

A STUDY OF FACTORS ASSOCIATED WITH INTESTINAL
IMMUNOGLOBULIN A IN CHILDREN WITH DIARRHOEA
ONLY AND THOSE WITH DIARRHOEA AND
MALNUTRITION

BY

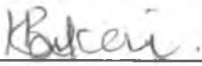
ROSE KAKAI

Thesis submitted in fulfilment for the degree of Doctor of
philosophy in the Department of Medical Microbiology,
University of Nairobi.

1994

DECLARATION:

I declare that this thesis is my original work, and has not been presented to any other University for degree work.

Signature 

Rose Kakai, BEd(Sc), MSc.

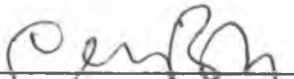
This thesis has been submitted for examination with our approval as University supervisors.

Signature 

Prof. I.A. Wamola, BSc., MSc., PhD.

Nairobi University

Medical Microbiology Department.

Signature 

Dr. J.J. Bwayo, MBChB., PhD.

Nairobi University

Medical Microbiology Department.

DEDICATION:

*This work is dedicated to my kid brother Caesar, who died
of diarrhoea at a tender age of two years.*

ACKNOWLEDGEMENT:

I wish to thank all those who assisted me in various ways to make this work possible.

First I would like to thank my supervisors, Prof. I. A. Wamola and Dr. J.J. Bwayo for their advise throughout the entire project. My regards to Dr. L.N. Mutanda, formally of Kenya Medical Research Institute, for his comments during preparation of the research proposal. I am indeed grateful to Dr. J.A. Ndinya-Achola of Nairobi University, Dr. T.K. Arap Siongok of National Public Health Laboratory Services, Dr. J.H. Ouma of Division of Vector-borne Diseases and Dr. F.A. Plummer of University of Manitoba without whose assistance this work would not have taken off the ground. I would also like to thank the Danish International Development Agency (DANIDA) mission in Kenya, particularly Ms. Karin Steffensen for financial assistance, Kenya Medical Research Institute (KEMRI) and Nairobi University, Medical Microbiology and Public Health Pharmacology and Toxicology Departments for allowing me to use their facilities. To Mr. Antony Mwatha, thanks indeed for your tireless efforts in computer expertise and to Dr. Nico J.D. Nagelkerke for statistical analysis. To the technical staff of Nairobi University Medical Microbiology Department, I cannot thank you individually but please accept this as an appreciation for the assistance

provided.

Special thanks go to my parents for their encouragement. Last but not least, sincere thanks to my husband Evans, my children Francis and Kenneth for allowing me a shoulder to lean on during the difficult moments.

TABLE OF CONTENTS

DECLARATION	ii
DEDICATION	iii ✓
ACKNOWLEDGEMENT	iv
LIST OF TABLES	viii
LIST OF FIGURES	xi
ABBREVIATIONS	xiii
DEFINITION OF TERMS	xvi
ABSTRACT	xxi
CHAPTER ONE: INTRODUCTION	1
1.1 Aims and objectives	5
CHAPTER TWO: LITERATURE REVIEW	6
2.1 Diarrhoea as a problem	6
2.2 Types of diarrhoea	10
2.3 Diarrhoea and nutrition	13
2.4 Diarrhoea and breast feeding	14
2.5 Aetiology of diarrhoea	18
2.6 Mechanisms of diarrhoea	30
2.7 Intestinal immune responses	35
2.8 Prevention and control	45
CHAPTER THREE: MATERIALS AND METHODS	47
3.1 Study population and selection criteria	47
3.2 Processing of stool for laboratory investigation	49
3.3 Enteroadhesive E. coli (EAEC) determination	50
3.4 Heat labile enterotoxigenic E. coli (LT)	51

3.5	Rotavirus antigen detection	53
3.6	HIV-1 determination	54
3.7	Specific immunoglobulin A determination	56 ✓
3.8	Total IgA determination	61
3.9	T-lymphocyte determination	62
3.10	Phagocytosis of killed <i>Candida albicans</i>	63
3.11	Statistical analysis	64
CHAPTER FOUR: RESULTS		66
4.1	Demographic information	66
4.2	Aetiology of diarrhoea	68
4.3	Diarrhoea and malnutrition	75
4.4	Mode of feeding	83
4.5	Duration of diarrhoea	90
4.6	Severity of diarrhoea	96
4.7	Human immunodeficiency virus (HIV) and diarrhoea	101
CHAPTER FIVE: DISCUSSION AND CONCLUSIONS		117
REFERENCES		127
APPENDIX		161

LIST OF TABLES:

Table 1	Distribution of nutritional status by age and sex	67
Table 2	Enteric pathogens isolated from normal and malnourished children	69
Table 3	Comparison of enteric pathogens with intestinal specific IgA	71
Table 4	Comparison of presence of specific IgA and pathogen	72
Table 5	Absolute count and percentage of CD4 and CD8 in normal and malnourished children	79
Table 6	Phagocytic activity in malnourished children with diarrhoea	81
Table 7	Phagocytic activity, total and specific IgA in relation to mode of feeding	84
Table 8	Mean duration of diarrhoea in relation to nutritional status	91

Table 9	Duration, malnutrition and severity of diarrhoea	97
Table 10	Comparison of total IgA, T-lymphocytes and phagocytosis with severity of diarrhoea	99
Table 11	Total IgA and phagocytosis in relation to HIV serostatus	103
Table 12	The effect of HIV on T-cell subsets and their relation to diarrhoea	105
Table 13	Enteric pathogens isolated from children in relation to HIV seropositivity	108
Table 14	Maternal HIV status and age of children with diarrhoea	110
Table 15	The effect of maternal HIV serostatus on duration of diarrhoea	111
Table 16	Maternal HIV serostatus and malnutrition	113
Table 17	Total IgA and phagocytosis in children born to HIV seropositive mothers	114

Table 18 T-lymphocytes in children born to HIV
seropositive mothers

LIST OF FIGURES:

Fig. 1	Distribution of rotavirus antigen and antibody by age	74	✓
Fig. 2	Nutritional status and total IgA	76	
Fig. 3	Nutritional status and specific IgA	77	
Fig. 4	Relation between phagocytic activity and duration of diarrhoea	82	
Fig. 5	Mode of feeding and specific IgA	85	
Fig. 6	Mode of feeding and T-lymphocytes	87	
Fig. 7	Relation between age and total IgA	89	
Fig. 8	Relation between total IgA and duration of diarrhoea	93	
Fig. 9	Relation between T-lymphocytes and duration of diarrhoea	95	
Fig. 10	Presence of specific IgA and severity of diarrhoea	100	

Fig. 11 Western blot bands of HIV positive children 102

Fig. 12 Severity of diarrhoea and T-lymphocytes
in HIV seropositive children 106

ABBREVIATIONS

ABTS	2,2'-azino-di [3-ethylbenzthiazoline-6-sulphate]	✓
ADCC	Antibody dependent cellular cytotoxicity	
AIDS	Acquired immunodeficiency syndrome	
BSA	Bovine serum albumin	
CDC	Centers for disease control	
CDD	Control of diarrhoeal disease	
CD4	T helper/inducer lymphocytes	
CD8	T suppressor/cytotoxic lymphocytes	
Corr	Correlation	
mm ³	Cubic millimetre	
CMI	Cell mediated immunity	
DD	Double immunodifusion	
DEAE	Diethyl aminoethyl	
DHSS	Department of health and social security	
E. coli	Escherichia coli	
EDTA	Ethylene diaminetetra acetic acid	
EIEC	Enteroinvasive <u>E. coli</u>	
ELISA	Enzyme linked immunosorbent assay	
EPEC	Enteropathogenic <u>E. coli</u>	
ETEC	Enterotoxigenic <u>E. coli</u>	
Fc	Fragment crystallisable	
g	Gravitational force	
gm	Gramme	
GIT	Gastrointestinal tract	

HBSS	Hank's balanced salt solution
HCL	Hydrochloric acid
HIV	Human immunodeficiency virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
K+	Potassium ions
KNH	Kenyatta National Hospital
LT	Enterotoxigenic <u>E. coli</u> heat labile toxin
ml	Millilitre
mm	Millimetre
MMWR	Morbidity and mortality weekly reports
Na+	Sodium ions
NaCO ₃	Sodium carbonate
NaIO ₄	Sodium periodate
nm	Nanometre
NS	Not significant
OD	Optical density
ORT	Oral rehydration therapy
PBS-T	Phosphate buffered saline with tween
PEG	Polyethylene glycol
PEM	Protein energy malnutrition
PMH	Pumwani maternity hospital
POW	Paediatric observation ward
RBC	Red blood cells
rpm	Rounds per minute

SD	Standard deviation
SE	Standard error
sIgA	Secretory IgA
SPA	Special programme on AIDS statement
SRID	Single radial immunodifusion
SS	<u>Salmonella-Shigella</u>
ST	Enterotoxigenic <u>E. coli</u> heat stable toxin
ul	Microlitre
VPH	Veterinary public health
WHO	World Health Organization
WHO Bull.	World Health Organization bulletin

DEFINITION OF TERMS

Active immunity - dependent upon stimulation of the subject's own immunological mechanisms.

Acute diarrhoea - passage of loose or watery stool three or more times per day for less than 14 days.

Adsorption - non specific adherence of substances in solution to cells or other substances of particulate matter.

Agglutination - clumping together of micro-organisms, on exposure to an appropriate antiserum.

Antibody - molecule produced by man and other animals in response to antigen which has the property of combining specifically with the antigen.

Antigen - molecule or particle which elicits the formation of and combines with antibody either directly, or through generation of activated lymphocytes.

Bottle fed - children who are fed entirely on supplementary food.

Breast fed - children who are fed entirely on breast milk.

Cellular immunity - pertaining to cell mediated immunity.

Diarrhoea - passage of loose or watery stool three or more ✓
times per day.

Enterotoxin - any exotoxin which when ingested or produced within the intestine, is absorbed by the gut and affects directly or indirectly the functioning of the intestinal mucosa.

Facultative - able to multiply in the presence or absence of oxygen.

HIV unexposed - children born to HIV seronegative mothers.

HIV exposed - children born to HIV seropositive mothers.

Humoral immunity - antibody dependent immunity.

Immune response - the specific response of the body to the introduction of an antigen.

Kwashiorkor - children who are 60-80% of expected weight for age with oedema.

Marasmic/kwashiorkor - children who are less than 60% of

expected weight for age with oedema.

Marasmus - children who are less than 60% of expected weight for age without oedema.

Mild diarrhoea - absence of blood or mucus in stool, with duration of 3 days or less.

Mixed fed - children who are fed on both supplementary food and breast milk.

Morbidity - the proportion of a population which has contracted a given disease at a given time, or during a specified period of time.

Moderate diarrhoea - absence of blood or mucus in stool, with duration of 4 days or more.

Mortality - the proportion of a population which has died of a given disease at a given time, or during a specified period of time.

Passive immunity - dependent upon injection of ready made antibodies and not upon the subject's own immunological mechanisms.

Persistent/chronic diarrhoea - passage of loose or watery stool three or more times per day for 14 days or more.

Phagocytosis - the process of ingestion into a cell by closing off an invagination of the protoplasm. Following ingestion, the contents of the phagosome are digested by the discharge of lysosomal enzymes into the phagosome.

Serotype - the identity of a bacterial strain as indicated by antigenic analysis, or a group of strains shown by serological tests to be antigenically identical.

Severe diarrhoea - presence of blood or mucus in stool irrespective of duration.

Specific - pertaining to a species.

Titre - the highest dilution of a serum or antigen preparation which gives a positive reaction under defined conditions.

Toxoid - toxin rendered harmless but still effective as an antigen

Weight for age - this is a nutritional parameter used to provide an indication of whether or not a child is

malnourished or essentially normal in body size. It does not give the duration of malnutrition.

ABSTRACT

A cross sectional study was undertaken to determine the role of intestinal immunoglobulin A (IgA) in prevention from, or limitation of diarrhoea disease among normal, malnourished and human immunodeficiency virus (HIV) seropositive children.

Stools were collected from children aged less than 5 years old, from which total IgA was determined by radial immunodiffusion and specific IgA by Enzyme-linked immunosorbent assay (ELISA). Intestinal parasites were identified by wet preparation and microscopy. Enteric bacteria namely Salmonella, Shigella, Escherichia coli and Campylobacter were isolated by culture on selective media then identified by biochemical tests and slide agglutination serotyping. Rotavirus was determined by ELISA test. Human immunodeficiency virus was determined by an ELISA screening test and confirmed by Western Blot.

Peripheral blood was collected from the children for determination of T-helper (CD4) and T-suppressor (CD8) lymphocytes by flow cytometry and phagocytic activity by killed Candida albicans yeast cells.

A total of 556 children were included in the study, mean age 11.5 months, standard deviation 14.8 months and range 5 days

to 60 months. Malnourished children had diarrhoea of longer duration ($p=0.001$) and more severity ($p<0.04$) than those well nourished. Similar results have been reported in other studies. Apart from total IgA which was higher in marasmic children ($p=0.01$), specific IgA levels, phagocytic activity and T-lymphocyte counts were independent of nutritional status. While there was no association between severity of diarrhoea and either total IgA, T-lymphocytes or phagocytic activity, Rotavirus specific IgA ($p = 0.05$) and EPEC 086A:K61 ($p = 0.01$) was significantly higher in mild compared to severe diarrhoea. It is therefore probable that prolonged and severe diarrhoea in malnutrition may be a result of impaired immune system.

There was a trend for increased total IgA levels ($p=0.2$) and T-helper cell count ($p=0.06$) in breast fed children compared to those mixed fed or bottle fed. Breast feeding may provide a direct localized protective function in the gut or via primed T-cells which regulate committed B-cells to produce IgA.

When the presence of specific IgA was compared with enteric pathogens, children had diarrhoea caused by different organisms other than the one they had intestinal IgA antibody to ($p<0.05$). This suggests that intestinal IgA may have a protective role to play in the host's resistance to diarrhoea

disease. The occurrence of enteropathogens in neonates suggest that passive immunity may be inadequate.

Total IgA, ($p=0.3$) and phagocytic activity, ($p=0.1$) were reduced, though not significantly in HIV seropositive children. The occurrence of enteropathogens was independent of HIV serostatus and CD4 cell depletion. Absolute CD4 lymphocyte counts were low in HIV seropositive children with severe diarrhoea ($p=0.01$). This suggests that CD4 lymphocytes may have a role to play in limiting severity of diarrhoea in HIV infection. Children of HIV seropositive mothers had prolonged diarrhoea than those of HIV seronegative mothers ($p=0.06$) regardless of their HIV status, and diarrhoea was more common among those aged less than six months ($p=0.02$, Odds ratio=4.75). Probably HIV infection prevents passive transfer of maternal immunity.

The results of this study suggest that the presence of intestinal IgA may have a role to play in protection against diarrhoea in both normal and malnourished children. The role of passive transfer of maternal immunity in HIV seropositive mothers needs further investigation.

CHAPTER ONE

INTRODUCTION

Diarrhoea remains a major cause of morbidity and mortality worldwide, especially in developing countries, largely due to poor socio-economic status. Worldwide, diarrhoeal diseases are second only to cardiovascular diseases as a cause of death, and the leading cause of childhood death (Snyder et al. 1982). Most investigators estimate that each year, 4.5 to 6 million children die from diarrhoea illness in Asia, Africa and Latin America (Sima et al. 1991, Snyder et al. 1982). In the United States of America, estimates exceed 10,000 deaths per year from diarrhoea (CDC 1992^b, Lew et al. 1991). In Kenya although many of the cases are not reported, morbidity rate is estimated at 4.9 episodes of diarrhoea per child per year, and death rates of 3.4 per 1,000 children per year (Omond-Odhiambo et al. 1984). On average, diarrhoea is responsible for 20% of all episodes of childhood illness (Lew et al. 1991, Kirkwood 1991, Mutanda 1980^b). These numbers may be rising because of the increasing deterioration of hygienic living conditions in overcrowded slums in urban centers.

A proportion of episodes of diarrhoea that begin acutely become persistent, the proportion being greatest in children

under two years of age. About 20% of all deaths in children less than 5 years old are due to diarrhoea, and more than 50% of these are attributed to persistent diarrhoea (Ebrahim ✓ 1990). This results in progressive deterioration in the nutritional status and general health of the patient with the risk of death (WHO/CDD 1988). Persistent diarrhoea in children remains a major health problem in developing countries and is usually associated with malnutrition, often of severe degree (Behrens 1991, Sullivan et al. 1990). Prevention of malnutrition is required in order to reduce the risk of acute diarrhoea becoming persistent and to offset the negative effect of diarrhoea on growth. Once persistent diarrhoea has developed, nutritional management is the mainstay of treatment.

Diarrhoea is one of the major signs in Acquired immunodeficiency virus (AIDS) patients. Most patients with AIDS (50-98%) either present with, or later have diarrhoea that is often life threatening (Colebunders et al. 1987). With the increasing frequency of AIDS and immunosuppressive chemotherapy, diarrhoea in immunocompromised patients present a growing challenge.

The role of immune response in limitation from, or prevention of diarrhoea is not well understood. Mucosal surfaces are bathed in secretions which have a host defence function to

remove potential pathogens (Tomasi 1972). Specific host defense in secretions is provided primarily by immunoglobulins, mainly secretory IgA because of its ability to bind to antigens, thus reducing the ability of pathogens to attach to mucosal cells (Roitt 1988). There is evidence that T-cells are required to eliminate some parasitic worm infestations from the gut (Zu et al. 1992). It therefore seems that T-cells can exert effector functions in the gastrointestinal tract as in other parts of the body. Phagocytic cells (neutrophils), which are essentially the first line of defense, have a critical role in the host defense against a variety of microbial pathogens. Recent studies have indicated that the increased frequency and severity of infections in malnourished populations may have an immunological basis (Chandra 1983).

Measures to prevent enteric infection emphasize the need for improved sanitation and hygiene to reduce person-to-person spread of pathogens (Feacham 1984^a). In developing countries where poverty and low hygienic standard of living is prevalent, results from several studies provide support for efforts to promote exclusive breast feeding during the first 4 to 6 months of life and partial breast feeding thereafter as a means of reducing considerably the risk of severe life threatening diarrhoea (Ketsela et al. 1990, Baltazar et al 1993, Feacham 1984^b). The protective effects do not appear to

continue after cessation of breast feeding.

Although oral rehydration is the cornerstone of treatment for all diarrhoea illnesses, the severity of certain inflammatory and persistent diarrhoea may warrant specific antimicrobial therapy (Kinoti et al. 1985). Because of severe morbidity and significant mortality caused by gastroenteritis and the difficulties in providing effective treatment especially for infections due to antibiotic-resistant strains, there is a need for effective vaccines. Efforts for vaccine development have not been successful. Knowledge about the mechanisms of immunity to enteric infections may have a bearing on strategies for vaccine development.

It may be hypothesised that increased susceptibility to persistent and severe diarrhoea in malnourished children may be due to reduced IgA in the gastrointestinal mucosa or T-lymphocytes. The purpose of this study is to determine the role of local and systemic immunity to diarrhoeal diseases. The results of this study are likely to give a better understanding of the host response to intestinal infections.

1.1 Aims and objectives

1.1.1 General objective

To determine the role of local and systemic immunity to diarrhoeal diseases.

1.1.2 Specific objectives

To determine the levels of intestinal IgA, peripheral blood T-lymphocytes and phagocytic activity in normal and malnourished children and relate it to:

1. Causative agents of diarrhoea.
2. Mode of feeding.
3. Duration of diarrhoea.
4. Severity of diarrhoea.
5. HIV serostatus.

CHAPTER TWO

LITERATURE REVIEW2.1 Diarrhoea as a problem

2.1.1 Developed and developing countries.

Worldwide, diarrhoea remains one of the most common illness among children. In United States of America (USA), children less than 5 years of age experience more than 20 million episodes of diarrhoea leading to approximately 200,000 hospitalizations and 400 deaths per year (CDC^b 1992). Most hospitalizations (65%) and death (85%) due to diarrhoea occur in the first year of life. Between 1979 and 1987 in USA, 11% of all diarrhoea deaths occurred in children aged less than 5 years (Lew et al. 1991). In many developing countries, 1.5 billion episodes of diarrhoea and 4 million associated deaths occur among children each year (CDC^b 1992, Snyder et al. 1982, Sima et al. 1991, Mata et al. 1967). In the tropics, nearly 1.5 million children are believed to be dying each year from diarrhoea caused by organisms other than cholera (Mata 1981). The incidence increases with the age up to a period marked in developing countries by the end of the weaning period. It is not clear whether this is due to lack of maternal antibody or

increased opportunity of exposure to enteric pathogens (Mata 1981). Often antibiotics fail to control, or even perpetuate the illness (Wamola et al. 1981, Kakai et al. 1987, Kunin ✓ 1993). Moreover, the interaction between diarrhoea, protein energy malnutrition (PEM), and impaired immune state, all acting in vicious cycle, further lead to the downhill course of the illness.

Gastroenteritis has been documented as the most common infection as compared to the total number of childhood infections in Nigeria, Zambia and Ethiopia, accounting for 44%, 25.3%, and 13.5% respectively (Winfred 1984, Patel et al. 1982, Bimal et al. 1988). Mortality rates as high as 41.7% in Ethiopia have been reported, owing probably to war and poverty which have prevailed in the country for a long time (Bimal et al. 1988). Rotavirus has been documented as the most common cause of acute childhood diarrhoea in Nigeria 15.2% (Olusanya et al. 1989) and Kenya 39% (Mutanda et al. 1980^a, Mutanda 1985^b) more so among in-patients than out-patients.

2.1.2 Kenya.

Diarrhoea accounted for 10.8% of all deaths thereby ranking third after respiratory diseases and congenital anomalies (Kirkwood 1991). A community based study in Machakos district

showed that 0.1% of all childhood diarrhoeal episodes end in death (Omondi-Odhiambo et al. 1984). This is considerably lower than the hospital based estimates of 11-19%, as would be expected, since it was based on all diarrhoeal episodes occurring in the community and not just on those severe enough to lead to hospital admission (Lew et al. 1991, Kirkwood 1991).

The poor sanitary facilities and the low standards of hygiene which still prevail in some parts of Kenya make diarrhoea due to Salmonella, Shigella and Escherichia coli likely to persist as an endemic disease (Wamola et al. 1974). Most studies have been centred around the urban and peri-urban areas. Data on the prevalence of diarrhoea in rural areas is therefore very scarce despite the fact that the rural population is the most disadvantaged due to poor personal and domestic hygiene practices (Mutanda 1980^b).

Diarrhoea has been reported in outbreaks either in hospitals (Mutanda et al. 1987^a, Mutanda et al. 1990) or within communities, cholera being the major causative agent in the latter especially in areas with poor hygiene such as Kariobangi and Kibera in Nairobi, Baringo and Kisumu districts. Sporadic cases have also been reported both in adults and children (Mutanda 1980^a, Mutanda 1987^b, Estambale et al. 1989). In a study in Kiambu District, mixed infections

were very common, notably Campylobacter with Giardia lamblia, while enterotoxigenic E. coli (ETEC) accounted for 33% of all the isolates (Chunge et al. 1989).

In Kenyatta National Hospital (KNH), gastroenteritis accounted for 20% of all the children admissions (Kalya et al. 1972). Similar results were found by Mutanda in KNH, Aga Khan hospital, and Getrude garden children's hospital, at the rate of 19.4%, 23.3% and 20.8% respectively (Mutanda 1980^b). Cryptosporidiosis alone accounted for 3.8% of the patients admitted to KNH with diarrhoea (Estambale et al. 1989). At the same Hospital, the most notorious among Salmonellae infections has been Salmonella typhimurium, occurring most commonly among children. In 1985, it took epidemic proportions and resulted in temporary closure of Paediatric Observation Ward (POW) (Mirza et al 1989). Since 1970, S. typhimurium has been on the increase, and in addition to Shigella and E. coli, it has become highly resistant to most of the drugs in use (Wamola et al. 1981, Kakai et al. 1987, Mutanda 1987^a, Kariuki et al. 1993). Mortality rates vary from one place to another, although it must be emphasized that unreported cases are numerous.

2.2 Types of diarrhoea

2.2.1 Acute

Acute diarrhoea is defined as the passage of loose or watery stools three or more times per day for less than 14 days (WHO/CDD^b 1987).

Acute diarrhoea is more frequent and more severe, especially among the malnourished (Mutanda et al. 1985^a). The incidence varies from 13.5% to 44% (Winfred 1984, Patel et al. 1982), particularly in infancy in poor countries including Kenya (Kalya et al. 1972, Wamola 1980). Results from a multicenter study suggest that microbe specific strategies for the control of childhood diarrhoeal diseases in developing countries should focus on Rotavirus, Shigella species and ETEC which are the major causative agents (Sima et al. 1991). In the same study, over 60% of cases of acute diarrhoea involving children under 3 years of age were among 0-11 months age group.

Death from acute diarrhoea is often due to dehydration which results from excessive loss of body fluids and electrolytes in diarrhoeic stools and vomitus, therefore management of acute diarrhoea is dependent upon the use of oral rehydration therapy (WHO/CDD^a 1987, Kinoti et al. 1985, Fauven et al.

