th>
<th>HIV –VE</th>
<th>HIV +VE</th>
<th>P-VALUE</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Spp.</td>
<td>21</td>
<td>26</td>
<td>0.665</td>
<td>1.24</td>
<td>0.58 – 2.63</td>
</tr>
<tr>
<td>Shigella Spp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cholera</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
### TABLE 9: INTESTINAL INFECTION BEFORE AND AFTER TREATMENT

**(a) SALMONELLA SPP.**

<table>
<thead>
<tr>
<th>SALMONELLA</th>
<th>HIV SEROSTATUS</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Present Before and After Treatment</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Present Before Treatment</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Present only After Treatment</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>NONE – Before and After Treatment</td>
<td>45</td>
<td>46</td>
</tr>
</tbody>
</table>

**P VALUE = 0.941**

**(b) CRYPTOSPORIDIUM PARVUM**

<table>
<thead>
<tr>
<th>CRYPTOSPORIDIUM</th>
<th>HIV SEROSTATUS</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Present Before and After Treatment</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Present Before Treatment</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Present only After Treatment</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>NONE – Before and After Treatment</td>
<td>59</td>
<td>55</td>
</tr>
</tbody>
</table>

**P VALUE = 0.0164**

**(c) GIARDIA INTESTINALIS**

<table>
<thead>
<tr>
<th>GIARDIA INTESTINALIS</th>
<th>HIV SEROSTATUS</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-VE</td>
<td>+VE</td>
</tr>
<tr>
<td>Present Before and After Treatment</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Present Before Treatment</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Present only After Treatment</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>NONE – Before and After Treatment</td>
<td>61</td>
<td>72</td>
</tr>
</tbody>
</table>

**P VALUE = 0.175**

26
MORTALITY

Out of the 200 patients recruited, 39 died giving a mortality of 20%. Four patients died before HIV test was performed, however, 25 (24%) were HIV positive and 10 (11%) were HIV negative (OR 2.47; 95% CI, 1.04 – 6.005; p-value = 0.04).

The highest number of those who died had Marasmic-Kwashiorkor 15/39 (35%; 10 HIV positive, 3 HIV negative and 2 not tested), followed by Marasmus 12/39 (31%; 10 HIV positive and 2 HIV negative), Kwashiorkor 10/39 (26%; 2 HIV positive, 6 HIV negative and 2 not tested) and underweight 2/39 (5%; both HIV positive) p-value 0.05. Only cryptosporidium infection was associated with high mortality 18/39 (46%; 10 HIV positive and 4 HIV negative, 4 not tested P=value 0.05).

When compared to other children, those who had cryptosporidium and died were more 18/51 than those without 21/148 (OR 3.30 CI 1.60 – 6.85 P-Value 0.00).
CHAPTER SIX

DISCUSSION

This study has demonstrated the high prevalence of enteropathogens in children with persistent diarrhoea and malnutrition on recruitment and after four weeks of treatment in hospital. However, this high prevalence was not influenced by the type of malnutrition or HIV serostatus of these patients (overall HIV seroprevalence found to be 54%). This observation supports the view that common gut pathogens do not occur at a higher frequency in HIV seropositive individuals (45).

The commonest enteropathogens isolated both on admission and after recovery (at end of study) were Salmonella Spp, Giardia Intestinalis and Cryptosporidium. The isolation rate of salmonella spp was quite high compared to what has been reported in Mirzapur, Bangladesh and Lima, Peru in children with persistent diarrhoea and malnutrition (26, 59). It is also interesting to note that salmonella spp were isolated more when patients had fully recovered, i.e. no diarrhoea and no evidence of malnutrition. A large proportion of the patients appear to have acquired the infection on the ward. This may reflect poor personal hygiene on part of the parents who were looking after these children in hospital. Many of the child caregivers had to be monitored by Health Workers to ensure that they observed basic principles of hand washing after cleaning their child who had defecated. The other factor may be that the infection could have been missed on admission and because of the use of antibiotics in the management of children with malnutrition, this encouraged higher carrier rate and hence prolonged excretion of the salmonella spp in patients who remained asymptomatic after recovery (71).

Giardia Intestinalis, the prevalence didn’t change. There was slight increase of 1.8% from 6% on admission to 7.8% on discharge. The results show that more patients had the infection isolated at the end of the study. This occurred despite the use of Metronidazole 200 mg three times a day for 7 days in those who had the infection isolated on admission. The possible explanation could be that detection of Giardia in stool is not a sensitive method. It could also have been acquired on the ward from other patients since most of them were sharing bed-cots due to limited bed capacity. Hence oral-feecal route becomes an important mode of transmission since children could easily put their hands in the mouth after touching the other child’s faeces in the same bed-
cot. This mode of transmission can be common to all enteropathogens. *Giardia Intestinalis* is also found in patients who are asymptomatic.\(^{72}\)

Even the results of cryptosporidium detection were quite high both on admission and at the end of the study although the prevalence at the end of the study was half that found on admission (26% on admission and 13% on discharge). These results are consistent with results of other studies which have demonstrated an association between malnutrition and cryptosporidium infection in children hospitalized with diarrhoea.\(^{32,33}\).

In Israel and Jamaica, studies carried out demonstrated that children with *cryptosporidium* infection were likely to be malnourished than were children without this infection.\(^{32,33}\)

Furthermore, in Guinea-Bissau, growth failure was observed in children infected with *cryptosporidium*, which may be highly prevalent in severely malnourished children.\(^{35}\) Both malnourished children and those with Paediatric AIDS have an abnormality in cellular immune function. In malnutrition unrelated to AIDS all aspects of immunity are diminished (lymph glands, tonsils and thymus are atrophied)\(^{68}\). Although Cell-mediated immunity is severely affected, Specific IgA antibodies in secretion are reduced, complement components are low and the ability of phagocytes to kill ingested bacteria efficiently is reduced.\(^{68}\)

This immune dysfunction may be the explanation for high prevalence rates of *cryptosporidium* infection similar to both HIV infected children and those not infected. This could also be true for other enteropathogens. In fact, in our patients the *cryptosporidium* isolation rate dropped by half at the end of the study. This may indicate that the immune system improved when patients recovered hence their ability to clear the infection. This can be supported by the observation made in this study were majority of the patients who appear to have had *cryptosporidium* on admission and at the end of the study were HIV seropositive.

*Cryptosporidium* infection has also been observed to be an important cause of severe, prolonged and life threatening diarrhoea, especially in AIDS patients.\(^{60,61}\). In our patients, it was associated with overwhelming watery diarrhoea with severe dehydration, difficult to treat and was associated with high mortality. This has also been demonstrated by Molbak et al in Guinea-Bissau where *Cryptosporidium* was associated with increased mortality.\(^{35}\). Mortality was also high in children with marasmus and marasmic-kwashiokor, especially those who were HIV
seropositive. One of the explanations is that probably these patients were suffering from terminal Paediatric AIDS and hence high mortality in these two types of malnutrition.

It has been known for a long time that *Isospora Belli* is an important cause of diarrhoea in HIV infected adults and not HIV infected children\(^{(69)}\). We were able to demonstrate this fact in our study. Chintu et al has also made a similar observation\(^{(45)}\).

It also is important to note that *Ascaris* and other helminthes were not detected at the end of the study. This may be an indication that the department’s policy of giving all children with malnutrition Mebendazole is good and is giving the required results.

The other interesting issue this study has demonstrated is that children with marasmus are more likely to be HIV seropositive than those with kwashiorkor. A similar observation has been made in HIV infected adults where they develop profound weight loss especially those who present with diarrhoea\(^{(55)}\). This profound weight loss is sometimes referred to as slim disease\(^{(55)}\). The slim disease in adults may be an equivalent of marasmus in children, hence a high HIV seroprevalence in this type of malnutrition. However, although HIV infection appears to have contributed significantly to the pathogenesis of marasmus, other causatives factors of malnutrition cannot be ignored as shown by the high rate of HIV seronegative cases in those with kwashiorkor. Factors such as lack of food due to persistent drought and high poverty levels which in Zambia is estimated at 80% in recent years remain important contributors to the problem of malnutrition\(^{(67)}\).
CHAPTER SEVEN

CONCLUSION

Children with persistent diarrhoea and malnutrition frequently are HIV infected with the seroprevalence of 54% at UTH today.

The dominant intestinal infections were Cryptosporidium parvum, nontyphoid salmonella spp and Giardia intestinalis before and after treatment for malnutrition. The prevalence of these enteropathogens was not influenced by HIV serostatus or type of malnutrition of the patients. During the study, many children cleared the intestinal infection, but some acquired it and a substantial number continued to be infected despite no diarrhoea. Pathogenic organisms were tolerated as commensals after nutritional rehabilitation. Clinical presentation of malnutrition was influenced by HIV infection with marasmic patients being more likely to be HIV positive and kwashiorkor patients HIV seronegative. Mortality was high in the children who had Cryptosporidium infection and in those HIV seropositive who were marasmic and marasmic-kwashiokor.
CHAPTER EIGHT

RECOMMENDATIONS

1. Drug trials should be conducted to find a cure for cryptosporidium because of its association with high mortality.

2. More studies should be conducted to determine:

(a) Prevalence of various strains of E.Coli which are known to be diarrhogenic in children with diarrhoea and malnutrition.