1991). Routine use of antimicrobials is not advised.

2.2.2 Chronic/persistent ✓

Chronic diarrhoea is defined as the passage of loose or watery stools three or more times per day for 14 days or more (WHO/CDD^b 1987).

A proportion of episodes of diarrhoea that begin acutely become persistent, the proportion being greatest in children under 2 years of age. About 20% of all deaths in children less than 5 years old are due to diarrhoea, and more than 50% of these are attributed to chronic diarrhoea despite treatment (Ebrahim 1990). This results in progressive deterioration in the nutritional status and general health of the patient with the risk of death (WHO/CDD 1988). There are no characteristic features in the mode of onset or in the symptomatology.

Reported incidence of persistent diarrhoea varies widely. Rates as high as two episodes per child per year in North East Brazil, 0.8 in Gambia and 0.3 in India have been reported (Ebrahim 1990). Since persistent diarrhoea is more frequent in children who have already suffered such episodes, the problem may be concentrated in a fairly small proportion of children. Persistent diarrhoea plays an important role in

the aetiology of PEM with wasting as a rapid outcome and marked stunting among the survivors (Sullivan et al. 1990). To date, however, no specific aetiological agents have been recognized as being important, although enteroadhesive E. coli (EAEC) and enteropathogenic E. coli (EPEC) appear to be common (WHO/CDD 1988, Mutanda et al. 1985'). Thus aetiological agents are similar to acute cases.

It would appear that in some cases of diarrhoea, the illness becomes prolonged because of either failure of intestinal mucosa to heal rapidly following an acute episode, or there is continuing injury in the intestinal epithelium. Risk factors for the development of persistent diarrhoea include pre-existing malnutrition, prior frequent episodes of diarrhoea or a prior episode of persistent diarrhoea, failure to breast feed during infancy and inappropriate management in form of antimotility drugs or antibiotics which may cause changes in gut flora.

The management of persistent diarrhoea is based on maintaining hydration and nutrition while intestinal damage is repaired. Routine use of antimicrobials is not advised. The present recommendation is to use antimicrobials only if a specific enteropathogen such as Shigella warranting treatment has been isolated. Research is underway to evaluate new antibiotic regimens that might be used for treating

shigellosis, especially in areas where Shigella are frequently resistant to currently available antimicrobials.

2.3 Diarrhoea and nutrition

Protein Energy Malnutrition in infancy and childhood is a major public health problem and is today the most serious nutritional problem in Africa and other developing countries. The causes of malnutrition are complex so that even in areas where food is plentiful, malnutrition may be evident. Illness and malnutrition are synergistically related in that malnourished children are more pre-disposed to illness of infectious origin and ill children are more likely to become malnourished. It is not clear whether diarrhoea pre-disposes to malnutrition or vice versa.

There is evidence to show that immune response is suppressed in malnutrition but the exact mechanism is not entirely clear (Schrimshaw et al. 1968, Chandra 1983). In Kenya and other parts of the world, workers have found that diarrhoea is more severe and lasts longer in malnourished children but no increased incidence has been demonstrated (Mutanda et al. 1985^a, Lloyd-Evans et al. 1983, Behrens 1991, Gordon et al. 1964, Oburra 1986, Mathur et al. 1985). Isolation of EPEC (Mutanda et al. 1985^a), ETEC (Mathur et al. 1985) and G. lamblia (Sullivan et al. 1990, Okeahialam 1982) was

significantly higher in malnourished children, indicating a greater bacterial and parasitic load in the gut. Chronic diarrhoea and malnutrition in Gambia children was associated with persisting damage to the mucosa of the small intestines (Lunn et al. 1991). There was extensive lymphocyte infiltration, especially the CD8 phenotype, suggesting the damage occurs as a result of local cytotoxic T-cell mediated reactions to luminal antigens, but the mechanism is unknown.

Nutritional status at admission is a major determinant of the prognosis. Mortality among children with diarrhoea is significantly higher among those presenting with signs of malnutrition (Oburra 1986, Beau et al. 1987).

2.4 Diarrhoea and breast feeding

It has been observed that breast feeding protects children from many illnesses (Khan 1984, Cunningham 1977, Gerard 1974, Jelliffe et al. 1976, Ketsela et al. 1990, Cunningham et al. 1991) may be due to immunological factors present in breast milk (Ruiz-Palacios et al. 1990, Cleary et al. 1991, Duffy et al. 1986). Active immunity as well as passive protection by breast milk antibody, lactoferrin, lysozyme, antibody and other factors help prevent many enteric infections. Breast feeding was associated with significantly less illness during the first year of life, especially if continued beyond four

and a half years of age (Cunningham 1977, Gerard 1974). Breast fed children are less likely to develop respiratory and gastrointestinal infections and allergic reactions (Regua et al. 1990, Oniang'o 1985). This may be due to the enormous amount of IgA present in breast milk which has an ability to bind to antigens, thus reducing the ability of pathogens to attach and penetrate the mucosal cells. Infants slowly develop their own immunologic defense in the months after birth, and breast feeding is a hygienic gradual method of protection during the transition to immunologic independence (Ajusi et al. 1989). Persistent diarrhoea syndrome tends to occur in non breast fed infants (Behrens 1991). A case-control study in Iraq showed that early introduction of bottle feeding was associated with greatly increased risk of severe diarrhoea during the first year of life. For example, the relative risk of hospitalization for diarrhoea for non breast fed versus exclusively breast fed was between 24 to 45 in the first 6 months of life (WHO/CDD 1988). Breast feeding was found to be similarly protective against diarrhoeal incidence, duration and severity, even after controlling for socio-economic status and weight for age in a study of infants during the first 6 months of life in an urban center in Brazil (WHO/CDD 1988). Thus the relative risk for having diarrhoea illness ranged from 1.1 to 2.6 for non breast fed versus exclusively breast fed infants.

It has been suggested that breast feeding protects against diarrhoea morbidity as a result of either one or a combination of the following factors: Less exposure to enteropathogens transmitted by contaminated food, protection due to humoral and cellular immune properties, promotion of intestinal flora that inhibits proliferation of enteric pathogens or enhancing infant's nutritional status thereby reducing susceptibility to diarrhoea.

Partially breast fed infants tended to occupy an intermediate position. Some workers have found that supplementation of breast feeding with additional foods increased significantly the risk of diarrhoea (Popkin et al. 1990). Scoub and co-workers suggest that the association of bottle feeding with gastroenteritis result from poor hygiene and greater opportunity for the spread of infection rather than lack of immune factors in artificial milk (Scoub et al. 1977). In Kenya, the peak age incidence of severe childhood gastroenteritis leading to admission to hospital is inversely related to the disappearance of maternal antibodies to Rotavirus, rather than to introduction of bottle feeding (Mutanda 1980°).

Contrary to most studies, workers in Nigeria found that breast fed children had a higher incidence of Rotavirus diarrhoea than those bottle fed (Gomwalk et al. 1990). A few

studies have also made the same observation and demonstrated that the presence of Rotavirus antibody in breast milk does not necessarily confer immunity (Totterdel et al. 1980, Gurwith et al. 1981).

Different recommendations have been offered as to whether human immunodeficiency virus (HIV) infected mothers should breast feed due to the risk of post-natal transmission of HIV via breast milk (WHO/SPA 1987, CDC 1985, DHSS 1988, Kennedy et al. 1990, Pizzo et al. 1991, Kennedy et al. 1989). It has not been established whether the usual nutritional and immunological benefits of breast feeding are maintained when the mother is infected or whether the child can be infected through breast milk (Kennedy et al 1990). In developing countries where poverty and low standards of hygiene prevail, the benefits of breast feeding out-weigh the risk of post-natal transmission of HIV via breast milk (Kennedy et al. 1990). World Health Organization aims at women in developing countries and recommends that where the alternatives are not safe, breast feeding should continue to be the method of choice regardless of mother's HIV infection status (WHO/SPA 1987). In developed countries where the alternatives may be safe, HIV infected women are advised against breast feeding to avoid post-natal transmission to a child who may not be infected (CDC 1985, DHSS 1988).

2.5 Aetiology of diarrhoea

Diarrhoea can be caused by a multiplicity of enteric agents, usually acquired by the faecal-oral-route as the main source of infection. Sources of infection in developing countries is mainly untreated river water due to faecal contamination while in developed countries it is piped water contaminated with sewage due to re-cycling of water. Outbreaks of diarrhoea have been reported in developed countries due to contaminated eggs and chicken traced back to their farms of origin (CDC^a 1992). Sporadic cases of traveller's diarrhoea from tourists who have recently visited developing countries have been reported (Mathewson et al. 1983). Some of the aetiological agents are listed below, although a large part of the agents remain unknown.

2.5.1 Rotavirus and other viruses

Rotavirus is a double stranded ribonucleic acid virus in the family, reoviridae. It is the most common cause of acute gastroenteritis in children (Olusanya et al. 1989, Mutanda 1980^a, Mutanda 1985^b, Mutanda 1980^c, Valman 1980, Asindi et al. 1991, Kakai 1984), most frequently in temperate countries during winter (Editorial 1977). In Kenya and Nigeria, Rotaviruses were more common during the dry season, especially among infants less than six months old (Olusanya

et al. 1989, Makino et al. 1983). Reports from Nigeria indicate that low relative humidity is an important environmental factor for Rotavirus survival and spread in this area (Paul et al. 1982). Studies in Kenya and Brazil have shown an inverse relationship between Rotavirus incidence and age, probably as a result of waning of maternal antibody or some other factors (Mutanda 1980^c, Makino et al. 1983, Tardelli et al. 1991). In rural and urban areas, incidence rates vary in Nigeria from 1.6% to 25.5% (Olusanya et al. 1989, Cocker et al. 1987), in Kenya from 13% to 40% (Mutanda 1985^b, Chunge et al. 1989), 11.5% in Saudi Arabia (Al-Freihi et al. 1993) and 14% in an urban area in Brazil (Tardelli et al. 1991). The high proportion of Rotavirus cases in hospital-based compared to community-based studies indicate that Rotavirus causes severe diarrhoea thereby resulting in patients seeking hospital assistance (Sima et al. 1991).

In Chile, nosocomial Rotavirus infections occurred in 20% of children less than 2 years old admitted to hospital without diarrhoea (WHO/CDD 1988). Thirty percent of the nosocomial infections were symptomatic, making Rotavirus the most common cause of nosocomial diarrhoea in this age group. It is speculated that Rotavirus may be airborne in this setting.

Other minor viral pathogens that causes diarrhoea include the

Adenoviruses, Norwalk-like agent, Caliciviruses, Coronaviruses, Enterovirus and small round viruses (Sima et al. 1991, Mutanda 1985^b, WHO/CDD 1988, Kapikian 1993).

Enteric Adenoviruses types 40 and 41 are responsible for about 5% of cases of childhood diarrhoea in developed countries, but the proportion in developing countries is unknown. In a study in Australia, Norwalk-like viruses were found in 32% of cases of acute gastroenteritis in adults but in none of 250 children with diarrhoea, suggesting that these agents may not be important diarrhoeal pathogens in young children (WHO/CDD 1988). The role of caliciviruses and enteric coronaviruses as causes of childhood diarrhoea in developing countries still need to be determined.

Measles-associated diarrhoea has been reported in several communities including Kenya with a multiplicity of aetiological agents (Sang et al. 1992). It is not clear if measles on its own is capable of causing diarrhoea.

2.5.2 Escherichia coli

This is a gram negative facultative anaerobe bacteria. Enteropathogenic E. coli were the first group of diarrhoea causing E. coli to be identified and they remain an important cause of diarrhoea worldwide (Cravioto et al. 1991, Gross

1983). The high number of EPEC in the first five months of life indicate extremely high level of faecal contamination of the environment (Chunge et al. 1989, Gross 1983, Mutanda et al. 1990). In Brazil, the incidence of EPEC in children less than 12 months old decreased with an increase in age (Tardelli et al. 1991). Hospitals may be the direct source of infection since EPEC serotype 0111 and serotype 0119 were isolated from the environment in several hospital nurseries (Mutanda et al. 1990, Mutanda et al. 1987^a, Trabulsi et al. 1985). The high number of EPEC isolates in asymptomatic children indicate that care should be exercised in assessing its significance in individual cases (Sima et al. 1991).

Enteroadhesive E. coli (EAEC), ETEC and enteroinvasive E. coli (EIEC) have not been extensively investigated. Whereas Mutanda found that there was no significant difference between children with diarrhoea and healthy controls with regard to frequency of ETEC isolation in KNH (Mutanda 1980^a), Waiyaki found heat labile toxigenic E. coli (LT) in 1.2% and heat stable toxigenic E. coli (ST) in 22% of children with diarrhoea and none from those without diarrhoea in Mombasa (Waiyaki et al. 1986). A hospital based study in Thailand has shown that EIEC account for only a small proportion of diarrhoea episodes in children under the age of 5 years, being identified in 1.5% of cases and 0.5% of healthy controls (WHO/CDD 1988).

2.5.3 Salmonella

This is a gram negative facultative anaerobe bacteria. It comprises of about 2000 serotypes. Salmonellosis most commonly results from ingestion of contaminated food and water. In industrialized countries outbreaks have occurred as a result of sewage contamination of water supplies. In USA, S. enteritidis is the most frequent serotype such that in 1990, it accounted for 21% of all Salmonella species in an outbreak associated with consumption of raw shell eggs (CDC^a 1992).

In the developing countries where water is often obtained from sources that are not purified or protected, water-borne salmonellosis is more common (WHO Bull. 1980^b). In Kenya Salmonella accounted for 3.7% and 5.6% of cases of diarrhoea in Mombasa and Nairobi (Waiyaki et al. 1986, Mutanda 1980^b), 34% in Saudi Arabia (Al-Freihi et al. 1993) and 7% in Brazil (Tardelli et al. 1991). S. typhimurium has been the most notorious in KNH and subclinical infections are fairly common.

2.5.4 Shigella

This is a gram negative facultative anaerobe bacteria. It comprises 4 species namely Sh. dysenteriae, Sh. flexneri, Sh.

sonnei, and Sh. boydii. Studies have shown that Sh. flexneri is the most common cause of endemic dysentery in developing countries while Sh. dysenteriae is frequently associated with severe epidemic disease (Mutanda 1987°, Northrup 1992). A review by Mutanda between 1975 to 1979 showed that Shigella species were the commonest pathogens encountered in KNH and accounted for 51 to 54% of all pathogens (Mutanda 1987°). In Bangladesh, Shigella accounted for 9.4% and 28.3% of urban and rural dysenteric stools respectively, with a peak prevalence occurring at 18-23 months, and 14.7% in Saudi Arabia (Henry 1991, Al-Freihi et al. 1993). Cases of dysentery are more likely to persist than cases of watery or mucoid diarrhoea.

2.5.5 Campylobacter

This is a gram negative microaerophilic curved rod bacteria. The introduction of a selective media by Skirrow in Britain facilitated isolation of Campylobacter in 7.1% of patients with diarrhoea and none from people without diarrhoea (Skirrow 1977). It was first isolated in Kenya in 1983 by Wamola at a frequency rate of 11.4% (Wamola et al. 1983). Later Waiyaki isolated it at a rate of 12.6% at Coast General Hospital in Mombasa and Chungu at the rate of 5.5% and 7.3% in Kakamega and Kiambu districts respectively (Waiyaki et al. 1984, Chungu et al. 1992). Incidence rates vary from one

country to another for example 19% in Gaza, 7.4% in Saudi Arabia and 6.6% in Italy for HIV related diarrhoea (Sallon et al. 1991, Bicocchi et al. 1992, Al-Freihi et al. 1993).

Information regarding the sources of infection are scarce but some studies identified chickens and dogs in the household and lack of running water as risk factors for acquiring C. jejuni infection (Skirrow 1977, Simango et al. 1991). The clinical picture varies from asymptomatic excretion or mild to severe diseases.

2.5.6 Vibrio cholera

V. cholera causes diarrhoea by virtue of their ability to produce enterotoxins. The disease has been endemic in India and the Ganges basin for centuries, from where epidemics have spread to other parts of the world. In 1971, cholera was first reported in the North Eastern part of Kenya affecting only nomadic people, after which there have been several epidemic and sporadic cases spread all over the country (Mngola 1974).

A series of outbreak investigations in village, hospital and prison settings in Tanzania led to interesting hypotheses that person-to-person transmission may occur among patients admitted to overcrowded, unsanitary health facilities, and

that nosocomial transmission can play an important role in the maintenance of cholera outbreaks in the community (WHO/CDD 1988). Man usually contracts cholera from water or food which has been contaminated with faeces of a cholera victim.

In severe cases, liquid faeces referred to "rice water stools" may lead to extreme dehydration, shock and death within hours. The organisms do not invade the epithelium.

2.5.7 Other bacteria

The role played by the bacteria named below as causative agents of diarrhoea have not been extensively investigated in Kenya.

The cause of diarrhoea associated with antibiotic use remains elusive in many cases. Apart from Clostridium defficille, no other intestinal pathogen has been consistently associated with nosocomial (hospital acquired) diarrhoea. Antibiotics may pre-dispose to diarrhoea by suppressing intestinal bacteria and disrupting local microbial flora (Danna et al. 1991). C. defficille produces a toxin causing pseudomembranous enterocolitis.

Bacillus cereus food poisoning is characterized by diarrhoea,

abdominal pain and cramps, 8-16 hours after ingestion of food contaminated with enterotoxin (Davis et al. 1980). Only rarely is illness accompanied by vomiting.

Staphylococcus aureus food poisoning, caused by preformed enterotoxin, is characterized by vomiting and diarrhoea commencing 1-6 hours after consumption of contaminated food especially dairy produce (Davis et al. 1980). Symptoms usually last less than 24 hours and death is extremely rare.

Yersinia enterocolitica, Vibrio parahaemolyticus, Aeromonas hydrophila and Aeromonas (Plesiomonas) shigelloides have not been extensively studied. Aeromonads are frequently isolated from stools of both healthy and diarrhoeic individuals but their role in diarrhoea is controversial (Davis et al. 1980).

2.5.8 Parasitic diarrhoea

A wide range of intestinal parasitic infections have been reported as causative agents of diarrhoea, some of which are discussed below:

Cryptosporidium, a coccidial protozoa has a wide distribution and is well known in veterinary medicine as a causal agent of enteritis in a number of animal species (Tzipori et al. 1981). The disease in man is probably acquired from the

surroundings such as fruits and vegetables, or from animals and humans with a recent infection (Collins 1984). Cryptosporidial infections often occurs in AIDS patients as a complicating factor, and appears to thrive in the absence of control of the host's immune system. However, the disease has also been diagnosed in otherwise normal immunocompetent individuals. Probably in these instances, it has resulted either from intake of large numbers of oocysts or that the patient has suffered temporary lowering of resistance because of a concurrent illness or other stress which has allowed low level infection to establish and develop unchecked (Jokipii et al. 1983).

In temperate countries, cryptosporidiosis is responsible for between 1-4% of cases of diarrhoea in children. In tropical and less well developed countries, it is much more highly prevalent, accounting for between 1-17% of cases of diarrhoea (Mersha et al. 1992). In Kenya, Cryptosporidium may not be a major pathogen in diarrhoea disease since it was isolated from only 1.1% of children with diarrhoea in 1986 at KNH (Mutanda et al. 1986). Later in 1992, it was isolated from 2.7% and 3.8% in Kakamega and Kiambu districts respectively (Chunge et al. 1992).

Trichomonas hominis is frequently observed in loose stools and has been suspected of being a possible cause of chronic

diarrhoea, especially in malnourished children (Mutanda et al. 1986, Chungue et al. 1988).

Giardia lamblia is a flagellate protozoa which is known to thrive in the lower part of the human bowel. It gives rise to symptoms of persistent diarrhoea, malabsorption and failure to thrive in some children, particularly those living in poor socio-economic environment (Okeahialam 1982, Amin 1975). Infection rates are high but illness due to Giardia is rare. Some workers have found G. lamblia more frequently from HIV infected patients but its role is not clearly understood (Bicocchi et al. 1992, Robinson et al. 1991).

Ascaris lumbricoides is not normally thought to be a cause of diarrhoea in children, it may cause diarrhoea and acute inflammation in some instances.

Entamoeba histolytica is the causative agent of amoebic dysentery.

Helminths such as Schistosoma mansoni, Taenia saginata and Taenia solium have been found in diarrhoeic stools.

Malaria infection is often accompanied by diarrhoea but is not clear if this is caused by the malaria parasites or other known causative agents of diarrhoea.

2.5.9 Fungal agents

Candida is generally regarded as a commensal in the gastrointestinal tract but some workers have isolated it more often in diarrhoea patients than in controls, under circumstances of profound immune suppression (Danna et al. 1991). It is not clear if the disease is invasive or non-invasive. Risk factors include low birth weight, prolonged antibiotic therapy and long hospitalization (Danna et al. 1991, Adam et al. 1991).

2.5.10 Human Immunodeficiency Virus

It is not clear if HIV infection alone is capable of causing diarrhoea although recent studies suggest that HIV may be a primary pathogen in the gut (Kotler 1989). Most patients with AIDS (50-98%) either present with, or later have diarrhoea that is life threatening. In Zaire, 84% of patients presenting with diarrhoea of one month's duration were found to be seropositive for HIV, and 40% of patients with AIDS presented with persistent diarrhoea (Colebunders et al. 1987). In several other studies, patients often present with persistent diarrhoea as one of the major signs of AIDS but no specific aetiological agent has been implicated (Datta 1989). Moreover, chronic diarrhoea, weight loss and malnutrition may also contribute to the overall immunodeficiency (Edwards et

al. 1990).

2.6 Mechanisms of diarrhoea

A clear understanding of the pathological behaviour and virulence factors of enteropathogens is important for the development of specific measures for treatment and prophylaxis of the diarrhoeal illnesses they cause. Some of the virulence factors are briefly discussed hereunder.

2.6.1 Toxin production

It has long been known that certain strains of E. coli can cause cholera-like acute diarrhoea in man by fluid accumulation in the lumen (Stephen et al. 1981). Further work has revealed that most enteropathogenic strains of E. coli produce a heat-labile enterotoxin (LT) and sometimes also a heat-stable enterotoxin (ST). Production of both toxins is mediated by transmissible plasmid.

The LT acts by stimulating adenylate cyclase activity and so, superficially resembles cholera toxin but the extent to which LT resembles cholera toxin is not fully understood. The LT is antigenically similar to cholera toxin. The ST is non-antigenic, has a very low molecular weight and has been found to be superficially similar to LT and cholera toxin, but it

causes diarrhoea by activating guanylate cyclase in the intestinal mucosa. Although enterotoxins of E. coli are very important factors in the production of enteropathogenic lesions, they are by no means the only factors in virulence.

2.6.2 Adhesion

Bacteria may adhere to epithelial surfaces by fibrils or pili to overcome mechanical clearance from the system by intestinal motility. Since adhesion of EPEC (as in V.cholera and ETEC) to the intestinal epithelium is essential for the development of enteric disease, the antibody mediated inhibition of bacterial adhesion will be one of the potential defence mechanisms of the host against the pathogens (Gaasta et al. 1982, Knutton et al. 1987). In a study by Cravioto, E. coli strains with localized adherence were significantly associated with acute non-bloody diarrhoea, aggregative adherence with persistent diarrhoea, and diffuse adherence with mixed etiological agents (Cravioto et al. 1991). Similar results were found by other workers in HIV infected children in Tanzania (Ciegelski et al. 1992).

The attachment of the protozoa Cryptosporidium parvum to the surface of the mucosa result in disruption of the microvilli. Heavy infection cause stunting, fusion and damage to, and degeneration of enterocytes. This leads to reduced ability to

digest and absorb food, resulting in profuse watery diarrhoea, the main clinical sign of the disease (Bird et al. 1980).

2.6.3 Invasive and non-invasive

In invasive pathogens, plasmid-encoded virulence marker antigen (VMA) is involved in mucosal penetration of epithelial cells of the gastrointestinal tract (GIT). Non-invasive bacteria will cause pathological changes without mucosal penetration.

It is certain that to cause dysentery, Shigella must penetrate and multiply within the epithelial cells of the colon (Northrup 1992, WHO Bull. 1987). This process leads to epithelial cell death, mucosal inflammation and epithelial ulceration and haemorrhage, which are the pathological hall marks of the disease (Northrup 1992). Little is known about the ways in which Shigella (which are acid sensitive) survive in the environment of the stomach.

Dysentery is characterized by frequent passage of loose stools with blood and mucus, and often is accompanied by fever, severe abdominal cramps and tenesmus (rectal pain following defecation). Shigella is not the only cause of bacillary dysentery, but is the most common. Other

aetiological agents include C. jejuni, EIEC and probably A. hydrophila and P. shigelloides.

Enteroinvasive E. coli have a pathological behaviour similar to that of Shigella by invading the epithelium (WHO Bull. 1980). A high molecular weight plasmid corresponds with virulence and is similar in both EIEC and Shigella (Harris et al. 1982).

2.6.4 Immunological reactions

It has been suggested that persistent diarrhoea syndrome may result from EPEC or EIEC which damage mucosal barrier allowing the passage of bacterial or dietary proteins through the mucosa, which may then initiate a hypersensitivity reaction, producing severe mucosal inflammation and thus further mucosal damage (Behrens 1991).

2.6.5 Unknown mechanisms

The exact mechanism by which G. lamblia produces its pathogenic effects are not clear. It has been shown to be associated with non-specific changes in the mucosal lining of the small bowel, and electron microscopic studies reveal the powerful sucking disc of the organism close to the microvilli. This probably leads to mechanical irritation and

damage of the microvilli, with subsequent malabsorption. The microvillus changes and resultant disaccharide deficiency revert to normal after eradication of the infections shown by clinical response of the children (Hoskins et al. 1967).

There have been suggestions that Rotavirus infection leads to production of immature crypt cells thus reducing the Na⁺, K⁺ ATPase which facilitates glucose absorption, but there is no evidence of toxin production. It is thought that vomiting may be due to a reflex arising from inflammatory changes in the jejunum and duodenum (T.H. Flewett - personal communication).

The frequent occurrence of profuse watery stools in many cases of campylobacteriosis suggest that an enterotoxin may be involved in pathogenesis (WHO/VPH 1984). On the other hand, the finding of dysenteric stools suggest that mucosal damage due to an invasive process analogous to that seen in shigellosis may be important in pathogenesis (WHO/VPH 1984).

Absence of leucocytes in Candida associated diarrhoea suggest non-invasive disease and so it may be secretory in origin but the mechanism is not clearly understood (Danna et al. 1991).

Human Immunodeficiency Virus infected patients present with diarrhoea but the mechanism is not known (Bicocchi et al. 1992). As the largest lymphoid organ in the body, the GIT is

a potential reservoir for HIV and it is an important site for HIV induced immunodeficiency. The resulting defects in cellular and humoral defense mechanisms pre-dispose the GIT to a spectrum of viral, fungal, bacterial and protozoan pathogens that cause relentless morbidity, in some cases, death (Bicocchi et al. 1992, Fauci 1988, Garcia-Rodriguez et al. 1991, Roilides et al. 1991).

2.7 Intestinal immune responses

It appears that immunity to diarrhoeal disease develops following previous infection as demonstrated by the different patterns of infection in countries where these diseases are endemic contrasted to those where only occasional epidemics occur. Thus there is a predominance of children being affected in the former and a much higher rate of infection in adults in the latter (Adam et al. 1991). It is not known if immunity to enteric infections is not solid but will break in the face of sufficiently high dose of infecting organisms.

The intestine is the largest immunological organ in the body, containing about half of the total number of lymphocytes, the majority of which are T-lymphocytes. Some of the mechanisms which may be involved in intestinal immune response are discussed below.

2.7.1 Humoral and cell-mediated immunity

The first line of defence, local immunity, consists of a combination of non-immunological (gastric juices, proteolysis, mucus coat) and immunological (secretory IgA, intraepithelial lymphocytes) factors in the gut lumen. These factors comprise an effective mucosal barrier against both invasive and non-invasive organisms, thus preventing infection and development of disease. If an infectious agent succeeds in penetrating the mucosal barrier, the second line of defence, systemic immunity, comes into play. This consists of cell-mediated immune response in which stimulated T-lymphocytes activate macrophages that eliminate intracellular organisms, or humoral immune response in which stimulated B-lymphocytes evoke antibody production that eliminate extracellular organisms by phagocytosis (Roitt 1988).

Many workers have investigated the immune status in healthy and malnourished children. Mucosal surfaces are bathed in secretions which have a host defence function to remove potential pathogens (Tomasi 1972). These host defence factors are found in intestinal secretions and maternal milk in the child's intestines. Specific host defence in secretions is provided primarily by immunoglobulins, mainly secretory IgA (sIgA) and to a lesser extent by IgG. It is thought that sIgA in secretions has an important host defence function because

of its ability to bind to pathogens, thus reducing the ability of the pathogens to attach to the mucosal cells. Subsequently, the rapid flow of the secretions washes away the antibody-pathogen complex.

Bacteria may adhere to epithelial surfaces by fibrils or pili which can be neutralized by antibody directed against them. One of the main effector pathways of antibody at secretory surfaces is the prevention of adherence of the organism (Williams et al. 1972). The ability of antibody to neutralize toxin has been demonstrated in several studies, and this may therefore play a part in providing immunity against infections with ETEC and Cholera in which the action of toxin play a central role (Rowley et al. 1986). It is possible that effector mechanisms involved must act at the surface of the gut or within its lumen. Studies have shown that IgA, the predominant immunoglobulin in the gut, which efficiently activate the alternative pathway of complement possesses protective activity against cholera in a baby mouse model (Steel et al. 1975).

Raised levels of faecal and pharyngeal IgA in gastrointestinal infections have been reported in several studies (Haneberg et al. 1975, Stals et al. 1984), but there is a controversy as to whether or not the high concentration can reduce the duration of diarrhoea (Stals et al. 1984,

Sonza et al. 1980, Riepenhoff-Talty et al. 1981). Haneberg and co-workers found that in prolonged diarrhoea, IgA levels were low or absent while IgM levels rose (Haneberg et al. 1975).

Studies suggest that Campylobacter immunity may be associated with mucosal IgA antibodies although patients with Campylobacter enteritis mount both an intestinal and systemic immune response to infection. Most patients had intestinal anti-C. jejuni IgA 1-5 days post onset with a peak titre 6-10 days then rapidly declined (Lane et al. 1987). This may have a potential as a rapid diagnostic for Campylobacter enteritis. In another study, there was a sharp increase of serum IgA antibody titre to Sh. flexneri 18 days post-infection (Bohemien et al. 1985). May be the specific IgA in serum stem from local production in the gut mucosa near the site of infection. This could point to a role for intestinal lymphocytes in protecting the host against infectious agents at mucosal level.

Sonza and co-workers found that anti-Rotavirus IgA, IgM and IgG in stools all reached peak titre between two and four weeks after infection then dropped back to undetectable levels after two months (Sonza et al. 1980). Rotavirus infection in the newborn, in contrast to the older infants and children is generally mild or asymptomatic and breast

feeding appear to play a protective role. It has also been suggested that the neonate may be protected by maternal antibody during the first few months of life (WHO/CDD 1988). In contrast, other workers have found that passively acquired serum antibodies do not protect the neonate against Rotavirus infection, as there was no correlation between the amount of virus excreted and cord blood Rotavirus antibody titre (Totterdel et al. 1980). Furthermore, prospective family studies have shown that protection among adults do not correlate with serum antibody levels (Wenman et al. 1979). Intestinal antibody responses may be important in preventing re-infection, but it is not known whether Rotavirus infection induces serum or local immune responses in newborn infants. The reasons for mild or asymptomatic infections among neonates remains unresolved. T-cell derived cytokines such as interferon and reactive oxygen intermediates liberated from primed macrophages may be active against Rotavirus (Roitt 1988).

The intestinal IgA response is of relatively short duration, lasting for a few weeks to a few months, but exhibits immunological memory. The response is greatest at the site of antigen exposure.

The demonstration that T-lymphocytes activate macrophages and thus eliminate typhoid bacilli which are otherwise capable of

multiplying within the macrophages provide an example of a T-lymphocyte effector mechanism (Ashcroft et al. 1967). It is likely that a similar strategy plays a role in eliminating other bacteria which invade and multiply within the wall of the gut. Other T-lymphocyte effector mechanisms such as cytotoxic killing of epithelial cells infected with virus may be important in specific forms of gastroenteritis. Human colostrum leucocytes have been reported to mediate both natural killer cytotoxicity and antibody dependent cellular cytotoxicity (ADCC) on Shigella organisms (Morgan et al. 1984). A study by Taglibue showed that intestinal lymphocytes could specifically co-operate with sIgA antibodies in ADCC reactions against Sh. flexneri / E. coli hybrid organisms (Taglibue et al. 1983). Intestinal IgA antibody responses may serve as a good marker for T-lymphocyte immunity in the intestines.

The main phagocytic cells are polymorphonuclear leucocytes (neutrophils) and macrophages (Roitt 1988). Organisms adhere to their surface, activate the engulfment process and are taken inside the cell where they fuse with cytoplasmic granules. This is followed by degranulation and release of proteolytic enzymes which are bactericidal. Finally, the killed organisms are digested by hydrolytic enzymes and degradation products released to the exterior. The complement system, a multicompetent triggered enzyme cascade, is used to

attract phagocytic cells to microbes. Presumably some organisms try to avoid undue provocation of phagocytic cells by adhering to, and colonizing the external mucosal surfaces of the body. Alternative pathway of complement can be stimulated by gram negative bacterial endotoxins and aggregated IgA, resulting in production of neutrophil chemotactic factors such as C5a and C3a. In some gut infections, the possible role of antibody immunity through stimulation of phagocytosis or through classical and alternative pathways of complement fixation is supported by the protection demonstrated following parenteral immunization with killed typhoid organisms (Ashcroft et al. 1967). In forms of gastroenteritis such as Salmonella, Campylobacter and EPEC infection in which there is invasion of the wall of the gut, these mechanisms could be called into play.

There is very little information on the role of non-protein (such as iron) deficiencies in phagocytosis. Kulapongs found that severe iron deficiency anaemia reduced bacterial killing capacity of neutrophils in only one of the eight Thai children (Kulapongs et al. 1974). Protein deprivation has been reported to enhance some viral infections. Coxsackie B virus infection in mice receiving low protein diets after weaning was increased as seen by more severe lesions and prolonged viral persistence.

2.7.2 Acquired immunodeficiency syndrome

Laboratory diagnosis in the absence of AIDS defining symptoms has been complicated by the persistence of passively acquired maternal antibody in infants up to 15 months of life (CDC 1987). Progression of immunological abnormalities related to HIV-1 infection in adults is well known, but has been rarely studied in children.

CD4 lymphocytes play an important role in the immune system by providing help for antibody production, co-ordinating immune response and specifically lysing virus infected target cells bearing type II histocompatibility antigens (Davis et al. 1980). Since the beginning of AIDS epidemic, progressive depletion of CD4 lymphocytes has been recognized as a central event in the pathogenesis and progression of this disease, probably leading to persistent diarrhoea in AIDS patients (Smith et al. 1992, Datta 1989, Garcia-Rodriguez et al. 1991, Roilides et al. 1991, Blanche et al. 1989). Patients with AIDS and diarrhoea have reduced CD4 counts suggesting that they have a greater degree of immunosuppression than those who do not develop diarrhoea (Smith et al. 1992). CD8 lymphocytes are cytotoxic and suppress the functions of CD4 lymphocytes. Studies have shown that in persistent diarrhoea, there was extensive lymphocyte infiltration, especially the CD8 phenotype, suggesting that intestinal damage occurs as a

result of local cell mediated reactions (Lunn et al. 1991). Other studies suggest that vulnerability to intestinal infections in AIDS is related more to local immune deficiency ✓ than to malnutrition (Reka et al. 1991).

T-lymphocyte abnormality appear to be accompanied by B-lymphocyte abnormality which manifests in AIDS patients as reduced IgA plasma cells in the mucosa, with or without gastrointestinal disease, but which may lead to the appearance of opportunistic infections (Smith et al. 1992, Trajman et al. 1992).

2.7.3 Malnutrition

There is evidence to show that immune response is suppressed in malnutrition but the exact mechanism is not entirely clear (Schrimshaw et al. 1968, Chandra 1983). Recent studies have indicated that increased frequency and severity, of infections in malnourished populations may have an immunological basis (Tomkins 1981, Cunningham-Rudles, 1982, Woodruff et al. 1970). The mucosal surface of children and animals with protein energy malnutrition are particularly prone to infections (Watson et al. 1980). Phagocytic activity is reportedly diminished (Watson et al. 1980, Chandra et al. 1972). The release of neutrophils from the spleen or bone marrow by either adrenalin or bacterial polysaccharide is

reduced in severely malnourished children (Chandra 1976). Chandra found reduced frequency of rosette forming thymus dependent T-lymphocytes in peripheral blood of malnourished infants and children (Chandra 1974). This may affect the T- and B-cell co-operation in local immunity.

Diarrhoea in malnourished children is associated with high mortality, especially in HIV infected children (Oburra 1986, Luta et al. 1989). Measles and poliovirus IgA was significantly reduced in nasopharyngeal secretions of malnourished patients (Chandra 1975). The impaired secretory antibody induction after immunization suggest that immunity after infections such as measles and gastroenteritis may be inadequate in malnourished children. Recovery would be slower and incomplete, which would permit severer illness. This may account for the increased susceptibility of malnourished patients to frequent and severe infections.

Whether persistence of diarrhoea and some aetiological agents in malnourished children is due to reduced immunoglobulin levels in the gastrointestinal tract or T-lymphocytes is yet to be established. The present study aims to determine the role of intestinal immunoglobulins and T-lymphocytes in prevention from, or limitation of gastrointestinal infections in malnourished children. The results of this study are likely to give a better understanding of the host resistance

to intestinal infections.

2.8 Prevention and control

Diarrhoea is spread mainly by the faecal-oral route, with food and water as the main sources of infection. The importance of personal and food hygiene, effective excreta disposal and safe water supply in preventing diarrhoea and other intestinal infections need to be constantly emphasized, as does the protective effect of breast feeding, which fortunately is widely prevalent in the African region (Ketsela et al. 1990, Cunningham et al. 1991, Feacham 1984^a, Feacham et al. 1984^b, Feacham 1986, Baltazar et al. 1993). In developing countries, there are still many misconceptions and beliefs on supernatural causation of diarrhoea among the rural inhabitants. In Kenya diarrhoea is believed to be a "God's disease" (Mania et al. 1979), thus making it very difficult to control through proper hygienic practices. Similar beliefs were observed in Ethiopia and Bangladesh (Bimal et al. 1988, Sahid et al. 1983). Sociological studies on the habits and attitudes of people towards diarrhoeal disease may help find adequate and acceptable control measures especially where vaccination holds no solution (Mutanda 1980^a). Effective control may eventually be achieved through use of vaccines.

The cornerstone for treatment of diarrhoeal illness is the replacement of lost fluid and electrolytes (Kinoti et al. 1985). Not only is this solution life saving in severe diarrhoea in which the logistics of intravenous fluids are difficult, but it is also less painful, safer and less costly. In addition to oral rehydration therapy, one should consider specific antimicrobial therapy for symptomatic patients with inflammatory or parasitic diarrhoeal. The increasing resistance of enteric pathogens to commonly used antimicrobial agents continues to narrow the range of available effective agents (Wamola et al. 1981, Kakai et al. 1987, Mutanda et al. 1987^a, Kunin 1993, Kariuki et al. 1993).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study population and selection criteria

The first three children aged 0-5 years, who presented to Kenyatta National Hospital (KNH) - Paediatric Observation Ward (POW) and Pumwani Maternity Hospital (PMH) - Paediatric AIDS clinic with acute or chronic diarrhoea from Monday to Friday were included in the study. After verbal consent from the mothers the children were enrolled into the study. Mothers were interviewed using a pre-tested standard questionnaire (Appendix) on demographic information, medical history including current duration of diarrhoea and mode of feeding. Children who had received oral rehydration or antibiotic therapy were excluded. Stools were obtained from the children by inserting a cotton wool swab in the anus of the child and tapping the faeces in a plastic polypot. Blood was collected from children by venipuncture into heparinized tubes for phagocytosis test, EDTA tubes for T-lymphocyte enumeration and into plain tubes for HIV serology. The specimens were forwarded to the laboratory and processed within 3 hours of collection. Children were classified according to their nutritional status following the Wellcome

criteria (Editorial 1970). The following categories of children were enrolled into the study:

- a. Diarrhoea alone - well nourished children (control).
- b. Marasmus - children less than 60% of the expected weight for age without oedema.
- c. Kwashiorkor - children 60-80% of expected weight for age with oedema.
- d. Marasmic/kwashiorkor - children less than 60% of expected weight for age with oedema.

3.1.1 Sample size

Prevalence (p) of diarrhoea at KNH, Paediatric Observation Ward (from clinic records) is 3%. If an error of 10% is allowed in achieving this rate, Z_a is a standard normal deviate of detecting a significant difference (a) and Z_b is the normal deviate to the one tailed test, then the sample size of the group to be included in the cases and controls is:

$$n = \frac{2(Z_a + Z_b)^2 (p) (1-p)}{(a)^2}$$

$$n = \frac{2(1.96 + 1.64)^2 (0.03) (0.97)}{(0.05)^2} = 302$$

3.2 Processing of stool for laboratory investigation

Wet preparation of fresh stools were made in normal saline and Lugol's iodine solution and examined by direct microscopy for pus, red blood cells, ova and cysts of intestinal parasites. For detection of Cryptosporidium oocysts, thin slide smears were stained using a modified Ziehl-Neelson method (Henriksen et al. 1981). Briefly, the smears were air dried, fixed in methanol for 2-5 min followed by further fixation in formalin vapour at 37°C for 20 min. These were stained with carbol fuchsin for 5-10 min, differentiated in 3% acid alcohol then counter stained with 0.25% malachite green for 0.5 min. With this stain, Cryptosporidium oocysts appear as bright pink spherules on a pale green background when examined under oil immersion with a light microscope.

Methods described by Cowan and Steel (Cowan et al. 1979) were used for isolation and identification of enterobacteriaceae. The specimens were inoculated onto MacConkey agar, selenite broth and Salmonella-Shigella agar (SS) for isolation of E. coli, Salmonella and Shigella respectively. These were incubated at 37°C for 24 hours. Lactose fermenting colonies suspected for E. coli were identified by slide agglutination using specific antisera (Wellcome). Non lactose fermenting colonies growing on SS agar were inoculated onto triple sugar iron (TSI) agar slants and incubated at 37°C overnight for the

identification of Salmonella and Shigella by slide agglutination using specific antisera (Wellcome).

Blaser-Wang, a selective supplement (Oxoid, code SR 98) in Oxoid blood agar base with 7% lysed horse blood was inoculated for isolation of Campylobacter (Blaser et al. 1978). This was incubated at 42°C for 48 hours in a candle jar. Small, grey or swarming colonies suspected for Campylobacter were confirmed by basic fuchsin stain. Campylobacter appear as curved gram negative rods.

A portion of the faecal specimens were diluted to 10% suspensions in phosphate buffered saline (PBS), centrifuged for 10 min at 1500 x g and the supernatant used in ELISA test for Rotavirus antigen detection (WHO kit).

The remaining stool specimens were diluted to 50% suspensions in PBS, centrifuged for 10 min at 1500 x g and the supernatant used for total and specific IgA determination.

3.3 Enteroadhesive E. coli (EAEC) determination

Tests to determine the ability of E. coli to adhere to monolayer of Hep-2 cells was done as follows (Cravioto et al. 1991). Monolayer of Hep-2 cells were grown for 2-3 days on coverslips to form a confluent layer. The test strain of E.

coli was grown overnight at 37°C in 5 ml peptone water with added D-mannose (1% W/V), from which 0.5 ml (10^5 cells/ml) was added to each monolayer dish and incubated at 37°C for 3 hours. The monolayer were washed three times with Hank's balanced salt solution (HBSS) and fixed in methanol for 5-10 min. The coverslips were stained with Giemsa stain (10% w/v PBS) for 30 min and washed twice in PBS. These were examined under light microscope at power 40 magnification.

E. coli strains were positive for Hep-2 cell adhesion when there were at least 10 attached bacteria per cell.

3.4 Heat labile enterotoxigenic E. coli (LT)

This was done using a co-agglutination technique which utilizes the binding of fragment crystallisable (Fc) part of the IgG molecule to protein A of Staphylococcus aureus (Christensen et al. 1973, Kronvall 1973).

3.4.1 Preparation of rabbit anti-LT

Methods described by Cruickshank were used (Cruickshank 1970). To detoxicate LT, 0.3 ml of 5% formalin was added to 2 mg of LT powder (Biken, Japan) to make up to 2 units per ml and incubated at 37°C for 2 weeks. A six week old rabbit was inoculated intramuscularly with 0.2, 0.4, 0.6, 0.8 and 1 ml

of 0.5 units per ml detoxicated LT on days 1, 3, 5, 7 and 9 respectively. On day 12 and 15, 0.2 ml of detoxicated LT with added 0.01% magnesium sulphate adjuvant was inoculated into the rabbit intravenously. Ten millilitres of blood was drawn from the rabbit on day 18 and the serum used in the coagglutination test.

3.4.2 Growth and stabilization of Staph. aureus

Staph. aureus Cowan I strain was grown in trypticase soy broth overnight at 37°C and centrifuged at 1500 x g at 4°C for 15 min. The pellet was washed 3 times in PBS by centrifugation as above, resuspended in 0.5% formaldehyde solution and kept at room temperature for 3 hours. The washing procedure was repeated 3 times, the pellet resuspended in 10% (V/V) PBS and heated at 80°C for 10 min. Finally, the cells were washed twice with PBS and resuspended in 10% PBS. Bacteria prepared and stabilized in this way can be stored at 4°C for 2 weeks.

3.4.3 Coating

To 2 ml of formaldehyde and heat stabilized staphylococci was added slowly to 0.1 ml of anti-LT with constant shaking for 3-5 min at room temperature. After mixing the suspension, staphylococci were washed twice as before and suspended to 1%

in PBS containing 0.1% sodium azide. The suspension is stable for months when stored at 4°C.

3.4.4 Co-agglutination test

A few colonies of the test strain of E. coli was emulsified in a drop of 0.05% triton X-100 on a glass slide and a drop of 1% anti-LT coated Cowan Staphylococci aureus added. A known positive and negative control sample were included. The strain was positive when agglutination occurred.

3.5 Rotavirus antigen detection

A Rotavirus ELISA kit (WHO reference laboratory, Birmingham, UK) was used following the instructions provided. Briefly, wells of flat-bottom microtitre plates were coated with 100 ul of anti-Rotavirus and incubated at room temperature for 1 hour. After adsorption, the plates were washed 5 times in PBS containing 0.5% Tween 20 (PBS-T). A volume of 100 ul, 1:10 suspension of stool was added to the wells and incubated at room temperature for 1 hour before washing in PBS-T. To positive and negative control wells was added Rotavirus antigen and PBS respectively. Rabbit anti-human Rotavirus conjugate was added to all the wells and incubated at room temperature for 1 hour. Finally, the plates were washed 5 times, enzyme substrate added and incubated at room

temperature for 15 min. The reaction was terminated by addition of 2 M sulphuric acid. The samples were considered positive when there was a colour change in the well by the naked eye and negative when there was no colour change.

3.6 HIV-1 determination

An ELISA (Organon Teknika, Belgium) test was used to screen for presence of HIV-1 antibody in patient's serum. Double ELISA positive samples were confirmed by Behring ELISA (Behring Enzygnost, Germany) or Western blot (Biotech/Dupont kit).

3.6.1 Screening ELISA (Organon) test

This is a screening test for detection of HIV. Microelisa wells were coated with recombinant HIV-1 antigen. Undiluted test serum was added to microtitre wells and incubated at 37°C for 1 hour, aspirated and washed 4 times in buffer. Positive and negative controls were included. Peroxidase conjugated goat anti-human immunoglobulin was added to the wells and incubated at 37°C for 30 min then washed 4 times. ABTS substrate was added to each well, incubated at room temperature for 10 min and the reaction stopped by a fluoride solution. The results were read by a spectrophotometer (Organon teknika microwell system, Reader 510, Belgium) at a

wavelength of 450 nm.

3.6.2 Behring ELISA

This was used in an enzyme immunoassay for detection of antibodies to HIV-1, following instructions provided by the manufacturer. Briefly, each microtitre well pre-coated with synthetic HIV-1 peptides was washed with wash solution followed by addition of test serum sample. Positive and negative controls were included. These were incubated at 37°C for 30 min and washed 5 times. Anti-human IgG peroxidase conjugate was added and incubated at 37°C for 30 min. After washing, substrate was added then incubated at room temperature in the dark. The enzyme reaction was terminated by the addition of 1 N sulphuric acid. The results were read by a spectrophotometer (Organon teknika microwell system, reader 510, Belgium) at a wavelength of 450 nm.

3.6.3 Western blot

This was used as a confirmatory test for HIV infection. The procedures described by the manufacturer was followed. Briefly, nitrocellulose strips (Biotech/ Dupont kit), pre-coated with HIV-1 proteins, were incubated with 100 ul serum specimens. The strips were washed to remove unbound material, reacted with 100 ul goat anti-human IgG conjugated with horse

radish peroxidase (HRP), followed by HRP substrate. Samples were considered positive for antibody to HIV-1 if any two or more of the following bands were present: p 24, gp 41 and gp 120/160. Samples were considered negative if no bands appeared. Samples were indeterminate if only p 24 band was present.

3.7 Specific immunoglobulin A determination

Specific IgA to Rotavirus and bacteria were determined by using an ELISA technique with rabbit anti-human IgA glucose oxidase conjugate as described below.