(b) The role of viruses in the pathogenesis of persistent diarrhoea and malnutrition in Zambia.

3. Health providers need to do more counselling related to HIV infection since a large proportion of the patients in the study population were HIV seropositive. This will help parents have a better understanding of their children’s illnesses and allow those who can afford to buy antiretroviral drugs.

4. Cross infection is common in hospital wards and ways of preventing this should be found.
CHAPTER NINE

LIMITATIONS

1. The isolation of intestinal infection due to bacteria was limited to *salmonella Spp*, *shigella Spp* and *vibro cholera*.

2. E.Coli specimens which were sent to London for serotyping failed to yield any results due to probably poor storage facilities in the laboratory and poor preparation for transportation.

3. It was not possible to determine the prevalence of enteroviruses because there was no technologist to analyse the stool samples in the virology laboratory during the study period.
REFERENCES


17. Diarrhoea Disease Household Case Management Survey, Zambia (October to December 1992), Ministry of Health; 1 – 36.


59. Etiologic Agents in Acute VS Persistent Diarrhoea in Children under three years of age in Peri-Urban Lima, Peru...


APPENDIX I

QUESTIONNAIRE

I. PATIENT INFORMATION:

1. Age: .......... months
2. Sex: (1 = male; 2 = female)
3. Birth Weight (grams) ............ Grammes
4. Gestational Age: -- months
5. Place of Birth: (1 = Hospital; 2 = Home)
6. Immunization: (0 = none; 1 = partial; 2 = completed) --
7. Place of Residence since Birth: (1 = Rural; 2 = Urban --
8. Current Address: ........................................................................
   Compound: ........................................................................
   House N: ........................................................................
   Section: ........................................................................
   Place of Reference: ....................................................................

   9. How many adults live in your house? ............
   10. How many contribute to income? ............
   11. How many children live in the house? ............

II. PREVIOUS ADMISSIONS:

   Any previous hospitalizations? (0 = No; 1 = Yes)
   Age on Admission 1 : -- months
   Duration 1 : -- days
Reason 1 :  1 = Acute Diarrhoea
           2 = Persistent Diarrhoea
           3 = Pneumonia
           4 = Malaria
           5 = P.E.M.
           6 = Skin
           7 = P.T.B.
           8 = Anaemia
           9 = Other: ......................

Age on Admission  2 :  --- months
Duration of Admission  2 :  --- days
Reason for Admission  2 :  --- minutes / hours

Age on Admission  3 :  --- months
Duration of Admission  3 :  --- days
Reason for Admission  3 :  ..............................................

Age on Admission  4 :  --- months
Duration of Admission  4 :  --- days
Reason for Admission  4 :  ..............................................

III. PRESENT ILLNESS:
1. DIARRHOEA
   (a) How long has this episode of diarrhoea been going on?  ------ days
   (b) How long since diarrhoea problem first began?  ------ days

2. (a) Is the patient coughing now?  -
     (0 = none;  1 = productive;  2 = non productive)
   (b) What is the duration of cough?  --- days
3. Has the patient got a rash?
   (a) (0 = none; 1 = localized; 2 = generalized)
   (b) Type of rash (1 = examination; 2 = peeling skin
       3 = ulcers; 4 = zoaster) --
   © When did the rash develop? --- days
4. Is the patient jaundiced? (0 = none; 1 = yes) --
   (a) How long has he/she been jaundiced? --- days
   (b) Does the patient have itchiness? (0 = none; 1 = yes)
5. (a) Does the patient have sores in the mouth? (0 = none; 1 = yes)
6. Other Symptoms: ..............................................................
7. What would you call this illness? ........................................

IV. MEDICATIONS TAKEN DURING PREVIOUS ONE MONTH
1. ANTIBIOTICS : (0 = none; 1 = Septrin’
   2 = Ampicillin; 3 = Cloxacillin
   4 = Gentamycin; 5 = Metronidazole
   6 = Cefotaxine; 7 = Antihelminths;
   8 = Procaine Penicillin; 9 = X-Pen
   10 = Chloramphenicol; 11 = A.T.T.;
   12 = Other: ..............................................................
2. Anti-Diarrhoeals: (0 = none; 1 = yes)
3. O.R.T. (1 = yes; 0 = none)
4. Traditional Medicine: (0 = none; 1 = Muleza
   2 = Guava Leaves 3 = Charcoal
   4 = Lukungua 5 = Other: ...............