3.7.1 Preparation of anti-human IgA conjugate

An IgG fraction of a rabbit anti-human IgA serum (code 105R-Medical and Biological Laboratories, Japan) was concentrated by ultra-filtration using a DIAFLO pm 30 millipore filter with a cut off point of 30,000 daltons. Optical density (OD) of the concentrated rabbit anti-human IgA was read on a spectrophotometer (Beckman model 25, USA) at 280 nm wavelength. The amount of rabbit IgG fraction was estimated using the formula given below:

$$E 1\% = 13.5 \text{ at } 280 \text{ nm}$$

The procedure adopted for conjugation was that of Wilson and

Nakane (Wilson et al. 1978, Nakane et al. 1974). A conjugation efficiency of 80% was assumed resulting in a 1:1 molar ratio of IgG to glucose oxidase. Hence 50 mg glucose oxidase enzyme (Type VII, Sigma chemical Co. St. Louis, USA) was dissolved in 9 ml distilled water. To this solution of glucose oxidase was added 1 ml of freshly prepared 0.15 M sodium periodate solution (32 mg periodate per ml distilled water). The resulting mixture was kept at room temperature in the dark for 30 min under slow magnetic stirring. This was dialysed against 200 ml of 1 mM acetic acid buffer, pH 4 at 4°C with buffer changes at 10 min intervals; the first two changes dialysing buffer contained 0.1% ethylene glycol, followed by further four changes. This was then centrifuged at 1500 x g for 15 min, and the pH of the supernate adjusted to 8 using 0.2 M sodium carbonate.

Five millilitres of IgG fraction equivalent to 50 mg IgG was pipetted into a glass beaker followed by addition of 2 ml of 0.2 M carbonate buffer pH 9, under magnetic stirring. Then 5 ml of (50 mg) dialysed glucose oxidase solution was slowly added to IgG solution in carbonate buffer and kept in the dark room for 2 hours at room temperature. After adjusting the pH to 7.5 using 1 mM hydrochloric acid (HCL), conjugation was allowed to continue at 4°C overnight. Fourty milligrammes of lysine was added to the IgG-glucose oxidase conjugate and further conjugation was allowed to proceed at room

temperature under magnetic stirring for 2 hours. After centrifugation at 1500 x g for 15 min, 1 ml normal rabbit serum and 5 ml glycerol were added to 4 ml of the supernatant and stored at -20°C in 1 ml aliquots. The optimal dilution for use was determined by ELISA checkerboard titration.

3.7.2 Preparation of rotavirus antigen

Rotavirus antigen for ELISA test was prepared from a pool of 15 ml faecal samples positive for Rotavirus antigen and purified by the method of Qifang and co-workers (Qifang et al. 1980). Briefly, 5 gm of semi-solid faecal samples were diluted to 15 ml with PBS, homogenized and centrifuged at 1500 x g for 10 min. One percent (W/V) of nonidet P-40 was added to the supernatant and kept at 4°C overnight before centrifuging at 2500 x g for 30 min. An 8% (w/v) polyethylene glycol-6000 was added and kept at 4°C overnight. After centrifugation at 2500 x g for 30 min, the pellet was resuspended in 2 ml PBS, carefully layered on 3 ml of 45% sucrose solution and centrifuged at 24,000 x g for 90 min. The pellet was resuspended in 5 ml PBS to remove residual sucrose and centrifuged at 24,000 x g for 1 hour. The pellet was suspended in PBS containing 0.1% sodium azide and used in ELISA test.

3.7.3 Rotavirus specific IgA determination

Methods described by Yolken and co-workers were followed (Yolken et al. 1978). Briefly, wells of flat-bottom microtitre plates (NUNC) were coated with rabbit anti-human Rotavirus (WHO kit) in 0.05 M carbonate bicarbonate buffer (pH 9.6), incubated at 4°C overnight and washed 5 times in PBS-T. Rotavirus antigen at a dilution of 1:40 was added to the wells and incubated at 37°C for 1 hour then washed as before. Faecal samples diluted to 1:40 in PBS-T containing 1% bovine serum albumin (BSA) was added to each well and incubated at 37°C for 1 hour. After washing, 1:300 dilution of glucose oxidase rabbit anti-human IgA conjugate was added to each well and incubated at 37°C for 1 hour. Finally, the plates were washed, ABTS enzyme substrate added and incubated at room temperature for 1 hour. The reaction was stopped by addition of 1 M HCL. The results were determined by reading OD using a 590 mini reader (Dynatech) at 410 nm. The results were expressed as positive/negative (P/N) ratio. Positive samples were titrated and the titre expressed from a cut off point obtained from regression line of negative controls.

3.7.4 Preparation of bacteria antigens

The antigens for ELISA procedure were prepared from cultures of the bacteria (S. typhimurium, EPEC 0111:K58, EPEC

086A:K61, and Sh. flexneri 2) in tryptic soy broth at 37°C overnight (Goodman et al. 1981). The culture was washed twice in 0.05 M carbonate buffer (pH 9.6) and resuspended in 10 ml carbonate buffer then incubated at 100°C for 20 min to remove flagella (H) and virulence (Vi) antigens. The suspension was placed in an ice bath and sonicated (Bronwill Scientific Inc., New York). The suspension was then centrifuged at 12,000 x g for 30 min, and the supernatant was stored in 2 ml aliquots at -20°C and used for specific IgA detection. Optimal dilution for use was determined by ELISA checkerboard titration.

3.7.5 Determination of specific IgA

The procedure described by Goodman and co workers were followed (Goodman et al. 1981). Wells of flat-bottomed microtitre plates (NUNC) were coated with an appropriate bacterial antigen (S. typhimurium and others) in 0.05 M carbonate-bicarbonate buffer (pH 9.6) at 4°C overnight. After washing, faecal samples diluted to 1:40 in PBS-T and 1% BSA was added to each well and incubated at 37°C for 1 hour. Positive and negative controls were included. After washing, 1:300 dilution of glucose oxidase rabbit anti-human IgA conjugate was added to each well and incubated at 37°C for 1 hour. Finally, the plates were washed, ABTS enzyme substrate added and incubated at room temperature for 1 hour. The

reaction was stopped by addition of 1 M HCL. Results were determined by reading OD using a 590 mini reader (Dynatech) at 410 nm. The results were expressed as positive/negative (P/N) ratio. Positive samples were titrated and the titre expressed from a cut off point obtained from regression line of negative controls.

3.8 Total IgA determination

3.8.1 Single radial immunodifusion

Total IgA levels were quantified by single radial immunodifusion (SRID) technique using monospecific rabbit anti-human IgA antisera (Mancini et al. 1965). In this study, 5% rabbit anti-human IgA, alpha chain specific antiserum (Jackson Immuno Research laboratories) was incorporated into 1.2% agar on a glass slide. Two millimetre diameter wells, 12 mm apart were punched into the agar. Five microlitres of 100%, 50%, 25% and 12.5% of standard human colostrum IgA control (Sigma Chemical Company, USA) and 5 ul of stool suspension were added to the wells and incubated in a moist chamber at 4°C overnight. Diameter of the precipitin rings were measured and IgA levels in stools estimated from the standard calibration curve.

3.8.2 Staining precipitin lines

For permanent records, the slides were dried, stained with ✓
coomasie brilliant blue.

3.9 T-lymphocyte determination

Peripheral blood samples were collected in EDTA tubes. Lymphocyte subsets were determined using standard techniques for simultaneous, direct, two-colour immunofluorescence staining of whole blood. Commercially available murine anti-human antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) dyes (Beckton-Dickinson immunocytometer systems, San Jose, Carlifonia) including isotype controls, CD4 (Leu-3a FITC) and CD8 (Leu-2a PE) helper and suppressor lymphocytes were used. Briefly, 10 ul of monoclonal antibody was added to 100 ul whole blood and incubated at room temperature for 30 min. After centrifugation, red blood cell were lysed and cells washed twice with isoton. The cells were fixed with 1% buffered para-formaldehyde prior to T-cell subset enumeration by flow cytometry.

The percentage of fluorescently labelled cells were determined by multiparameter analysis using a fluorescent activated cell scanner (FACScan) flow cytometer (Beckton-

Dickinson immunocytometry systems, San Jose, California). Results were expressed as the percentage of cells positive for each phenotype. Absolute subset counts were obtained as the product of the total lymphocyte count and the percentage of lymphocyte subset population of interest.

3.10 Phagocytosis of killed *Candida albicans*

3.10.1 Preparation of *C. albicans*

C. albicans were grown in 2% sabouraud dextrose broth for 48 hours at 37°C to obtain the organisms in the yeast phase. This was centrifuged at 350 x g for 10 min, the deposit washed twice in PBS and the concentration adjusted to 5×10^6 cells/ml in a Naubauer chamber. The test cells were heat killed at 100°C for 30 min in a water bath and stored in batches of 5 ml at -20°C until needed.

3.10.2 Phagocytosis test

Five millilitres of RPMI 1640 was added to 0.5 ml of heparinized patient's blood and centrifuged at 600 x g for 10 min. The pellet was mixed with 0.5 ml heat killed *C. albicans* and incubated at 37°C for 1 hour then centrifuged at 600 x g for 10 min. Thin slide smears were prepared from the pellet and stained by May Grunwald-Giemsa stain. Slides were fixed

in methanol for 2-3 min, stained with May-Grunwald for 10 min followed by Giemsa stain for 10 min. They were washed in tap water, air dried and examined under oil immersion using a light microscope. Two hundred neutrophils were counted and the phagocytic index (PI) calculated as follows:

$$\text{PI} = \frac{\text{Number of yeast cells per neutrophil}}{\text{neutrophils engaged in phagocytosis}} \times 100$$

3.11 Statistical analysis

All information obtained was stored in a filemanager data file in a computer (unix system). Data analysis was done using a computer software for statistical analysis (SPSS-PC, EPISTAT). Statistical results were obtained using n x n contingency tables with Yate's corrected Chi-square and unpaired student-t-test. Graphical presentations were done by using Havard Graphics version 3.0 computer programme.

Chi-square statistic

Chi-square tests were used to find associations where data were dichotomous (either or) or multichotomous (two or more classifications). Data were presented as the number of subjects who exhibited an attribute in the control and test subjects. In numerical discrete data, when observations were

integers which correspond to a count histograms were used. A chi-square statistic provided a test to the hypothesis that the proportion of subjects with a certain attribute in the control and test groups was same.

Frequency distribution

Frequency distribution was done for both discrete and continuous variables to summarize and present data. It consisted of a series of pre-determined class intervals together with counts of the number of observations whose values fell within the intervals for each class. For the histograms, the ordinates began at zero and the height of each bar pertained percentage of subjects in that class.

Student's t test

The null hypothesis in this study was that the sample means of both the control and test subjects were equal. It was rejected if the observed significance level was <0.05 . The data were given as mean and standard deviation of observations. Two tailed tests were used to detect the difference in the means between test and control subjects regardless of the direction of the difference. Inference was that the true population could either be above or below null hypothesis.

CHAPTER FOUR

RESULTS**4.1 Demographic information**

Demographic characteristics of the population are summarized in Table 1. A total of 556 children were recruited, 51% (284/556) of whom were males and 49% (272/556) females giving male:female ratio of 1:1. The mean age for the entire population was 12.8 (SD 14.8) months and ranged from 5 days to 60 months. Sixty nine percent of the children were well nourished, 29% were malnourished and nutritional status was unknown in 2%. Children with kwashiorkor had a higher mean age than those in other nutritional categories (18.7 vs 9.1, $p < 0.001$). Most of the malnourished children presented with diarrhoea (26.4% vs 4.7) compared to those with normal nutrition (33.5% vs 35.5%), $p = 0.001$. The mean age for malnourished children with diarrhoea was lower than those without diarrhoea (12.0 ± 9.7 vs 25.5 ± 15.4 months, $p < 0.002$). Mean age of well nourished children was similar in those with diarrhoea and those without (10.1 ± 11.8 vs 15.4 ± 18.3 months, $p = 0.3$, NS).

Table 1. Distribution of nutritional status by age (months) and sex.

Nutritional status	Mean age	M (%) n=284	F (%) n=272	Total (%) n=556
Normal	12.8 ± 15.7	34.7	34.0	68.7
Marasmus	9.1 ± 8.2	5.0	4.9	9.9
Kwashiorkor	18.7 ± 13.7	7.6	7.1	14.7
Maras/kwash	11.3 ± 5.5	2.9	1.8	4.7
Not recorded	12.1 ± 11.4	0.9	1.1	2.0

Key: M= male, F= female, normal= well nourished children, marasmus= less than 60% of expected weight for age without oedema, kwashiorkor= 60-80% of expected weight for aged with oedema, Maras/kwash= less than 60% of expected weight for age with oedema.

Twenty seven percent (73/271) of the children were born to HIV seropositive mothers, sixteen percent (44/271) of whom had diarrhoea.

4.2 Aetiology of diarrhoea

4.2.1 Aetiological agents

Aetiological agents isolated depended on nutritional state of the children (Table 2). Enteropathogenic E. coli was more significantly isolated from normal children, (30.1% vs 14%, $p < 0.05$), while Campylobacter either alone, in combination with Rotavirus or with EPEC-LT ($p = 0.02$) were more common in malnourished children with diarrhoea. Rotavirus, Salmonella and Shigella were equally isolated from both normal and malnourished children. Cryptosporidium oocyst were not detected in any of the patients.

Intestinal parasites either alone or in combination with bacteria were responsible for diarrhoea in a small proportion (8.9%) of children. Ascaris lumbricoides (1.3%) and G. lamblia (0.6%) were found in normal children while T. hominis either alone (4.7%), or in combination with Rotavirus and G. lamblia (2.3%) were found in malnourished children. It appears that T. hominis was an opportunistic infection in immunosuppression due to malnutrition.

Table 2. Enteric pathogens isolated from normal and malnourished children.

Pathogen	Nutritional status:				p
	Normal (%) n=154	Maras (%) n=50	Kwash (%) n=43	M/Kwash (%) n=26	
Viral					
Rota	14.7	6.0	4.7	16.0	.1
Bacterial					
EPEC	30.1	6.0	14.0	12.0	<.05*
Shig	2.5	2.0	2.3	0	.8
Camp	0	4.0	4.7	8.0	.02*
ETEC-LT	0.1	6.0	2.3	8.0	.06
Salm	1.9	6.0	4.7	0	.3
Parasitic					
T.homin	0	2.0	4.7	4.0	.6
Ascaris	1.3	0	0	0	.6
G.lamb	0.6	0	0	0	.8
Mixed aetiology					
Rota + ETEC-LT	1.3	0	0	0	.7
Rota + salm	0.1	0	0	0	.8
Rota + Camp	0	0	0	4.0	.02*
Rota + EPEC	4.5	2.0	4.7	4.0	.8
Shig + EPEC	1.3	0	0	0	.6
Shig + ETEC-LT	0.1	0	0	0	.8
Salm + ETEC-LT	7.7	2.0	0	4.0	.2
Camp + EPEC	1.3	0	0	0	.6
Camp + ETEC-LT	0	0	0	4	.02*
EPEC + ETEC-LT	0.6	6.0	2.3	0	.08
EPEC + E.histo	0	0	2.3	0	.1
EPEC + G.lamb	0.6	0	0	0	.8
Rota + LT + EPEC	0.6	0	0	0	.8
Rota + Salm + EPEC	0.6	0	0	0	.8
Rota + Camp + EPEC	0.6	0	0	0	.8
Rota+G.lamb+T.homin	0	2.0	0	0	.2
Salm + Shig + EPEC	0	2.0	0	0	.2
Unknown	26.3	54.0	53.5	40.0	<.03*

Key: * = significant isolation rate in normal vs malnourished children by Chi square test, Rota = Rotavirus, EPEC = Enteropathogenic E. coli, Shig = Shigella, Camp = Campylobacter, ETEC-LT= Heat labile enterotoxigenic E. coli, Salm = Salmonella, T.homin = Trichomonas hominis, G.lamb = Giardia lamblia, E.histo = Entamoeba histolytica, 0 = No aetiological agents isolated, Normal = Normal nutritional status, Maras = Marasmus, Kwash = Kwashiorkor, M/Kwash = Marasmus Kwashiorkor.

There were few well nourished children with diarrhoea of unknown aetiology than malnourished children (26.3% vs 47.8%, $p = 0.03$). May be diarrhoea in the latter group was caused by aetiological agents that were not determined in this study.

4.2.2 Aetiological agents and intestinal IgA

The presence of specific IgA was inversely correlated with isolation of aetiological agents. In some instances, there was complete absence of aetiological agent in the presence of specific antibody probably indicating previous infection (Table 3). Except in 2 cases with Rotavirus, detection of aetiological agents was associated with absence of specific antibody. This is an indication that once specific IgA to a certain pathogen has been formed, it may protect against future infections with that particular pathogen but not against other enteric pathogens. This is further supported by results in Table 4 in which children with specific IgA to Rotavirus (23.0 vs 2.0, $p = 0.05$) and Sh. flexneri 2 (16.0 vs 0.0, $p = 0.04$) did not have respective pathogens in their stool.

Table 3. Comparison of enteric pathogens with intestinal specific IgA in children presenting with diarrhoea.

Pathogens isolated	% of children with specific IgA to:				
	Rota n=23	Shig.2 n=16	O111:K n=26	O86A:K n=10	S.typhim n=14
Rotavirus	8.7	6.3	23.1	10.0	28.6
S. typhim	4.3	-	7.7	-	0.0
Shig.2	4.3	0.0	-	-	-
EPEC	17.4	37.5	15.4*	30.0**	28.6
EPEC-LT	13.0	6.3	15.4	-	-
Salm+EPEC	-	12.5	-	10.0	7.1
None	52.2	37.5	38.5	50.0	35.7

Key: - = Not tested, 0 = No antibody detected, EPEC = enteropathogenic E. coli, O111:K = EPEC serotype O111:K58, O86A:K = EPEC serotype O86A:K61, * = children with EPEC of serotypes other than EPEC O111:K58, ** = children with EPEC of serotypes other than EPEC O86A:K61, Shig.2 = Shigella flexneri 2.

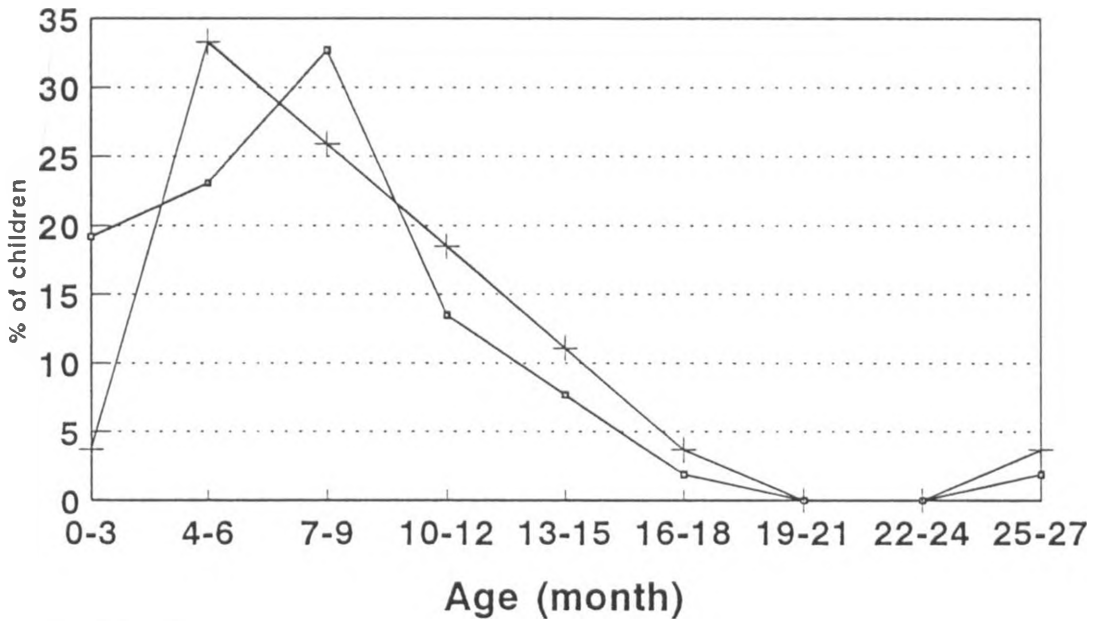
Table 4. Comparison of the presence of specific IgA and pathogen.

Pathogen	Number of children with:			P-value
	Path only n=75	IgA** only n=89	IgA and Pathogen n=2	
Rota	43	23	2	<0.05*
Shig.2	4	16	0	0.04*
O111:K58	9	26	0	0.07
O86A:K61	9	10	0	0.8
S. typhim	10	14	0	0.7

Key: 0 = No antigen or specific antibody detected, * = Significant by Chi square test, ** = some children had antibody to more than one pathogen, Path = pathogen.

There was an increase in Rotavirus specific antibody with age (Fig. 1). Rotavirus was detected from a large proportion of children aged less than three months who presented with diarrhoea (19.2%). This correlated to the low levels of Rotavirus specific IgA (3.7%) in the intestinal tract. The prevalence of Rotavirus detection in children less than 3 months old could probably also be due to immaturity of immune system and/or low levels of maternal antibody transfer. Children aged between 4-6 months seem to develop their own immunity against Rotavirus which is reflected in a decline in virus isolation (23.1%) and rapid increase in intestinal IgA (33.3%).

Fig. 1
Distribution of rotavirus and anti-rotavirus IgA by age.



KEY: \square Rotavirus n=52, \times anti-rotavirus n=27

4.3 Diarrhoea and malnutrition.

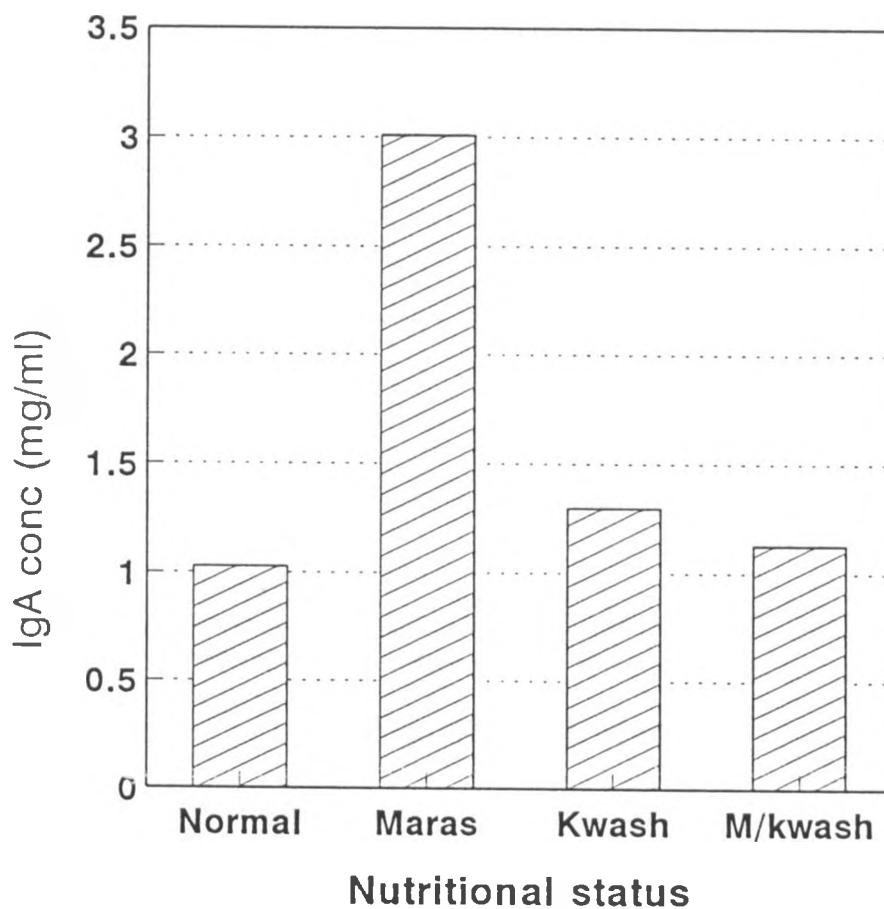
4.3.1 Morbidity and mortality

More than half (64.4%, 358/556) of the children presented with diarrhoea, with a mortality rate of 3.2% (9/285). Fifty five percent (5/9) of the children who died, mainly due to salmonellosis 44.4% (4/9), were malnourished.

4.3.2 Intestinal total and specific IgA antibodies

Total IgA varied with nutritional state of the children. Results in Figure 2 show that children with marasmus had significantly higher levels of total IgA (3.0 mg/ml) than those with normal nutritional status (1.0 mg/ml), kwashiorkor (1.3 mg/ml) or marasmic/kwashiorkor (1.1 mg/ml), $p = 0.01$. It is probable that children with marasmus develop high levels of intestinal total IgA due to repeated infections. In contrast, specific IgA titres were independent of nutritional status (Fig. 3). There was no significant difference between nutritional status and specific IgA titre to Rotavirus, S. typhimurium, EPEC serotype 0111:K58 and Sh. flexneri 2. However, intestinal specific IgA to Rotavirus was high compared to S. typhimurium and Sh. flexneri 2 (53.1 vs 6.9 vs 5.6, $p = 0.05$) respectively, may be as a result of repeated infections with Rotavirus. This indicates that malnourished children were able to mount humoral immune response.

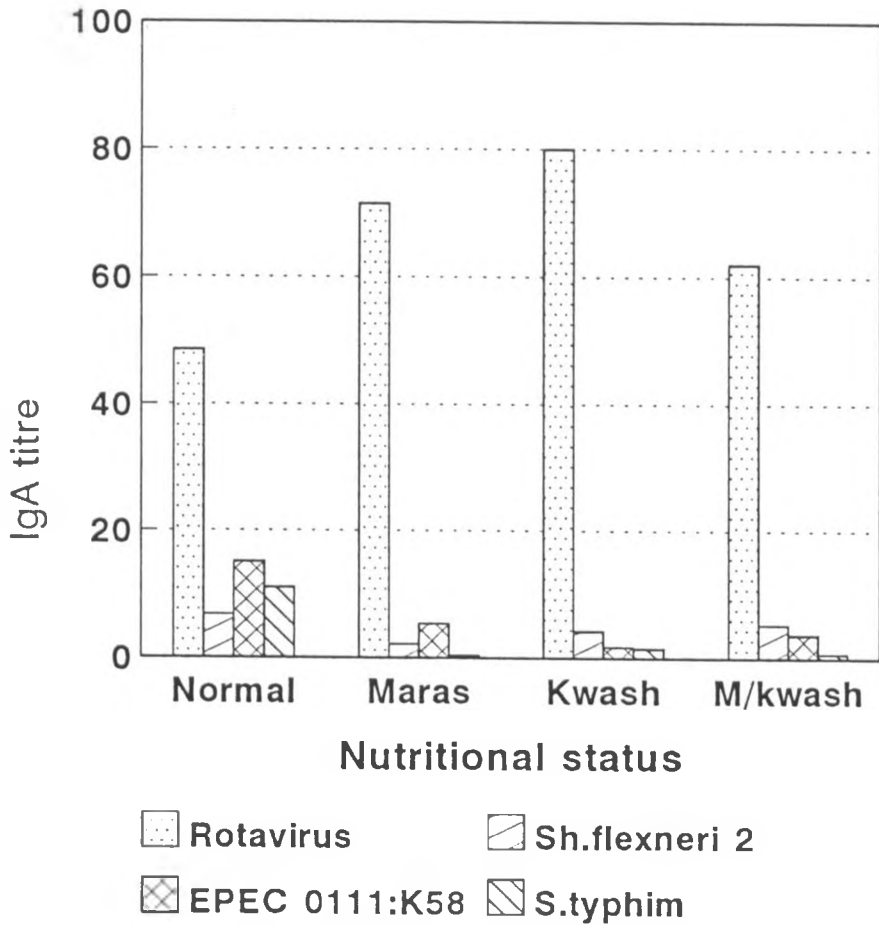
Fig. 2
Nutritional status and total IgA



KEY: $p = 0.01$ between normal and malnourished
IgA conc = IgA concentration (mg/ml)

Normal = normal nutritional status, maras = marasmus, kwash = kwashiorkor, m/kwash = marasmus kwashiorkor.

Fig. 3
Nutritional status and specific IgA



KEY: $p = 0.7$, NS between normal and malnourished
 $p = 0.05$ between rotavirus, S.typhimurium
 or Sh.flexneri 2 IgA and malnutrition

4.3.3 T-lymphocytes and nutritional status

Percentage of CD4 was reduced while CD8 percent and count increased in children who were malnourished. Malnourished children (Table 5) had significantly lower CD4 percentage (25.9% vs 32.0%, $p = 0.001$) and higher CD8 percentage (38.3% vs 30.1%, $p < 0.001$) than those of normal nutrition. In addition CD8 counts were increased in the malnourished (1583.8 cells/mm³ vs 2286.9 cells/mm³, $p = 0.004$). In contrast, there was no significant difference in absolute CD4 counts between those malnourished and normal nutrition (1506.0 cells/mm³ vs 1691.5 cells/mm³, $p = 0.4$, NS). In general, there was reduced CD4/CD8 ratio among the malnourished than those with normal nutrition (0.7 vs 1.1, $p = 0.01$). This indicates that in effect there was significant increase in CD8 count but normal CD4 count levels.

Reduced CD4 percentage and increase CD8% correlated with diarrhoea (Table 5). Children with diarrhoea had a significantly lower CD4 percentage (27.7% vs 33.2%) than those without diarrhoea, especially among the malnourished (23.8% vs 27.6%), $p < 0.001$).

Table 5. Mean absolute count (cells/mm³) and percentage of CD4 and CD8 in normal and malnourished children with diarrhoea.

T-cell	Normal nutrition			Malnourished		
	Diarrhoea			Diarrhoea		
	Yes n=35	No n=132	Total n=167	Yes n=13	No n=15	Total n=48
CD4%	27.7±8.5	33.2±8.7	32.0±8.9	23.8±11.0	27.6±10.7	25.9±10.8
CD8%	32.5±11.1	29.4±9.3	30.1±9.8	38.7±13.0	37.9±11.2	38.3±11.8
CD4C	1583.6±935.4	1720.1±1078.6	1691.5±1049.0	1300.1±1094.1	1584.5±848.3	1506.0±971.2
CD8C	1869.6±1420.1	1508.0±1047.1	1583.8±1140.2	1910.8±1281.0	2612.8±1589.1	2286.9±1471.6
CD4/CD8	0.8±0.7	1.1±1.0	1.1±0.9	0.7±0.9	0.6±0.5	0.7±0.7

Key: CD4% = CD4 percent, CD8% = CD8 percent, CD4C = CD4 count, CD8C = CD8 count.

CD4% p = 0.001 between normal and malnourished; p = <0.001 between with and without diarrhoea.

CD8% p < 0.001 between normal and malnourished; p = 0.02 between with and without diarrhoea.

CD4C p = 0.4 between normal and malnourished; p = 0.02 between with and without diarrhoea.

CD8C p = 0.004 between normal and malnourished; p = 0.2 between with and without diarrhoea.

Similarly, there was a significant difference in CD4 absolute counts between children with diarrhoea and those without irrespective of whether they were of normal nutrition (1583.6 cells/mm³ vs 1720.1 cells/mm³) or malnourished (1300.1 cells/mm³ vs 1684.5 cells/mm³), $p = 0.02$. This was also true of CD8 percentage which was significantly higher in children with diarrhoea than those without whether they were normal (32.5% vs 29.4%) or malnourished (38.7% vs 37.9%), $p < 0.001$.

4.3.4 Phagocytic activity and nutrition

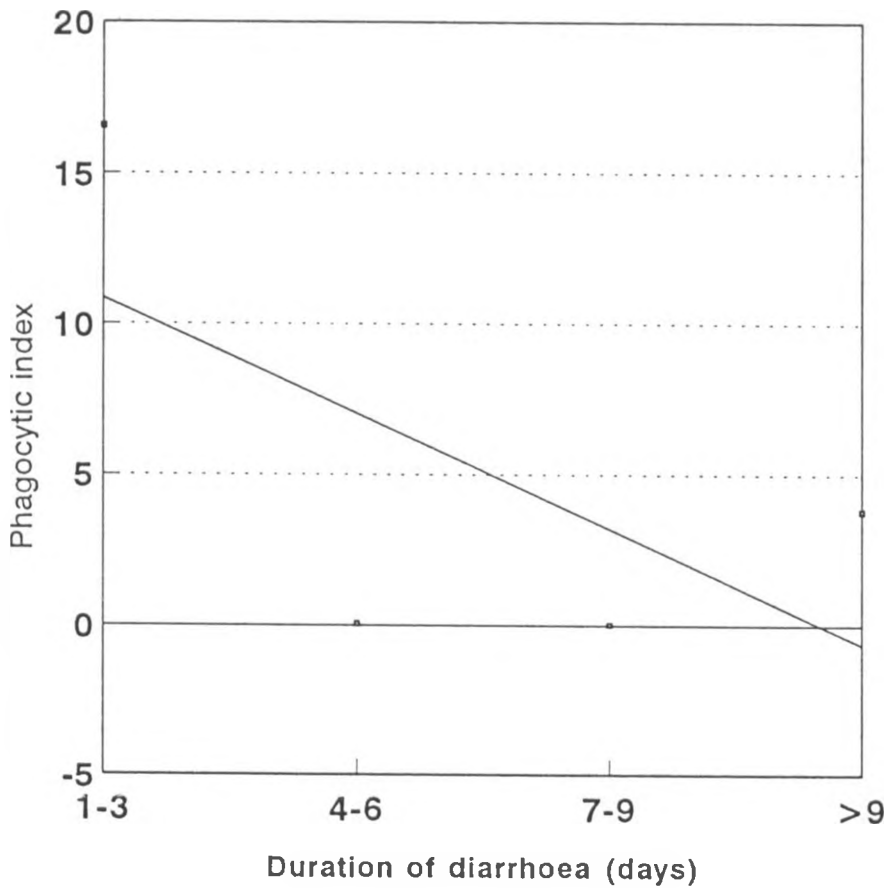
Phagocytic activity was independent of nutritional status. There was no significant difference (Table 6) in phagocytic activity between normal and malnourished children (phagocytic index = 28.9 vs 21.3, $p = 0.5$, NS). However, there was reduced phagocytic activity in children presenting with diarrhoea compared to those without (phagocytic index = 10.8 vs. 24.6, $p = 0.02$). There was a trend for inverse relationship between duration of diarrhoea and phagocytic activity (Correlation = -0.085, $p = 0.5$, NS) (Fig. 4). Although phagocytosis was depressed in children with diarrhoea, those with shorter duration of diarrhoea had higher phagocytic index. This indicates that high phagocytic activity may limit duration of diarrhoea.

Table 6. Phagocytic activity in malnourished children with diarrhoea.

	PI in children with nutritional status*	
	normal n=88	malnourished n=11
Diarrhoea (n=13)	21.3 ± 48.4	10.8 ± 27.9
No diarrhoea (n=86)	24.6 ± 50.9	28.9 ± 25.5

Key: Phagocytic index (PI) = product of the mean number of yeast cells ingested and the percentage of neutrophils engaged in phagocytosis, normal = well nourished children, * = excluding marasmic/kwashiorkor, p= 0.5, NS between normal and malnourished, p = 0.02 between children with and without diarrhoea.

Fig. 4
Relation between phagocytic activity and duration of diarrhoea



KEY: Inverse relationship between phagocytic Index and duration of diarrhoea (Corr. = -0.085, p = NS)

Phagocytic index = product of mean number of yeast cells ingested and the percentage of neutrophils engaged in phagocytosis.

4.4 Mode of feeding

4.4.1 Intestinal total and specific IgA antibody

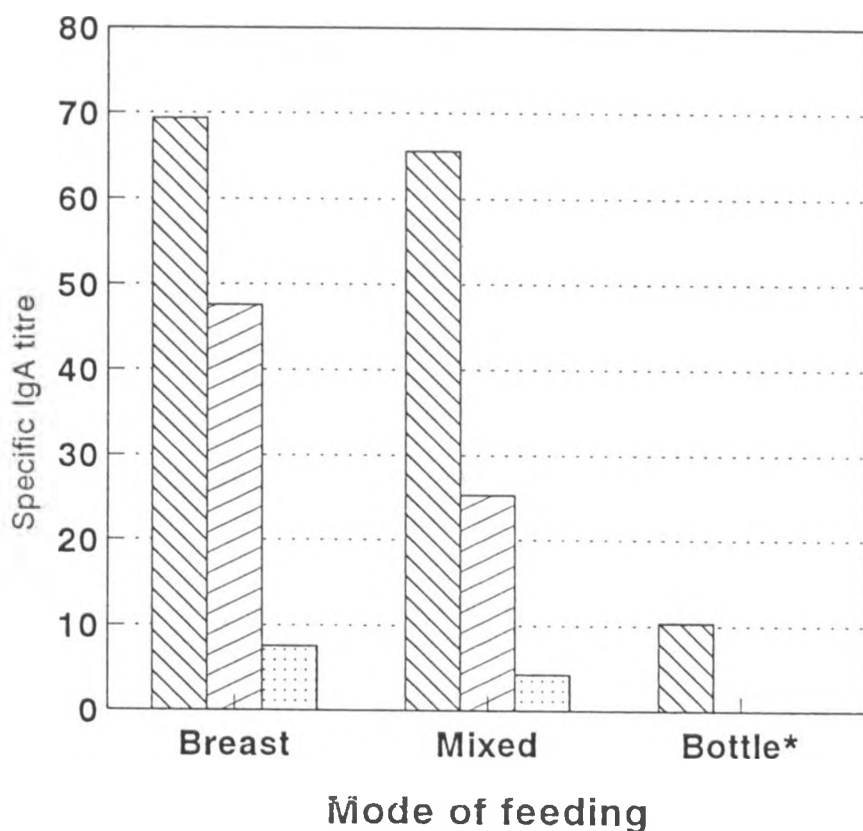
Breast feeding appears to increase total IgA. There was a trend (Table 7) for higher total IgA in children who were breast fed (1.7 mg/ml), than those mixed fed (1.5 mg/ml) or bottle fed (0.8 mg/ml), ($p = 0.2$, NS). These results suggest that this may be due to the IgA present in breast milk. It is probable that children who were breast fed had slightly higher IgA levels due to large volume of breast milk they receive compared to those mixed fed. This was more obvious with specific IgA which varied according to the mode of feeding. Specific IgA titre against Rotavirus was significantly higher in breast fed (69.4 vs 10.4, $p < 0.001$) and mixed fed (65.4 vs 10.4, $p = 0.03$) children than those bottle fed (Fig. 5). Similarly, IgA titres to EPEC serotype 086A:K61 (47.6 vs 25.3 vs 0.0) and Sh. flexneri 2 (7.6 vs 4.3 vs 0.0) tended to be higher in breast fed and mixed fed than those bottle fed respectively. These results suggest that specific IgA was acquired via breast milk.

Table 7. Phagocytic activity, total and specific IgA in relation to mode of feeding

Parameter	Mode of feeding:		
	Breast n=48	Mixed n=110	Bottle n=30
Total IgA	1.7 ± 2.6	1.5 ± 2.5	0.8 ± 2.0
Rotavirus IgA	69.4 ± 49.8	65.4 ± 23.3	10.4 ± 4.2
086A:K61 IgA	47.6 ± 34.3	25.3 ± 22.7	0.0
Sh.flex.2 IgA	7.6 ± 25.7	4.3 ± 24.6	0.0
PI	22.5 ± 60.1	11.7 ± 21.3	0.0*

Key: Total IgA (mg/ml) p = 0.07 between breast and bottle feeding, p = 0.08 between mixed and bottle feeding, p = 0.6 between breast and mixed feeding, * = only one child tested, Sh. flex.2 = Shigella flexneri 2, 086A:K61 = EPEC serotype 086A:K61, PI = phagocytic index.

Fig. 5
Mode of feeding and specific IgA



 Rotavirus
  EPEC 086A:K61
  Sh.flexneri 2

KEY: $p < 0.001$ between rotavirus breast and bottle feeding

$p = 0.03$ between rotavirus mixed and bottle feeding

* excluding IgA to EPEC 086A:K61 and Sh.flexneri 2

Breast fed = children entirely fed on breast milk, mixed fed = children fed on both breast milk and supplementary food, bottle fed = children entirely fed on supplementary food.

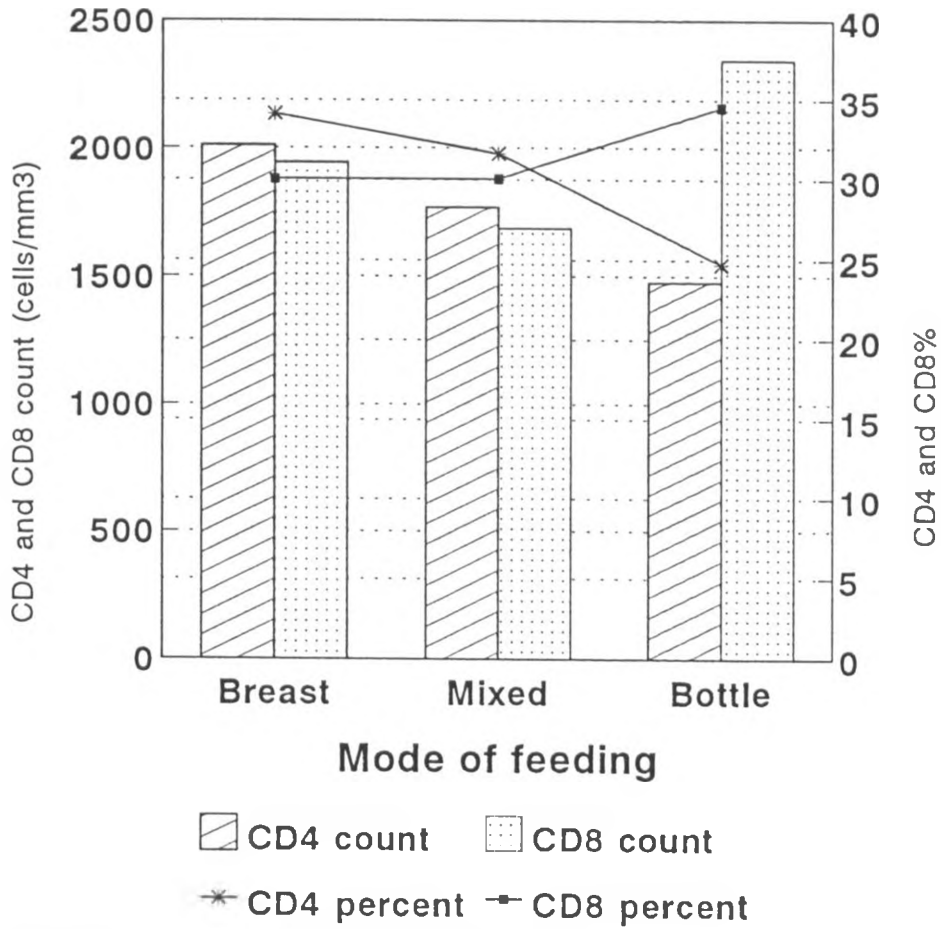
4.4.2 T-lymphocytes and mode of feeding

The mode of feeding influenced the levels of CD4. As shown in Figure 6, breast fed children had significantly higher CD4 percentage (34.15%) than those mixed fed (31.65%) or bottle fed (24.75%), $p = 0.002$, with a strong linear relationship, $p = 0.02$. This trend was also true of CD4 absolute counts which were higher in children who were breast fed (2010.8 cells/mm³) and mixed fed (1769.9 cells/mm³) than in those who were bottle fed (1477.5 cells/mm³), $p = 0.06$, NS. However, there was no relationship between mode of feeding and CD8 percent or CD8 absolute cell count.

4.4.3 Phagocytosis and mode of feeding

Again breast feeding appeared to improve phagocytic activity. Phagocytic activity was significantly higher in breast fed children than those mixed fed or bottle fed (phagocytic index = 22.5 vs. 11.7, $p < 0.001$) (Table 7). Phagocytic neutrophils may be transferred by breast milk. Results for bottle fed could be biased because there were too few children in this category.

Fig. 6
Mode of feeding and T-lymphocytes

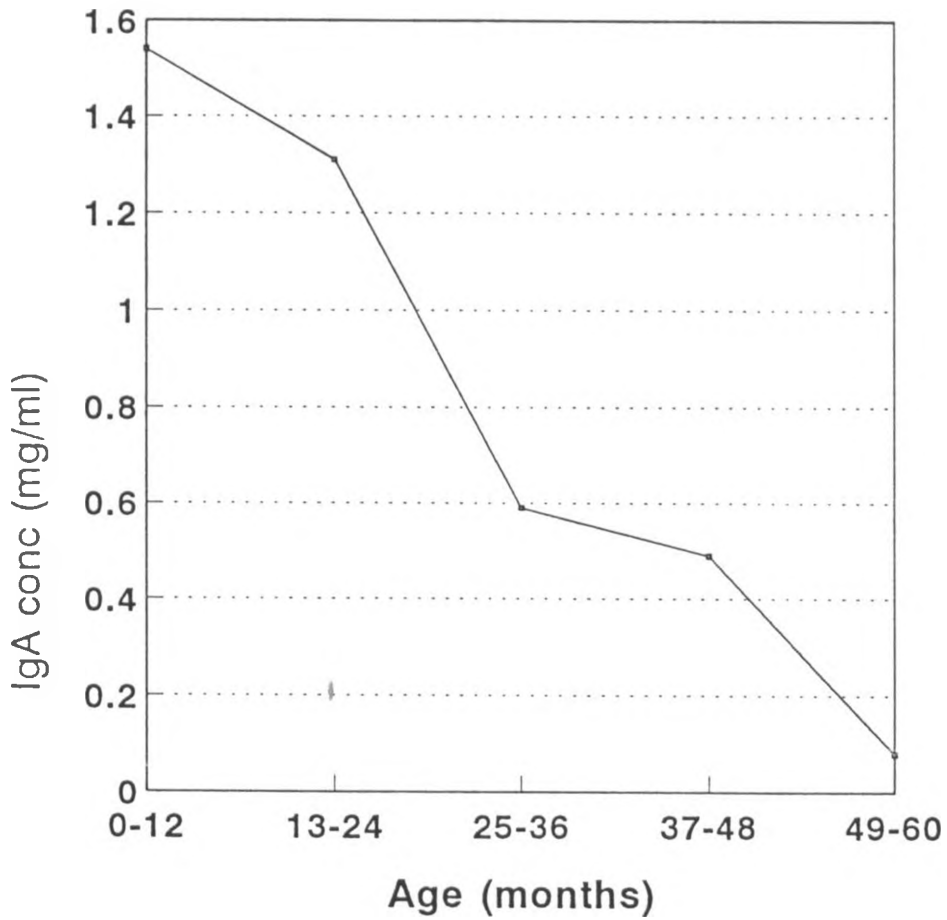


KEY: CD4% $p < 0.002$ between breast and bottle
 CD4 count linearity $p < 0.02$
 CD8% and CD8 count $p = NS$

4.4.4 Age, total IgA and mode of feeding

Total IgA was correlated with age of the children. There was an inverse relationship between age and total IgA levels (correlation = -0.1844 , $p = 0.01$), with a peak in those aged less than 12 months old (Fig. 7). Since this is the age at which most children are breast fed as indicated above, these results suggest that the high levels of IgA may be acquired via breast milk. To examine the effect of age and breast feeding on total IgA, multiple regression was used. There was no effect of age on IgA levels (regression coefficient = 0.16 , $SE = 0.3$, $p = 0.7$), but the effect of feeding on IgA was marginally significant (regression coefficient = -0.94 , $SE = 0.55$, $p = 0.09$).

Fig. 7
Relation between age and total IgA



KEY: Correlation $p = 0.01$ between age and total IgA
IgA conc (mg/ml) = IgA concentration

4.5 Duration of diarrhoea

4.5.1 Nutritional status and duration of diarrhoea

Duration of diarrhoea was related to nutritional status of the children (Table 8). Children with normal nutritional status had a shorter duration of diarrhoea (2.1 days) than those with marasmus (3.2 days), kwashiorkor (3.0 days) or marasmic/kwashiorkor (3.5 days), $p < 0.001$. There was a strong positive correlation between duration of diarrhoea and nutritional status (corr. = 0.1947, $p < 0.01$), with the highest duration among those with marasmic/kwashiorkor.

4.5.2 Mode of feeding and duration of diarrhoea

There was a relation between mode of feeding and duration of diarrhoea. When duration of diarrhoea was compared with the mode of feeding, breast fed children had a tendency for shorter mean duration, though insignificant, than those mixed fed (8.3 days vs. 9.8 days, $p = 0.6$) or bottle fed (8.3 days vs 11.2 days, $p = 0.2$). Reduced duration of diarrhoea in breast fed children is probably related to passive transfer of immunity from the mother's milk.

Table 8. Mean duration of diarrhoea in relation to nutritional status.