V. HEALTH OF OTHER FAMILY MEMBERS
1. (a) Mother: (1 = Alive and well; 2 = Alive but ill; 3 = dead)
   (b) If 3, cause of death: .....................................................
2. (a) Father: (1 = Alive and well; 2 = Alive but ill; 3 = dead)
   (b) If 3, cause of death: .....................................................

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3. (a) How many siblings: --
   (b) Any deaths? - Reason
   (Which birth order) -

                      
                      
                      
                      
                      
                      
                      
                      
                      

© Any Disability? (Y / N) Specify: ........................................


4. (a) Is there anyone in the family suffering from diarrhoea
   (0 = none; 1 = presently; 2 = during past one month)
   (b) If there is, relation to patient: -- (1 = Mother; 2 = Father;
                      3 = Sib; 4 = Uncle/Aunt; 5 = Grandpar;
                      6 = Cousin; 7 = Lodger

© When did diarrhoea start in this person? -- days

5. (a) Is there any member of the family with HIV/AIDS or TB?
   (0 = No; 1 = yes) --
   (b) If yes, relation to patient: -- (1 = Mother; 2 = Father;
                      3 = Sib; 4 = Uncle/Aunt; 5 = Grandpar;
                      6 = Cousin; 7 = Lodger)

© Is this person symptomatic? --
   (0 = Asymptomatic; 1 = Ill; 2 = dead)

VI. DIETARY HISTORY:

1. How many months was patient fed only on breast milk? --- months

2. When was other milk introduced?
   (a) Cow’s milk --- months
   (b) Formula milk --- months

3. At what age was solid food introduced in patient’s diet? --- months
VII. CURRENT DIET:
1. Milk: - (0 = none; 1 = breast; 2 = formula; 3 = cow’s
2. Porridge: - (0 = none; 1 = maize meal only;
   2 = maize meal + milk; 3 = maize meal + groundnuts;
   4 = maize meal + kapenta; 5 = Soya
3. Eggs: - (0 = none; 1 = yes)
4. Nshima with available relish: - (0 = none; 1 = yes)
5. How many times is patient fed per day? -
6. Other feeds offered: .................................................................
   ........................................................................
   ........................................................................

VIII. PHYSICAL EXAMINATION:
1. height ‘ Length ---- ( cm )
2. Weight ---- ( grams )
3. Mid Arm Circumference --. ( cm )
4. Axillar 1 ( oC ) --. ( degree °C )
5. Hair Texture - (1 = normal’ 2 = sparse hypopigmented;
   3 = alopecia)
6. Dermatoses - (0 = none; 1 = localized; 2 = generalized)
7. Oedema - (0 = none; 1 = localized; 2 = generalized)
8. Hepatomegaly - (0 = none; 1 = yes)
9. Lymphadenopathy - (0 = none; 1 = localized;2 = generalized)
10. Oral Thrush - (0 = no; 1 = yes)
11. Skin eruption: - (0 = none; 1 = erythematous;
   2 = macular; 3 = popular; 4 = vesicular)
12. Other identifiable conditions:
   (a) Condition 1 .................................................................
   (b) Condition 2 .................................................................
   (c) Condition 3 .................................................................
   (d) Condition 4 .................................................................
IX. PROBLEMS IDENTIFIED:

1. Diagnosis: (1 = Underweight; 2 = Marasmus
   3 = Kwashiorkor; 4 = Marasmic-Kwashiorkor)

2. Diarrhoea:
   (a) No. of stools in past 24 hours: --
   (b) Type of stool: (1 = watery; 2 = bloody;
   3 = loose; 4 = foamed)

3. Tuberculosis (0 = none; 1 = past pulm; 2 = present pulm;
   3 = past extra pulm; 4 = present extra pulm)

4. Associated symptoms in past 24 hours:
   (a) Abdominal pains: (0 = none; 1 = yes)
   2 = mild; 3 = moderate; 4 = severe)
   (b) Vomiting : (0 = none; 1 = yes)
   © Perianal Inflammation: (0 = none; 1 = yes)

X. CURRENT TREATMENT

...........................................................................................................
...........................................................................................................
...........................................................................................................
...........................................................................................................
...........................................................................................................
...........................................................................................................

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