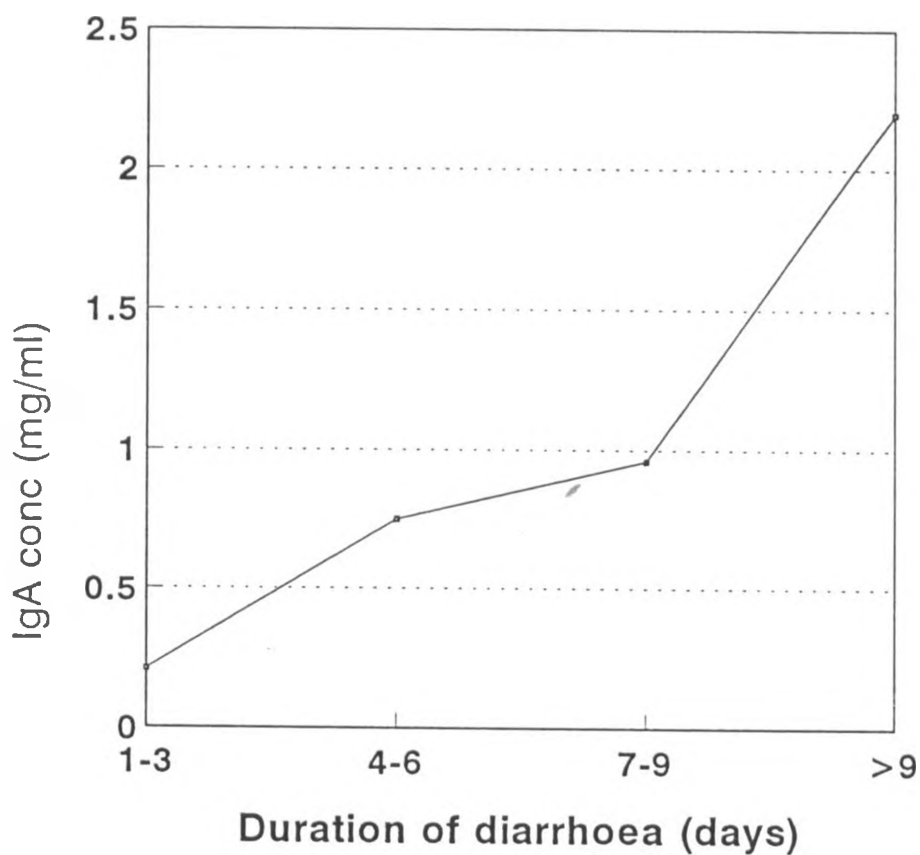
Nutritional status	Mean duration of diarrhoea (days)
Normal (n=281)	2.1 ± 1.4
Marasmus (n=47)	3.2 ± 1.7
Kwashiorkor (n=51)	3.0 ± 1.8
M/kwash (n=26)	3.5 ± 1.5
Total	2.6 ± 1.6

Key: p < 0.001 between normal and malnourished, p < 0.001 for linear association between duration of diarrhoea and nutritional status, Correlation = 0.1947, p < 0.001 between nutritional status and duration of diarrhoea, M/Kwash = Marasmic/kwashiorkor.

4.5.3 Total, specific IgA and duration of diarrhoea

Infections of the gastrointestinal tract result in local intestinal immune response. Children with diarrhoea had significantly higher levels of total IgA than those without (1.6 mg/ml vs 0.8 mg/ml, $p = 0.005$). There was a tendency (Fig. 8) for intestinal total IgA to increase with an increase in duration of diarrhoea ($p = 0.06$). Children with short duration of diarrhoea lasting 1-3 days had significantly lower levels of total IgA compared to those with prolonged diarrhoea lasting more than 9 days (0.2 mg/ml vs 2.2 mg/ml, $p = 0.05$). This was also true of specific IgA. There was a positive correlation between duration of diarrhoea and specific IgA to S. typhimurium (correlation = 0.4178, $p = 0.01$) but not to Rotavirus. This is probably a result of the present infection that may have limited the current infection and/or will protect against future infections.

Fig. 8
Relation between total IgA and duration of diarrhoea



KEY: $p = 0.05$ between 1-3 days and greater than 9 days
IgA conc = IgA concentration (mg/ml)

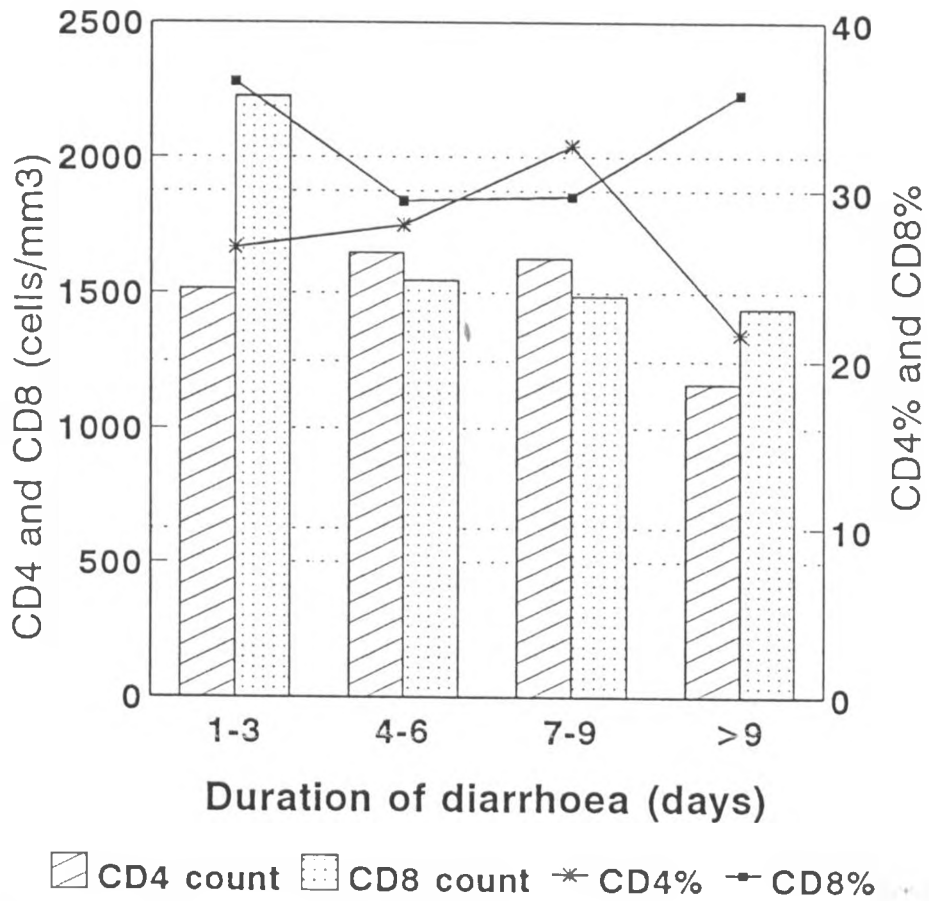
4.5.4 T-lymphocytes and duration of diarrhoea

The CD4 lymphocyte levels were independent of duration of diarrhoea. There was no significant difference between CD4 percent (26.7% vs 21.6%, $p = 0.3$, NS) or count (1514.9 cells/mm³ vs. 1165.7 cells/mm³, $p = 0.7$, NS) and duration of diarrhoea (Fig. 9). However, CD8 count was significantly higher in short duration of diarrhoea lasting less than 3 days compared to that lasting more than 9 days (2225.0 cells/mm³ vs 1441.9 cells/mm³, $p = 0.01$).

4.5.5 Phagocytosis and duration of diarrhoea

Phagocytic activity was correlated with duration of diarrhoea. Phagocytic index (Fig. 4) was high in diarrhoea lasting less than 3 days compared to that lasting 4 days and above (phagocytic index = 16.6 vs. 0.1, $p = 0.002$). These results suggest that phagocytosis may limit duration of diarrhoea or that phagocytosis was depressed in children with long duration of diarrhoea. Increased phagocytosis in children with short duration of diarrhoea could have limited diarrhoea.

Fig. 9
Relation between T-lymphocytes and duration of diarrhoea



KEY: $p = 0.01$, CD8 counts between diarrhoea lasting 1-3 days and greater than 9 days

4.6 Severity of diarrhoea

4.6.1 Duration and severity of diarrhoea

There was a strong positive correlation between duration and severity of diarrhoea (Correlation = 0.1983, $p = 0.001$) (Table 9). Mild diarrhoea lasted a shorter time of between 1-3 days compared to moderate and severe diarrhoea (66.0% vs 11.0% vs 34.2%, $p = 0.01$). In contrast, severe diarrhoea lasted more than 9 days compared to moderate and mild diarrhoea (36.1% vs 31.0% vs 3.0%, $p < 0.01$). Similarly, there was an association between severity of diarrhoea and malnutrition (Table 9). Whereas the percentage of children with normal nutrition decreased with severity of diarrhoea (73.5% vs 54.3% vs 49.4%), that of malnourished children increased with severity of diarrhoea (26.5% vs 45.7% vs 50.6%) for mild, moderate and severe diarrhoea respectively (Correlation = 0.1999, $p < 0.001$, OR = 2.8, CI = 1.6-5.1). This indicates that prolonged and severe diarrhoea may be related to the nutritional status.

Table 9. Duration (days), malnutrition and severity of diarrhoea.

% of children according to severity** of diarrhoea:				
	Mild n=100	moderate n=72	severe n=158	p-value
duration				
1-3	66.0	11.0	34.2	<0.01*
4-6	25.0	20.8	16.5	0.2
7-9	6.0	31.9	18.4	<0.05*
>9	3.0	36.1	31.0	<0.01)*
nutrition				
Normal	73.5	54.3	49.4	<0.001*
Malnutr	26.5	45.7	50.6	<0.001*

Key: Correlation = 0.1983, $p < 0.001$ between duration and severity of diarrhoea, Correlation = 0.1999, $p = 0.001$ between nutritional status and severity of diarrhoea * = statistically significant by chi square test, Malnutr = malnourished, Mod = moderate, ** = severity was determined according to the presence or absence of blood or mucus in stool (Gordon et al. 1964).

4.6.2 Total and specific IgA antibody

While total IgA was independent (Table 10), specific IgA was higher in children with mild diarrhoea. Rotavirus specific IgA titre was significantly higher (Fig. 10) in children with mild than those with severe diarrhoea (143.2 vs 34.0, $p = 0.05$). A similar trend was observed in specific IgA titre to EPEC 086A:K61 (41.8 vs 24.1, $p = 0.1$, NS) and EPEC 0111:K58 (27.5 vs 5.9, $p = 0.06$, NS). It would appear from the results that the presence of specific IgA limits severity of diarrhoea.

4.6.3 T-lymphocytes and severity of diarrhoea

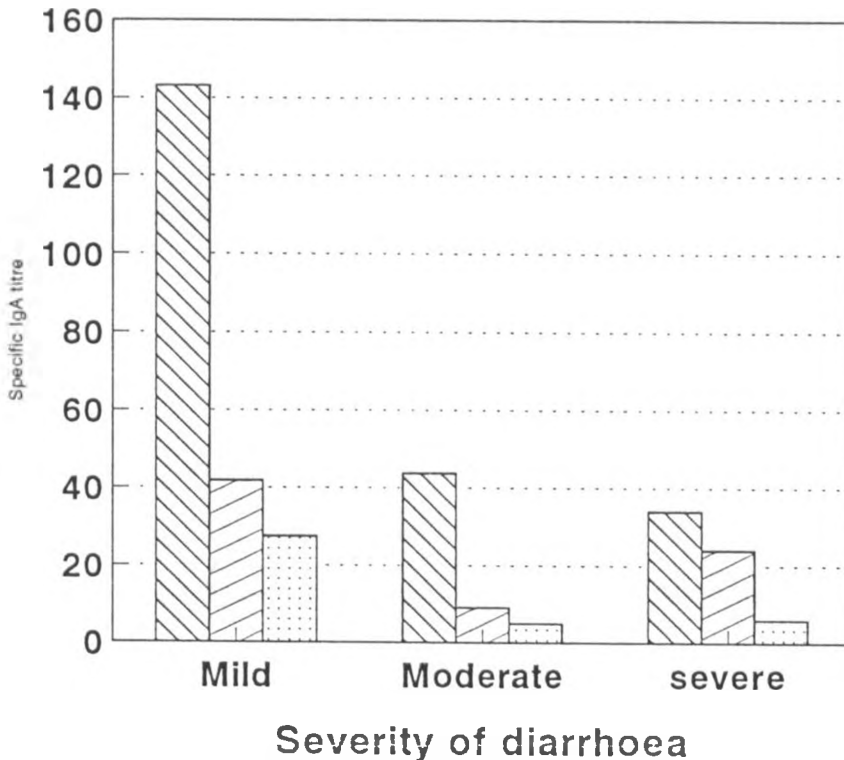
There was no association between severity of diarrhoea and T-lymphocytes. The CD4 counts (1566.6 cells/mm³ vs 1642.6 cells/mm³ vs 1396.4 cells/mm³, $p = 0.8$, NS) and CD8 counts (1928.9 cells/mm³ vs 2465.2 cells/mm³ vs 1592.6 cells/mm³, $p = 0.4$, NS) were similar in mild, moderate and severe diarrhoea respectively (Table 10).

Table 10. Comparison of total IgA, T-lymphocytes and phagocytosis with severity of diarrhoea.

Parameter	Severity of diarrhoea:			p-value
	mild n=39	moderate n=28	severe n=56	
CD4%	27.6 ± 9.3	28.0 ± 5.9	24.7 ± 10.7	0.6
CD8%	33.9 ± 13.6	34.6 ± 10.1	34.7 ± 10.3	1.0
CD4C	1566.5 ± 988.1	1642.6 ± 530.1	1396.4 ± 1156.9	0.8
CD8C	1928.9 ± 1627.0	2465.2 ± 1378.0	1592.6 ± 788.8	0.4
CD4/CD8	0.8 ± 0.6	0.7 ± 0.4	0.9 ± 1.5	0.6
Total IgA	1.5 ± 2.3	1.9 ± 2.5	1.6 ± 2.9	0.8
PI	4.8 ± 10.2	1.6 ± 2.2	1.9 ± 3.7	0.5

Key: CD4C = mean CD4 absolute count (cells/mm³), CD8C = mean CD8 absolute count (cells/mm³), Total IgA= mean total IgA (mg/ml), PI = phagocytic index (mild n=6, moderate n=2, severe n=4).

Fig. 10
Presence of specific IgA and severity of diarrhoea



Rotavirus
 EPEC 086A:K61
 EPEC 0111:K58

KEY: $p=0.05$, rotavirus IgA between mild and severe

$p=0.01$, EPEC 086A:K61 IgA between mild and moderate

$p=0.06$, EPEC 0111:K58 IgA between mild and severe

Mild = absence of blood or mucus in stool with duration less than 4 days, moderate = absence of blood or mucus in stool with duration 4 days or more, severe = presence of blood or mucus in stool irrespective of duration (Gordon et al. 1964).

4.6.4 Phagocytosis and severity of diarrhoea

Results shown in Table 10 indicate higher phagocytic index in children with mild diarrhoea compared to those with either moderate or severe diarrhoea. However the results may be biased because children tested for phagocytic activity were too few for comparison.

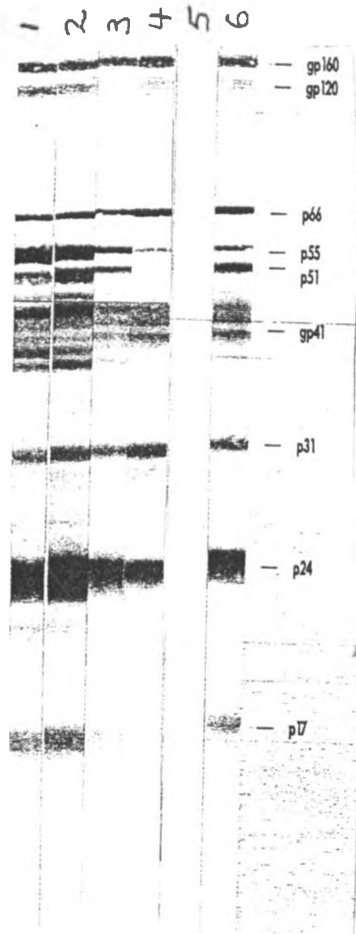
4.7 Human immunodeficiency virus (HIV) and diarrhoea

4.7.1 Total IgA and HIV

HIV serostatus was a significant determinant of humoral immune response in children. Western blot bands of HIV seropositive children (determined at 15 months of age) are shown in Fig. 11. Total IgA was reduced in HIV seropositive children (Table 11) than those who were seronegative, (0.2 mg/ml vs 0.7 mg/ml, $p = 0.04$). This was irrespective of whether they had diarrhoea (0.4 mg/ml vs 0.2 mg/ml) or not (1.4 mg/ml vs 1.5 mg/ml), $p = 0.3$, NS, and whether they were malnourished (0.4 mg/ml vs 1.4 mg/ml) or not (0.2 mg/ml vs 0.5 mg/ml), $p = 0.3$, NS. This reflects depressed humoral immune response in HIV seropositive children.

Fig. 11

Western blot bands showing HIV seropositive children.



Key: 1-4 = HIV positive patient's serum, 5 = negative control, 6 = positive control.

Table 11. Total IgA and phagocytosis in relation to HIV serostatus.

	% HIV serostatus:		p-value
	pos n=16	neg n=72	
Total IgA	0.2 ± 0.3	0.7 ± 2.1	0.04*
PI	15.1 ± 60.5	29.4 ± 28.6	0.1

Key: pos = positive, neg = negative, PI = phagocytic index, * = statistically significant by student's t-test.

4.7.2 T-lymphocytes and HIV

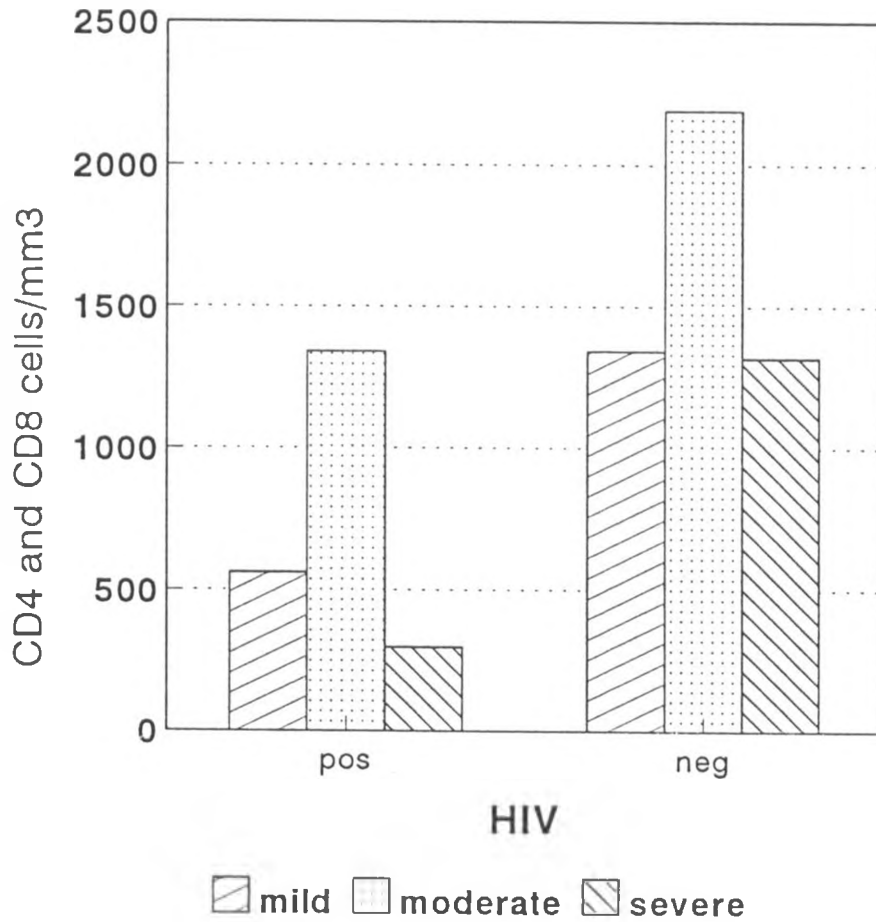
HIV seropositivity and diarrhoea were associated with reduced CD4 and increased CD8 counts. HIV seropositive children had reduced CD4 counts (840.0 cells/mm³ vs 1477.0 cells/mm³, $p = 0.04$) and increased CD8 counts (2050.5 cells/mm³ vs 1353.5 cells/mm³, $p = 0.004$), compared to HIV seronegative (Table 12). This resulted in significant reduction of CD4/CD8 ratio in the former group (0.4 vs 1.1, $p = 0.01$). Children presenting with diarrhoea and HIV had more depressed CD4 counts (624.2 cells/mm³ vs 1773.9 cells/mm³) than those HIV seronegative (1345.1 cells/mm³ vs 1589.1 cells/mm³), $p = 0.01$. Severity of diarrhoea was related to immunosuppression. CD4 counts were significantly reduced in HIV seropositive than HIV seronegative (298.7 cells/mm³ vs 1318.5 cells/mm³, $p = 0.01$) children who had severe diarrhoea (Fig. 12). These results suggest that HIV seropositive children who presented with diarrhoea had a greater degree of immunosuppression than those who did not present with diarrhoea.

Table 12. The effect of HIV on mean T-cell subsets and their relation to diarrhoea.

T-cell	HIV seropositive			HIV seronegative		
	Diarrhoea:			Diarrhoea:		
	yes n=8	no n=50	Total n=58	yes n=21	no n=65	Total n=86
CD4%	17.5±8.8	31.1±9.3	29.2±9.1	29.0±8.0	32.4±8.1	31.6±8.0
CD8%	42.2±14.6	33.1±11.2	34.0±12.9	31.2±7.4	29.4±9.0	30.0±8.2
CD4C	624.2±612.2	1773.9±824.5	840.0±711.6	1345.1±653.6	1589.1±1261.9	1477.0±957.8
CD8C	1250.0±392.4	2060.3±1517.2	2050.5±643.4	1505.5±711.8	1317.5±755.0	1353.5±733.4
CD4/CD8	0.5±1.6	0.9±0.5	0.4±1.1	0.9±0.9	1.2±1.7	1.1±1.3

Key: CD4% = CD4 percent, CD8% = CD8 percent, CD4C = CD4 count (cells/mm³), CD8C = CD8 count (cells/mm³), p= 0.04 for CD4C between HIV seropositive and seronegative, p = 0.004 for CD8C between HIV seropositive and seronegative, p= 0.001 for CD4C between HIV positive plus diarrhoea and HIV negative with diarrhoea, diarrhoea includes all categories irrespective of severity.

Fig. 12
Severity of diarrhoea and T-lymphocytes in
HIV seropositive children



KEY: $p = 0.01$ severity of diarrhoea between HIV pos and neg
 pos = HIV seropositive (N=16), neg = HIV seronegative (n=72)

4.7.3 Phagocytosis

Phagocytic activity was reduced (Table 11), though not statistically significant, in HIV seropositive than HIV seronegative children (phagocytic index = 15.1 vs 29.4, $p = 0.1$, NS). This was irrespective of whether they had diarrhoea (Phagocytic index = 2.0 vs 16.4) or not (Phagocytic index = 14.7 vs 32.4), $p = 0.1$, NS.

4.7.4 Enteric pathogens and HIV status

The occurrence of enteric pathogens was not dependent on the child's HIV state (Table 13). Enteropathogenic E. coli (31.3% vs 18.7%, $p = 0.4$, NS), enterotoxigenic E. coli (6.3% vs 5.3%, $p = 0.6$, NS), enteroadhesive E. coli (6.3% vs 12.0%, $p = 0.8$, NS) and Shigella (6.3% vs 6.6%, $p = 0.6$, NS) were equally isolated from HIV seropositive and seronegative children. Whereas T. hominis (6.3%) was isolated from an HIV seropositive child, Campylobacter (4%), Ascaris (14.7%), Hookworm (1.3%) and E. histolytica (1.3%) parasites were isolated from HIV seronegative children but this was statistically insignificant. The CD4 absolute counts were significantly lower in children with E. coli (1298.4 cells/mm³ vs 1717.9 cells/mm³, $p = 0.05$). Probably this is the reason for slightly higher isolation rate of enteropathogenic E. coli in HIV seropositive children.

Table 13. Enteric pathogens isolated from children in relation to HIV seropositivity

Pathogen	Children (%) with HIV status:		p-value
	pos n=16	neg n=75	
EPEC	31.3	18.7	0.4
ETEC	6.3	5.3	0.6
EAEC	6.3	12.0	0.8
Shigella	6.3	6.6	0.6
T. hominis	6.3	0.0	0.4
Camp	0.0	4.0	0.9
Ascaris	0.0	14.7	0.2
G. lamblia	0.0	9.3	0.5
Hookworm	0.0	1.3	0.4
E. histo	0.0	1.3	0.4
Unknown	50.0	48.0	0.9

Key: EPEC = enteropathogenic E. coli, ETEC = enterotoxigenic E. coli, EAEC = enteroadhesive E. coli, pos = positive, neg = negative, Camp = Campylobacter, E. histo = Entamoeba histolytica, G. lamblia = Giardia lamblia, T. hominis = Trichomonas hominis.

4.7.5 Diarrhoea and maternal HIV status

The influence of maternal HIV serostatus on diarrhoea in children depended on the age of the children. Twenty six percent (19/73) of children born to HIV seropositive mothers presented with diarrhoea during the first six months of life compared to 5.5% (4/73) of those born to HIV seronegative mothers (Table 14) ($p = 0.002$, OR = 5.2, 95% CI = 1.6-17.6), but after 6 months of life, 34.2% (25/73) HIV exposed and 34.2% (25/73) unexposed children equally presented with diarrhoea. Children of HIV seropositive mothers got diarrhoea earlier in life probably due to immunosuppression resulting from HIV exposure.

There was a significant difference in duration of diarrhoea between HIV exposed and unexposed children. HIV exposed children (Table 15) presented with prolonged diarrhoea lasting more than 9 days (11.0% vs 1.4%, OR = 8.7, 95% CI = 1.1-68.1, $p = 0.03$). In general, the mean duration of diarrhoea was longer in HIV exposed than unexposed children (7.8 vs 4.1 days, $p = 0.1$, NS). Probably maternal HIV infection affected transfer of passive immunity to the baby.

Table 14. Maternal HIV status and age (months) of children with diarrhoea.

Age	% maternal HIV serostatus:	
	pos n=44	neg n=29
0-6	26.0	5.5
>6	34.2	34.2

Key: pos = positive, neg = negative, p = 0.02 between HIV exposed and unexposed.

Table 15. The effect of maternal HIV serostatus on duration of diarrhoea (days).

Duration	% Maternal HIV serostatus:		p	OR	95% CI
	pos n=44	neg n=29			
1-3	26.0	23.3	0.2	0.6	0.2-1.4
4-6	15.1	9.6	0.8	1.0	0.4-2.8
7-9	8.2	5.5	1.0	0.9	0.2-3.2
>9	11.0	1.4	0.03*	8.7	1.1-68.1

Key: pos = positive, neg = negative, CI = confidence interval, OR = odds ratio, * = statistically significant by Chi square test, diarrhoea refers to all categories irrespective of severity.

4.7.6 Malnutrition and maternal HIV

Maternal HIV serostatus did not influence the nutritional state of the children. Normal (29.2% vs. 54.2%) and malnourished (5.2% vs. 11.4%), children (Table 16) were born to HIV seropositive and HIV seronegative mothers respectively, $p = 0.6$, NS.

4.7.7 Total IgA and maternal HIV

From this study, maternal HIV serostatus had no effect on total IgA. There was no significant difference in total IgA (Table 17) levels between HIV exposed and unexposed children (0.9 mg/ml vs. 0.8 mg/ml, $p = 0.8$). Specific IgA was not determined in this group of children.

4.7.8 T-lymphocytes and maternal HIV

CD8 percent and counts were increased in children born to HIV seropositive mothers. Whereas there was no difference (Table 18) in CD4% (30.8% vs 31.8%, $p = 0.5$, NS) and CD4 absolute counts (1690.4 cells/mm³ vs 1612.5 cells/mm³, $p = 0.6$, NS) between HIV exposed and unexposed children, CD8% (32.3% vs 29.0%, $p = 0.04$) the CD8 counts were significantly higher in HIV exposed (1837.0 cells/mm³ vs 1373 cells/mm³, $p = 0.01$).

Table 16. Maternal HIV serostatus and malnutrition

Nutritional status	% maternal HIV serostatus:	
	pos n=93	neg n=178
Normal	29.2	54.2
Malnourished	5.2	11.4

Key: pos = positive, neg = negative, p = 0.6, NS, OR = 1.2, 95% CI = 0.3 - 4.4 between maternal HIV and nutritional status.

Table 17. Total IgA and phagocytosis in children born to HIV seropositive mothers.

Parameter	Maternal HIV serostatus:		p-value
	pos n=117	neg n=52	
Total IgA	0.9 ± 1.9	0.8 ± 1.9	0.8
PI	18.7 ± 37.4	29.3 ± 62.7	0.3

Key: PI = phagocytic index, pos = positive, neg= negative.

Table 18. T-lymphocytes in children born to HIV seropositive mothers.

T-cell	Maternal HIV serostatus:		p-value
	pos n=132	neg n=64	
CD4%	30.8 ± 9.5	31.8 ± 9.2	0.5
CD8%	32.3 ± 10.7	29.0 ± 9.6	0.04*
CD4C	1690.4 ± 895.5	1612.1 ± 1286.6	0.6
CD8C	1837.0 ± 1274.4	1373.0 ± 1021.7	0.01*
CD4/CD8	0.9 ± 0.7	1.2 ± 1.3	0.3

Key: pos= positive, neg = negative, * = significant by student's t-test,
 CD4% = mean CD4 percent, CD8% = mean CD8 percent, CD4C = mean CD4 count,
 CD8C = mean CD8 count.

4.7.9 Phagocytosis and maternal HIV

There was no significant difference (Table 17) in phagocytic activity between HIV exposed and unexposed children (Phagocytic index = 18.7 vs. 29.3, $p = 0.3$, NS). This indicates that maternal HIV did not impair phagocytic activity in these children.

CHAPTER FIVE

DISCUSSION

Malnutrition continues to be a problem in many of the developing countries. A close association between diarrhoea and malnutrition is apparent, and by acting together, contribute to high rates of childhood morbidity and mortality (Beau et al. 1987, Mathur et al. 1985, Oburra 1986). In this study, 29.3% (163/556) of the children were malnourished, 84.0% (137/163) of whom presented with diarrhoea with a mortality rate of 0.03% (5/163). The mechanisms of host defense responsible for controlling diarrhoea infections are poorly understood. Diarrhoea is characteristically self limiting in immunocompetent hosts but prolonged and life threatening in immunocompromised such as AIDS or malnourished patients.

Total IgA was significantly higher among children with marasmus than the other nutritional categories (Fig. 2), may be as a result of repeated infections which these children get. It could however, not be established in this study what the infections were. These results agree with those of Saha in which he found raised stool total IgA levels in malnourished children with chronic diarrhoea (Saha et al.

1990). Malnutrition impairs the body's immune mechanisms, since malnourished children in this, and other studies had diarrhoea of longer duration and severity (Lloyd-Evans et al. 1983, Mutanda et al. 1985, Oburra 1986, Tomkins 1981, Chandra 1975). Intestinal specific IgA to Rotavirus, Sh. flexneri 2, S. typhimurium and EPEC 0111:K58 were unaffected by nutritional status (Fig. 3). Titres for Rotavirus specific IgA was significantly higher than the other enteric pathogens irrespective of nutritional state may be due to repeated infections with Rotavirus. Rotavirus has been reported to be the most common cause of childhood diarrhoea in developed and developing countries (Kapikian 1993, Mutanda 1980). Since total and specific IgA levels were independent of nutritional status, malnourished children are able to mount a humoral immune response. Reduced CD4 and increased CD8 seen in malnourished children may be a result of impaired immune system due to malnutrition (Chapel et al. 1986), which in turn may have led to susceptibility to enteric pathogens. When bacteria or other invading parasites penetrate the skin or mucus membranes, cellular defense mechanisms are immediately brought into play (Davis et al. 1980). Local and blood borne macrophages (mononuclear phagocytes) and neutrophils (polymorphonuclear leucocytes) accumulate around the invaders and initiate the phagocytic process. Contrary to studies by Chandra (1983), phagocytic activity was independent of nutritional status in this study (Table 6).

It is well documented that breast feeding protects children against gastroenteritis (Khan 1984, Cunningham 1977, Ketsela et al. 1990, Cunningham et al. 1990). Breast feeding appeared to increase phagocytic activity, total IgA and specific IgA (Table 7 and Fig.5). Rotavirus specific IgA levels ($p = 0.03$) and CD4 percentage (linearity $P = 0.02$) were significantly higher in breast fed than in mixed fed and bottle fed children. In addition, breast fed children had a shorter mean duration of diarrhoea compared to those mixed fed and bottle fed. This suggests that breast feeding protects children against diarrhoeal morbidity, thereby enhancing the body's resistance to gastrointestinal infections (Popkin et al. 1990). This is further supported by finding that there was an inverse relationship between age and total IgA (Corr. = - 1844, $P = 0.01$), with a peak in the 0-12 months age group, mainly due to the effect of breast feeding (Fig. 7). This effect may be reduced when artificial feeds are introduced in combination with breast feeding (Feacham et al. 1984^a) due to reduction in the volume of breast milk consumed or bacterial contamination in feeds especially in developing countries where there is poor hygiene.

Aetiological agents isolated depended on the nutritional state of the children (Table 2). Whereas EPEC was more significantly isolated from normal children, Campylobacter, either alone or in combination with Rotavirus or heat labile

toxigenic E. coli was more commonly isolated from malnourished children ($p = 0.02$). Similar results were reported in Gambia (Lloyd-Evans et al. 1983). Although Campylobacter is now recognized as an important enteric pathogen, especially in the malnourished, its presence in faeces from apparently healthy children from some communities makes it difficult to estimate its significance. It is not clear if Trichomonas hominis is a causative agent of diarrhoea (Mutanda et al. 1986, Chunge et al. 1988). In this study, T. hominis either alone or in combination with Rotavirus and Giardia lamblia were found in malnourished children. It is possible that Campylobacter and T. hominis are opportunistic infections in immunosuppressed malnourished children. Diarrhoea of unknown aetiology was found in 26.3% (41/154) of well nourished and 47.8% (56/119) of malnourished children. There is no clear reason for this but it is probably due to prior treatment before enrolment, inflammatory reaction in the gastrointestinal tract due to generalised systemic infection (Behrens 1991), hypersensitivity reactions, undetectable agents which could have been eliminated by immune response or enteric pathogens that were not determined in this study.

Children with diarrhoea had significantly higher total IgA than those without diarrhoea ($P = 0.05$), may be as a result of the current infection. Diarrhoea in children was caused by

different organisms other than the one they had specific intestinal IgA antibody to, especially in Rotavirus and Sh. flexneri 2 infections (Table 3 and 4). This suggest that intestinal IgA may have a protective role in the host resistance to diarrhoeal disease which is species specific. Only two children had both Rotavirus antigen and specific IgA antibody but it was not possible from this study to determine whether they had same or different serotypes, the infecting virus dose was high or merely reflecting initial stages of infection. Wyatt and co-workers, (1983) reported four different serotypes of human Rotaviruses. There was an increase in Rotavirus specific IgA with age, with a peak in the 4-9 months age group (Fig 1). This peak coincides with the peak age incidence of acute Rotavirus diarrhoea in children (Konno et al. 1978, Mutanda 1985^b, Mutanda-personal communication), suggesting that patients may be able to mount a local immunological response. This is further supported by detection of Rotavirus from a large proportion of children aged less than 3 months, after which it subsided as the Rotavirus IgA titre peaked. Prevalence of Rotavirus deection in children less than 3 months may be due to immaturity of the immune system, low level maternal antibody transfer or increased exposure to enteropathogens.

Duration of diarrhoea was dependent on nutritional status, (Table 8), suggesting that this may have an immunological

basis. Gastrointestinal infections may result in some form of local intestinal immune response in that total IgA increased with duration of diarrhoea (Fig. 8). This was also true of specific IgA in which there was a positive correlation between S. typhimurium specific IgA and duration of diarrhoea. Whereas CD4 was independent of duration of diarrhoea, CD8 counts and phagocytic activity were significantly higher in diarrhoea of short duration lasting less than 3 days. Probably CD8 and phagocytosis may be the most important defence mechanism in limiting duration of diarrhoea in children irrespective of aetiological agent and nutritional status (Fig. 9, Table 6). Other studies have shown that Rotavirus diarrhoea has a mean duration of 8 days (Mutanda 1985^b) after which it subsides probably owing to the immunological response of the patients.

Prolonged and severe diarrhoea in malnourished children may be a result of impaired immune system (Chapel et al. 1986). There was a positive correlation between severity of diarrhoea and both duration of diarrhoea nutritional state of children (Table 9). Rotavirus specific IgA may limit severity of diarrhoea because it was significantly higher in children with mild compared to those with moderate to severe diarrhoea (Fig. 10). However, there was no association between severity of diarrhoea and either total IgA, CD4 lymphocytes, CD8 lymphocytes or phagocytosis (Fig. 10, Table 10), suggesting

these may have no role in limiting severity of diarrhoea.

Infection with HIV results in destruction of immune system with subsequent development of AIDS. AIDS is characterised by severe depletion of CD4 lymphocytes, resulting in susceptibility to opportunistic infection (Fauci 1988, Emonyi et al. 1992). However, interpretation of CD4 absolute counts has certain limitations. Defects in T-helper function can be detected months to years before a critical reduction in CD4 cell numbers (Shearer et al. 1991). These defects involve selective loss of T-helper function to recall or autologous antigens, but not to T-cell mitogens or human leucocyte antigens (HLA). One possible mechanism that could account for the early defect in T-helper function is that the antigen processing cells (APC) such as macrophages and monocytes necessary for processing and presenting recall antigens to the T-helper cells are functionally defective, without there necessarily being a reduction in CD4 T-cell numbers. Perhaps loss of T-helper function to recall antigens is due to selective loss or inactivation of CD4 cells that express the memory phenotype in asymptomatic HIV positive individuals.

HIV seropositive children had reduced intestinal IgA and absolute CD4 counts compared to HIV seronegative while phagocytic activity was not affected (Table 11 and 12). Similarly, absolute CD4 counts were significantly lower in

HIV seropositive than HIV seronegative children presenting with diarrhoea (Table 12), more so in those with severe diarrhoea (Fig. 12). This suggests that CD4 lymphocytes may have an important role to play in limiting severity of diarrhoea in HIV seropositive children (Smith et al. 1992). A study by Zu and co-workers have shown that passive breast milk IgA antibody especially in high titre may be partially protective (Zu et al. 1992). Severe, persistent cryptosporidiosis in immunosuppressed rodents and humans with AIDS demonstrate that T-lymphocytes are essential for controlling Cryptosporidium infection. Probably HIV infection prevents passive transfer of maternal immunity, thereby resulting in prolonged diarrhoea seen in this group of children (Table 15). This is further supported by finding that children born to HIV seropositive mothers presented with diarrhoea mostly during the first six months of life (Table 14). This is possibly due to increased risk of transmission of enteric pathogens from the HIV seropositive mother, reduced passively acquired immunity, inability of the ill mother/father to properly care for the child or HIV infection of the child. Whereas nutritional status (Table 16) total IgA and phagocytic activity (Table 17) were independent of maternal HIV seropositivity, CD8% and CD8 counts (Table 18) were significantly higher in HIV exposed children probably due to repeated and/or prolonged gastrointestinal infections (Datta 1989).

CONCLUSIONS

The following conclusions were drawn from the results of this study:

1. IgA from the mother's milk exert a direct localised protective function in the gut of the child thereby limiting duration and severity of diarrhoea. This is important in stressing the value of breast feeding.
2. Cytotoxic T-lymphocytes and chemotactic factors arising from activated T-lymphocytes and complement may attract phagocytic cells leading to inflammation of the gastrointestinal tract and prolonged diarrhoea in malnourished and HIV exposed children in the absence of enteric pathogens.
3. Infections of the gastrointestinal tract results in IgA and T-cell immune response irrespective of nutritional status, which may limit current infection and protect against re-infection.
4. Specific intestinal IgA to pathogens suggested that a vaccine that induces local immunological response may be effective in conferring resistance in both normal and malnourished children.

5. HIV exposed children may be pre-disposed to increased morbidity regardless of their own HIV status due to impaired immune response by interfering with passive transfer of maternal antibody, increased risk of transmission of pathogens from the mother or poor economic status of the mother due to illness.

RECOMMENDATIONS

1. Since diarrhoea and malnutrition are synergistically related, the control of malnutrition in endemic tropical areas is likely to improve the outcome of diarrhoea.
2. Mechanisms that lead to increased morbidity in children born to HIV seropositive mothers need to be investigated.

REFERENCES

- Adam KAR, Rasheed SA. Invasive candidiasis in infants: experience from Saudi Arabia. *Ann. Trop. Paed.* 11: 271-275, (1991).
- Ajusi JD, Onyango FE, Mutanda LN, Wamola IA. Bacteriology of unheated expressed breast milk stored at room temperature. *East Afr. Med. J.* 66: 381-387, (1989).
- Al-Freihi H, Twum-Dansi K, Sohaibani M, Bella H, El-Mouzan M, Sama K. The microbiology of acute diarrhoeal disease in the Eastern province of Saudi Arabia. *East Afr. Med. J.* 70: 267-269, (1993).
- Amin N. Giardiasis: a common cause of diarrhoea. *Postgrad. Med.* 66: 151-154, (1975).
- Ashcroft MT, Singh B, Nicholson CC, Ritchie JM, Sobryan E, Williams F. A seven year field trial of two typhoid vaccines in Guyana. *Lancet.* 2: 1056-1059, (1967).
- Asindi AA, Ibia EO, Udo JJ. Mortality pattern among

Nigerian children in the 1980's. *J. Trop. Med. Hyg.* 94: 152-155, (1991).

Baltazar JC, Tiglao TV, Tempongko SB. Hygiene behaviour and hospitalized severe childhood diarrhoea: a case control study. *WHO Bull.* 71: 323-327, (1993).

Beau JP, Garenne M, Diop P, Briend A, Mar ID. Diarrhoea and nutritional status as risk factors for child mortality in a Dakar hospital (Senegal). *J. Trop. Paed.* 33: 4-9, (1987).

Behrens R. Persistent diarrhoea syndrome. *Africa Health* 13: 10-11, (1991).

Bicocchi R, Sighinolfi L, Bedetti A, Rossi MR. Epidemiological features of HIV related diarrhoea in North Eastern Italy. Abstr. VIII Int. Conf. on AIDS/III STD World Congress, Amsterdam, Netherlands. Vol. 3 pp.134, (1992).

Bimal KS, Dagnow MB. Beliefs and practices related to diarrhoeal diseases among mothers in Gondar region, Ethiopia. *Trop. Geogr. Med.* 40: 259-263, (1988).

- Bird RG, Smith MD. Cryptosporidiosis in man. Parasite life cycle and fine structural pathology. *J. Path.* 132: 217-233, (1980).
- Black RE, Brown KH, Becker S. Malnutrition is a determining factor in diarrhoeal duration but not incidence among young children in a longitudinal study in rural Bangladesh. *Am. J. Clin. Nutr.* 39: 87-94, (1984).
- Blanche S, Rouzioux C, Moscato MLG. A prospective study of infants born to women seropositive for human immunodeficiency virus type 1. *New Eng. J. Med.* 320: 1643-1648, (1989).
- Blaser M, Cravens J, Wang PB. Campylobacter enteritis associated with canine infection. *Lancet.* 2: 979-981, (1978).
- Bohemen CG, Nabbe AJM, Zanen HC. IgA response during accidental infection with Shigella flexneri. *Lancet.* 2: 673, (1985).
- CDC Classification system for human immunodeficiency virus (HIV) infection in children under 13 years of age. *MMWR.* 36: 225-

236, (1987).

- CDC Recommendation for assisting in the prevention of perinatal transmission of human T lymphatic virus Type III/ lymphadenopathy associated virus and AIDS. MMWR. 34: 721-732, (1985).
- CDC^a Outbreak of Salmonella enteritidis infection associated with consumption of raw shell eggs, 1991. MMWR. 41: 369-372, (1992).
- CDC^b The management of acute diarrhoea in children: Oral rehydration, maintenance and nutritional therapy. MMWR. 41: 1-20, (1992).
- Chandra RK. Iron and immunocompetence. Nutr. Rev. 34: 129-132, (1976).
- Chandra RK, Seth V. Opsonic activity, phagocytosis and bactericidal activity of polymorphs in undernutrition. Arch. Dis. Child. 47: 282-284, (1972).
- Chandra RK. Reduced secretory antibody response to live attenuated measles and poliovirus vaccines in malnourished children. Br. Med. J. 2: 583-585,

(1975).

- Chandra RK. Rosette forming T-lymphocytes and cell mediated immunity in malnutrition. Br. Med. J. 3: 608-609, (1974).
- Chandra RK. Nutritional regulation of immunity and infection in the gastrointestinal tract. J. Paed. Gastroent. Nutr. 2: 181-187, (1983).
- Chapel H, Haeney M. Essentials of clinical immunology. Blackwell Scientific publication. 1986.
- Christensen P, Kahlmeter G, Johnson S, Kronvall G. Method for serological grouping of streptococci with specific antibodies adsorbed to protein A-containing staphylococci. Infect. Immun. 1: 881-885, (1973).
- Chunge RN, Simwa JM, Karumba PN, Kenya PR, Kinoti SN, Muttinga J, Nagelkerke N. Comparative aetiology of childhood diarrhoea in Kakamega and Kiambu districts, Kenya. East Afr. Med. J. 69: 437-441, (1992).
- Chunge CN, Estambale BBA, Pampa HO, Chitayi PM, Munanga

P, Kangethe S. Pathogenicity of *Trichomonas hominis* in Kenya. *East Afr. Med. J.* 65: 532-534, (1988).

Chunge RN, Wamola IA, Kinoti SN, Muttunga J, Mutanda LN, Nagelkerke N, Muthami L, Muniu E, Simwa JM, Karumba PN, Kabiru P. Mixed infections in childhood diarrhoea. Results of a community study in Kiambu district, Kenya. *East Afr. Med. J.* 66: 715-723, (1989).

Ciegelski JP, Dukes CS, Msengi AE, Mbise R, Redding-Lallinger R, Levine MM. Pathogenic *E. coli* in HIV seropositive children with chronic diarrhoea in Tanzania. Abstr. VIII Int. Conf. on AIDS/III STD congress. Amsterdam, Netherlands. Vol.3 pp.74, (1992).

Cleary TG, West MS, Ruiz-Palacios G, Winsor DK, Calva JJ, Guerrero M, Van R. Human milk secretory immunoglobulin A to *Shigella* virulence plasmid coded antigens. *J Pediatr.* 118: 34-38, (1991)

Cocker AO, Dosunmu-Oguni O, Odugbeni T, Alabi SA, Macaulay SA. A study on the prevalence of *Rotavirus* diarrhoea in Ohoozaran local

government area, Imo state, Nigeria and Lagos University teaching hospital, Lagos, Nigeria. East Afr. Med. J. 64: 586-589, (1987)

Colebunders R, Francis H, Mann JM. Persistent diarrhoea strongly associated with HIV infection in Kinshasa, Zaire. Am. J. Gastroenterol. 82: 859-864, (1987).

Collins GH. Cryptosporidiosis. Med. J. Austr. 140: 516-517, (1984).

Cowan ST Steel KJ. Manual for the identification of medical bacteria. Second edition. London. Cambridge university press. 1979.

Cravioto A, Tello A, Navarro A, Ruiz J, Villafan H, Uribe F, Elsave C. Association of E. coli Hep-2 adherence patterns with type and duration of diarrhoea. Lancet. 337: 262-264, (1991)

Cruickshank R. Immunological and serological methods. In: Medical Microbiology. Ed. Cruickshank R, Duguid JP, Swain RHA. Edinburgh and London. Eleventh edition, 1970.

- Cunningham AS. Morbidity in breast fed and artificially fed infants. J. Pediatr. 90: 726-729, (1977).
- Cunningham AS, Jelliffe DB, Jelliffe EFP. Breast feeding and health in the 1980's: A global epidemiologic review. J. Pediatr. 118: 659-666, (1991).
- Cunningham-Rudles. Effects of nutritional status on immunological function. Am. J. Clin. Nutr. 35: 1202-1210, (1982).
- Danna PL, Urban C, Bellin E, Rahal JJ. Role of Candida in pathogenesis of antibiotic-associated diarrhoea in elderly patients. Lancet. 337: 511-514, (1991).
- Datta P. Infant morbidity and mortality. Abstr. V Int. Conf. on AIDS, Montreal, Canada, pp. 974, (1989).
- Davis BD, Dulbecco R, Eisen HN, Ginsberg HS. Microbiology. Third Edition. Harper and Row Publishers. 1980.
- DHSS. PL/CMO (88) 7. HIV infection, breast feeding and human milk banking. London. Department of

Health and Social Security. 27: 1988.

- Dufffy LC, Byers TE, Riepenhoff-Talty M, La Scolea LJ
Zielezny M. The effect of infant feeding on
Rotavirus induced gastroenteritis. A
prospective study. Am. J. Pub. Health 76: 259-
263, (1986).
- Ebrahim GJ. Editorial: Persistent diarrhoea. J. Trop.
Pediatr. 36: 50-51, (1990).
- Editorial. Epidemic winter viruses. Br. Med. J. 2: 465,
(1977)
- Editorial. Classification of infantile malnutrition.
Lancet. 2: 302-303, (1970).
- Edwards P, Wodak A, Cooper DA, Thompson IL, Penny R. The
gastrointestinal manifestations of AIDS.
Austr. New Zealand J. 20: 141-148, (1990).
- Emonyi WI, Bwayo JJ, Anzala A, Kreiss J, Plummer FA,
Ndinya-Achola J. Correlation of immune status
and clinical status. Abstr. VIII Int. Conf.
AIDS/III STD congress. Amsterdam, Netherlands.
19-24, July, pp 76, 1992.

- Estambale BBA, Bwibo CRA, Kangethe S, Chitayi PM. The occurrence of Cryptosporidium oocysts in faecal samples submitted for examination at Kenyatta National Hospital. 66:792-795, (1989).
- Fauci AS. The human immunodeficiency syndrome: infectivity and mechanisms of pathogenesis. Science 239: 617-622, (1988).
- Fauven V, Yunus M, Zaman K, Charkraborty J, Sarder AM. Diarrhoea mortality in rural Bangladesh children. J. Trop. Pediatr. 37: 31-36, (1991).
- Feacham RG. Preventing diarrhoea: what are the policy options? Health policy and planning 1: 109-117, (1986).
- Feacham^a RG, Koblinsky MA. Intervention for the control of diarrhoeal diseases among young children: promotion of breast feeding. Bull. WHO. 62: 271-291, (1984).
- Feacham^b RG. Intervention for the control of diarrhoeal diseases among young children: promotion of personal and domestic hygiene. Bull. WHO. 62:

467-476, (1984).

- Gaasta W, Graaf FK. Host specific fimbrial adhesins of non invasive enterotoxigenic E. coli strains. Microbiol. Rev. 46: 129-161, (1982).
- Garcia-Rodriguez MC, Omenaca MC, Ferreira A, Madero R, Zapico R, Muro M, De jose I, Fontan G. Immunological follow up in children born to HIV-1 infected mothers. Acta. Paediatr. Scand. 80: 1183-1191, (1991).
- Gerard JW. Breast feeding: second thoughts. Pediatr. 54: 757-764, (1974).
- Gomwalk NE, Gopsham LT, Umoh UJ. Rotavirus gastroenteritis in pediatric diarrhoea in Jos, Nigeria. J. Trop. Pediatr. 36: 52-55, (1990).
- Goodman E, Wort AJ, Jackson FL. Enzyme-linked immunosorbent assay for detection of pertussis immunoglobulin A in nasopharyngeal secretions as an indicator of recent infection. J. Clin. Microbiol. 13: 286-292, (1981).
- Gordon JE, Guzman MA, Ascoli W, Schrimshaw NS. Acute

diarrhoea disease in less developed countries: patterns of epidemiological behaviour in rural Guatemalan villagers. Bull. WHO. 31: 9-20, (1964).

Gross RJ. E. coli diarrhoea. J. Infect. 7: 177-192, (1983).

Gurwith MW, Wenman W, Hinde D, Feltham S, Greenberg H. A prospective study of Rotavirus infection in infants and young children. J. Infect. Dis. 144: 218-223, (1981).

Haneberg B, Aarskog D. Human faecal immunoglobulins in healthy infants and children, and in some with diseases affecting the gastrointestinal tract immune system. Clin. Expt. immunol. 22: 210-222, (1975).

Harris JR, Wachsmuth K, Davis BR, Cohen ML. High molecular weight plasmid correlates with Escherichia coli enteroinvasiveness. Infect. immun. 37: 1295-1298, (1982).

Henriksen SA, Pohlez SFL. Staining of Cryptosporidium by a modified Ziehl-Neelson technique. Acta. Vet.

Scand. 22: 594-596, (1981).

- Henry FJ. The epidemiologic importance of dysentery in communities. Rev. Infect. Dis. 13: S238-244, (1991).
- Hoskins LC, Winawer SJ, Broitman SA, Gottlieb LS, Zamcheck N. Clinical giardiasis and intestinal malabsorption. Gastroenter. 53: 265-279, (1967).
- Jelliffe DB, Jelliffe EFP. Breast is best. Lancet. 2: 635, (1976).
- Jokipii L, Pohjola S, Jokipii AMM. Cryptosporidium: a frequent finding in patients with gastrointestinal symptoms. Lancet. 2: 358-361, (1983).
- Kakai R, Immunology of viral gastroenteritis with special reference to Rotavirus - a review. Msc. Med. Microbiol. project, London School of Hygiene and Tropical Medicine, University of London, 1984.
- Kakai R, Mutanda LN, Masudi AM. Rapid acquisition of

gentamicin and nalidixic acid by enterobacteria in Kenyatta National Hospital. *Medicom.* 9: 133-137, (1987).

Kalya R, Oduori ML. The bacteriology of stools of children admitted to Kenyatta National Hospital suffering from diarrhoea. *East Afr. Med. J.* 49: 949-952, (1972).

Kapikian AZ. Viral gastroenteritis. *JAMA* 269: 627-630, (1993).

Kariuki S, Olsvik O, Mitema E, Gathuma J, Mirza N. Acquired tetracycline resistance genes in nosocomial Salmonella typhimurium infection in a Kenyan hospital. *East Afr. Med. J.* 70: 255-258, (1993).

Kennedy KI, Fortney JA, Bonhomme MG, Potts M, Lamptey P, Carswell W. Do the benefits of breast feeding outweigh the risk of post natal transmission of HIV via breast milk? *Trop. Doc.* 20: 25-29, (1990).

Kennedy KI, Fortney JA, Sokal DC. Breast feeding and HIV. *Lancet.* 1: 333, (1989).

- Ketsela T, Asfaw M, Kebede D. Patterns of breast feeding in Western Ethiopia and their relationship to acute diarrhoea in infants. *J. Trop. Pediatr.* 36: 180-183, (1990).
- Khan MU. Breast feeding, growth and diarrhoea in rural Bangladesh children. *Hum. Nutr. Clin. Nutr.* 38: 113-113, (1984).
- Kinoti SN, Maggwa ABN, Turkish J, Wassuna A. Management of acute childhood diarrhoea with oral rehydration therapy at Kenyatta National Hospital, Nairobi, Kenya. *East Afr. Med.J.* 62: 5-11, (1985)
- Kirkwood BR. Diarrhoea. In: Disease and mortality in sub-saharan Africa. Ed. Feacham RG, Jamison DT. A world bank publication. Oxford University press. 1991.
- Knutton S, Balddini MM, Kaper JB, McNeish AS. Role of plasmid coded adherence factor in adhesion of *E. coli* to Hep-2 cells. *Infect. Immun.* 55: 78-85, (1987).
- Konno K, Suzuki H, Imai A, Katsuzawa T, Ishida N,

- Katsushima N, Sakamoto M, Kitaooka S, Tsuboi R, Adachi M. A long term survey of Rotavirus infection in Japanese children with acute gastroenteritis. J. Infect. Dis. 138: 569-576, (1978).
- Kotler D. Clinical gastrointestinal diseases in AIDS. Abstr. 5th Int. Conf. on AIDS, pp 208, (1989).
- Kronvall G. Rapid slide agglutination method for typing pneumococci by means of specific antibody adsorbed to protein A-containing staphylococci. J. Med. Microbiol. 6: 187-190, (1973).
- Kulapongs P, Vithayasai V, Suskind R, Oslon RE. Cell mediated immunity, phagocytosis and killing function in children with severe iron deficiency anaemia. Lancet. 2: 689-691, (1974).
- Kunin CM. Resistance to antimicrobial drugs-a worldwide calamity. Ann. Int. Med. 118: 557-561, (1993).
- Lane EM, Batchelor RA, Bourgeois AL, Burr DH, Olson JG. Urine and faecal IgA response during naturally

acquired infection with Campylobacter jejuni.
Lancet. 1: 1141, (1987).

Lew JF, Glass RI, Gangarosa RE, Cohen IP, Bern C, Moe CL. Diarrhoeal deaths in the united States, 1979 through 1987. JAMA. 265: 3280-3284, (1991).

Lloyd-Evans N, Drasar BS, Tomkins AM. A comparison of prevalence of Campylobacter, Shigella and Salmonellae in faeces of malnourished and well nourished children in Gambia and Northern Nigeria. Trans. Roy. Soc. Trop. Med Hyg. 77: 245-247, (1983).

Lunn PG, Northrup-Clewes CA, Downes RM. Chronic diarrhoea and malnutrition in the Gambia: studies on intestinal permeability. Trans. Roy. Soc. Trop. Med and Hyg. 85: 8-11, (1991).

Luta M, Anabwami G, Braddick M. HIV in malnourished children at Kenyatta National Hospital, Nairobi, Kenya. Abstr. V Int. Conf. on AIDS, Montreal, Canada, pp. 218, (1989).

Makino Y, Matsumoto I, Chiba Y, Mohammed OA, Ogaja PO,

- Kibue AM, Muli JM, Nakitare GW. Virological survey of children in Nyeri and Mombasa monthly survey of Rotavirus in faeces. East Afr. Med.J. 60: 536-541, (1983).
- Mancini G, Carbonara AO, Heremans JM. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochem. 2: 235-241, (1965).
- Mania A. Machakos project studies. Beliefs and practices concerning treatment of measles acute diarrhoea among the Akamba. Trop. Geogr. Med. 31: 139-148, (1979).
- Mata LJ. Environmental determinants and origin of malnutrition. In: Suskind RM (ed.). Malnutrition and immune response. New York: Raven press, 9-19, (1981).
- Mata LJ, Urruttia JJ, Gordon JE. Diarrhoea disease in a cohort of Guatemalan village children observed from birth to two years. Trop. Geogr. Med. 19: 247-251, (1967).
- Mathewson JJ, Dupont HL, Morgan DR, Ericsson CC.

Enteroadherent E. coli associated with traveller's diarrhoea. Lancet. 1: 1048, (1983).

Mathur R, Reddy V, Naidu AN, Ravikumar, Krishnamachari KA. Nutritional status and diarrhoeal morbidity: a longitudinal study in rural Indian pre-school children. Hum. Nutr. Clin. Nutr. 39: 447-454, (1985).

Mersha D, Tiruneh M. Frequency of isolation of Cryptosporidium oocysts in Ethiopian children with acute diarrhoeal disease. East Afr. Med. J. 69: 314-315, (1992).

Mirza NB, Wamola IA. Salmonella typhimurium outbreak at Kenyatta National Hospital. East Afr. Med J. 66: 453-457, (1989).

Mngola EN. Cholera. In: Health and disease in Kenya. Ed. Vogel LC, Muller AS, Odingo RS, Onyango Z, De Geus A. East African Lit. Bureau. 1974.

Morgan DR, Dupont A, Gonik B, Kohl S. Cytotoxicity of human peripheral blood and colostral leucocytes against Shigella species. Infec.

Immun. 46: 25-33, (1984).

- Mutanda^a LN. Epidemiology of acute gastroenteritis in early childhood in Kenya: Aetiological agents. Trop. Geog. Med. 32: 138-141, (1980).
- Mutanda^a LN, Masudi AM, Kangethe SK, Patel A. Emergence of gentamicin resistant E. coli 0111:k58 in a nursery ward at Kenyatta National Hospital. East Afr. Med. J. 64: 241-247, (1987).
- Mutanda^b LN. Dysentery associated with invasive E. coli 0128ac in an adult. A case report. East Afr. Med. J. 64: 418-423, (1987).
- Mutanda^b LN. Epidemiology of acute gastroenteritis in early childhood in Kenya. I: Incidence in hospitals. J. Trop. Pediatr. 26: 172-176, (1980).
- Mutanda LN, Patel A, Masudi AM, Maina G. Aetiology of diarrhoea in pre-term neonates at Kenyatta National Hospital nursery, Nairobi, Kenya. East Afr. Med. J. 67: 223-230, (1990).
- Mutanda LN, Kangethe SK, Kakai R, Gathecha C. Diarrhoea and Trichomonas hominis. Medicom. 8: 11-13,

(1986).

- Mutanda^c LN. Epidemiology of acute gastroenteritis in early childhood in Kenya. V: An inverse relationship between the peak age incidence and the waning of Rotavirus maternal antibodies. East Afr. Med. J. 57: 545-548, (1980).
- Mutanda^d LN. Epidemiology of acute gastroenteritis in early childhood in Kenya VI. Some clinical and laboratory characteristics related to the aetiological agents. East Afr. Med. J. 57: 599-606, (1980).
- Mutanda^a LN, Kangethe SK, Juma R, Lichenga EO, Gathecha C. Aetiology of diarrhoea in malnourished children at Kenyatta National Hospital. East Afr. Med. J. 62: 835-841, (1985).
- Mutanda^c LN. Epidemiology of acute gastroenteritis in early childhood in Kenya. III: East Afr. Med. J. 57: 317-326, (1980).
- Mutanda^c LN. A prospective analysis of the occurrence of Shigella flexneri serotypes in some African countries. East Afr. Med. J. 64: 816-820,

(1987).

- Mutanda^b LN. A review of ten years of Rotavirus study in Africa. *Medicom* 7: 37-41, (1985).
- Nakane PK, Kawaoi A. Peroxidase labelled antibody: A new method of conjugation. *J. Histochem. Cytochem.* 22: 1084-1091, (1974).
- Northrup R. Technical literature update on diarrhoea. 7: 1, (1992).
- Oburra NM. A study of factors influencing the outcome of acute childhood diarrhoea at Paediatric observation ward of Kenyatta National Hospital. M. Med. Thesis, Nairobi University, (1986).
- Okeahialam TC. Giardiasis in protein energy malnutrition. *East Afr. Med. J.* 59: 765-770, (1982).
- Olusanya O, Taiwo O. Rotavirus as an aetiological agent of acute childhood diarrhoea in Ile, Ife, Nigeria. *East Afr. Med. J.* 66: 100-104, (1989).

- Omondi-Odhiambo, Voorhoeve AM, Van Ginneken JK. Age-specific infant and child mortality and causes of death. An epidemiological study. Ed. Van Ginneken JK and Muller AS. In: Maternal and child health in rural Kenya. London and Sydney: Croom Helm. (1984).
- Oniang'o RK. Trends in infant feeding - bottle feeding revisited. *Medicom* 7: 163-166, (1985).
- Patel IU, Bhushan V, Chintu C, Bathirumathan. Bacteriology study of diarrhoea in children at University teaching hospital, Lusaka, Zambia. *East Afr. Med. J.* 59: 793-797, (1982).
- Paul MO, Erinle EA. Influence of humidity on Rotavirus prevalence among Nigeria infants and young children with gastroenteritis. *J. Clin. Microbiol.* 15: 212-216, (1982).
- Pizzo PA, Butler KM. In the vertical transmission of HIV, timing may be everything. *New Eng. J. Med.* 325: 652-654, (1991).
- Popkin BM, Adair L, Akin JS, Black R, Briscoe J, Fliieger W. Breast feeding and diarrhoea morbidity.

Pediatr. 86: 874-882, (1990).

Qifang P, Fuxi Q, Furong Y, Schuzhen C, Yugins, Quiying L, Zimin H, Baoyun Z. Studies on the aetiology of autumnal infantile acute gastroenteritis-Rotavirus. Chin. Med. J. 93: 36-40, (1980).

Regua AH, Bravo VLR, Leal MC, Lobo Leite MEL. Epidemiological survey of enteropathogenic E. coli isolated from children with diarrhoea. J. Trop. Pediatr. 36: 176-179, (1990).

Reka S, Brooks E, Kotler DP. Effects of malnutrition and intestinal infection upon the mucosal inflammatory response. Abstr. 7th Int. Conf. AIDS, pp 246, (1991).

Riepenhoff-Talty M, Bogger GS, Li P, Patricia JC, Baret HJ, Ogra PL. Development of serum and intestinal antibody response to Rotavirus after naturally acquired Rotavirus infection in man. J. Med. Vir. 8: 215-222, (1981).

Robinson RD, Murphy EL, Wilks RJ, Neva FA, Terry SI, Hanchard B, Figueroa JP, Blattner WA. Gastrointestinal parasitic infection in

healthy Jamaican carriers of HTLV-1. J. Trop. Med. Hyg. 94: 411-415, (1991).

Roilides E, Clerici M, Deplama L, Rubin M, Pizzo PA, Shearer GM. Helper T-cell responses in children infected with HIV-1. J. pediatr. 118: 724-730, (1991).

Roitt IM. Essential immunology. Blackwell Scientific publications. 6th edition, 1988.

Rowley D, Brooy LJ. Intestinal immune responses in relation to diarrhoeal diseases. J. Diarr. Dis. Res. 4: 1-9, (1986).

Ruiz-Palacios GM, Calva JJ, Pickering LK. Protection of breast fed infants against Campylobacter diarrhoea by antibodies in human milk. J. Pediatr. 116: 707-713, (1990).

Saha K, Chopra K, Dua N. Raised stool and serum IgA levels in malnourished infants with chronic diarrhoea and associated parasitic infections. J. Trop. Pediatr. 36: 69-74, (1990).

Sahid NS, Mizanur Rahman ASN, Aziz KMA, Faruque ASG,

Bari MA. Beliefs and treatment related to diarrhoeal episodes reported in association with measles. *Trop. Geogr. Med.* 35: 151-156, (1983).

Sallon S, Showa R, Masri M, Khalil M, Blundel N, Hart CA. Cryptosporidiosis in children in Gaza. *Ann. Trop. Med.* 11: 277-281, (1991).

Sang FC, Kangethe SK, Orinda VA, Gatheru Z, Black RE, Waiyaki PG. Escherichia coli associated with acute measles at Kenyatta National Hospital. *East Afr. Med. J.* 69: 135-139, (1992).

Schrimshaw NS, Taylor CE, Gordon JE. Interactions of nutrition and infection: Geneva WHO monograph series. No. 57, (1968).

Scoub BD, Prozesky OW, Lecatsas G, Oothuizen R. The role of breast feeding in the prevention of Rotavirus infections. *J. Med. Microbiol.* 2: 25-31, (1977).

Shearer GM, Clerici M. Editorial review: T-helper cell defects in HIV infection. *AIDS (journal)* 5: 245-253, (1991).

- Sima H, Lu quang Zhen, Mathan MM, Mathew MM, Orlate J, Espejo R, Khim Maung U, Ghafoor MA, Khan MA, Sami Z, Sutton RG. Etiology of acute diarrhoea among children in developing countries: a multicentre study in five countries. Bull. WHO. 69: 509-642, (1991).
- Simango C, Rukure G. Potential sources of Campylobacter species in homes of farm workers in Zimbabwe. J.Trop. Med. Hyg. 94: 388-392, (1991).
- Skirrow MB. Campylobacter enteritis: A new disease. Br. Med. J. 2: 9-11, (1977).
- Smith PD, Quinn TC, Strober W, Tanoff EN, Masur H. Gastrointestinal infections in AIDS. Ann. Inter. Med. 116: 63-77, (1992).
- Snyder JD, Merson MH. The magnitude of the problem of acute diarrhoeal disease: A review on active surveillance data. Bull. WHO. 60: 605-613, (1982).
- Sonza S, Holmes IH. Coproantibody response to Rotavirus infection. Med. J. Austr. 2: 496-499, (1980).

- Stals F, Walther FS, Bruggenman CA. Faecal and pharyngeal shedding of Rotavirus and Rotavirus IgA in children with diarrhoea. J. Med. Vir. 14: 333-339, (1984).
- Steel EJ, Chaicumpa W, Rowley D. Further evidence of cross-linking as a protective factor in experimental cholera: properties of antibody fragment. J. Infect. Dis. 132: 175-180, (1975).
- Stephen J, Pietrowski RA. In: Bacterial toxins. 1981.
- Sullivan PB, Marsh MN, Phillips MB, Dewwit O, Neale G, Cevallos AM, Yamsdon P, Farthing MJG. Prevalance and treatment of giardiasis in chronic diarrhoea and malnutrition. Arch. Dis. Child. 65: 304-306, (1990).
- Taglibue A, Nencioni L, Villa L, Keren DF, Lowell GH, Boraschi D. Antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes with secretory IgA. Nature 306: 184-186, (1983).
- Tardelli GT, Rassi V, McDonald K, Silva Ramos SRT, Trabulsi

LR, Vieira AM, Guth BEC, Candeias JAN, Ivey C, Toledo MRF, Blake PA. Enteropathogens associated with acute diarrhoea disease in urban infants in Sao Paulo, Brazil. J. Infect. Dis. 164: 331-337, (1991).

Tomasi Jr. TB. Secretory immunoglobulins. New Eng. J. Med. 287: 500-506, (1972).

Tomkins A. Nutritional status and severity of diarrhoea among pre-school children in rural Nigeria. Lancet. 1: 860-862, (1981).

Totterdel BM, Chrystie IL, Banativala JE. Cord blood and breast milk antibodies in neonatal Rotavirus infection. Br. Med. J. 1: 828-830, (1980).

Trabulsi LR, Toledo MRF, Murahovschi J, Neto UF, Candeias JAN. Epidemiology of infantile bacterial diarrhoea disease in Brazil. In: Bacterial diseases (eds.) Takeda Miwatani, KTK Scc. Pub., Tokyo 1985.

Trajman A, Belo M, Oliveira A, Zaltam C, Elia C. Early changes of enteric humoral immunity in HIV disease. Abstr. VII Int. Conf. on AIDS/III STD

world Congress, Amsterdam, Netherlands, pp. 141, 19-24 July 1992.

- Tzipori S, Campell I. Prevalence of Cryptosporidium antibodies in ten species of animals. Clin. Microbiol. 14: 455-461, (1981).
- Valman HB. Diarrhoea: the first five years of life. Br. Med. J. 280: 701-703, (1980).
- Waiyaki PG, Kibue AM, Sang FC, Ichoroh CJ, Shimotori S, Echara M, Ichinose Y, Watanabe S. Occurrence of *C. jejuni* in diarrhoeal stools at Coast General Hospital and Mvita clinic, Mombasa. Abstr. 23/84 Proc. 5th Ann. Med. Sci. Conf. KEMRI/KETRI, Nairobi. 6th-10th Feb. 1984.
- Waiyaki PG, Sang FC, Ngugi JM. Enterotoxigenic Escherichia coli infection in childhood diarrhoea in Mombasa, Kenya. East Afr. Med. J. 63: 29-35, (1986).
- Wamola IA, Mirza NB. Problems of Salmonella infections in Kenya. East Afr. Med. J. 58: 677-701, (1981).
- Wamola IA, Itotia JN, Say PJ, Cruickshank B. Diarrhoeal

diseases due to Shigella, Salmonella and enteropathogenic E. coli. In: Health and diseases in Kenya, ed. Vogel LC, Muller AG, Odingo RS, Onyango S, De Guess A. pp 185-192, (1974).

- Wamola IA. Bacterial stool pathogens in Kenyatta National Hospital. East Afr. Med. J. 57: 867-871, (1980).
- Wamola IA, Mirza NB, Ngugi JM, Bwibo NO. Campylobacter gastroenteritis in Nairobi. East Afr. Med. J. 60: 146-149, (1983).
- Watson RS, McMurrey DN. The effects of malnutrition on secretory and cellular immune processes. Crit. Rev. Fd. Sci. Nutr. 12: 113-159, (1980).
- Wenman WM, Hinde D, Feltham S, Gurwith M. Rotavirus infection in adults. N. Engl. J. Med. 301: 303-306, (1979).
- WHO Bull. Development of vaccines against shigellosis: memorandu from a WHO meeting. WHO Bull. 65: 17-25, (1987).

- WHO Bull^a. Escherichia coli diarrhoea: WHO Scientific working group. WHO bull. 58: 23-36, (1980).
- WHO Bull^b. Enteric infections due to Campylobacter, Yersinia, Salmonella and Shigella: WHO Scientific working group. WHO Bull. 58: 519-537, (1980).
- WHO/CDD. Manual for treatment of acute diarrhoea. WHO/CDD/SER/30, 1980.
- WHO/CDD^a. Programme for control of diarrhoeal diseases. Interim report, 1986, WHO/CDD/87.26, pp 27-28, 1987.
- WHO/CDD. Programme for control of diarrhoeal diseases. Sixth programme report, 1986-1987, WHO/CDD/88.28, 1988.
- WHO/CDD^b. Persistent diarrhoea in children in developing countries. WHO/CDD/88.27, Geneva, WHO 1987.
- WHO/SPA. Special programme on AIDS statement, breast feeding/breast milk and HIV, SPA/INF/87.8. 1987.

- WHO/VPH. Report of the WHO consultation on veterinary public health aspects of prevention and control of Campylobacter infections. WHO: VPH/ CDD/ FCS/ 81.1, 1984.
- Williams RC, Gibbons RJ. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. *Science* 177: 697-699, (1972).
- Wilson MB, Nakane PK. Recent developments in the periodate method of conjugating horse radish peroxidase (HRPO) to antibodies. In: *Immunofluorescence and related staining techniques*. Eds. W. Knapp, K. Holubar, G. Wick. Elsevier/North Holland Biomed press. pp. 215-224, 1978.
- Winfred I. The incidence of neonatal infections in a nursery unit at the Ahmadu Bello University teaching hospital, Zana, Nigeria. *East Afr. Med. J.* 61: 197-202, (1984).
- Woodruff JF, Kibourne ED. The influence of postweaning undernutrition in Cocksackie B3 infection of adult mice. 1. Viral persistence and increased

severity of lesions. J. Infect. Dis. 121: 135-137, (1970).

Wyatt RG, Harvey DJ, Pittman AL, Yasutaka H, Greenberg HB, Kalica AR, Flores J, Kapikian AZ. Direct isolation in cell culture of human Rotaviruses and their characterization into four serotypes. J. Clin. Microbiol. 18: 310-317, (1983).

Volken RH, Wyatt RG, Kim HW, Kapikian AZ, Chanock RM. Immunological response to infection with human reovirus-like agent: Measurement of anti-human reovirus-like agent immunoglobulin G and M levels by the method of enzyme-linked immunosorbent assay. Infect. Immun. 19: 540-546, (1978).

Zu SX, Fang GD, Fayer R, Guerrant R. Cryptosporidiosis: Pathogenesis and immunology. Parasit. Today. 8: 24-27, (1992).

APPENDIX

QUESTIONNAIRE FOR DIARRHOEA STUDY

Date Day____Month____Year_____

Lab.No. _____Clinic.No. _____

Name of child _____

Age (months) _____

Weight (Kg) _____

Height (cm) _____

Sex 0 Male

1 Female

Name of mother _____Residence _____

Duration of diarrhoea (in days) _____

Mode of feeding 0 Breast feeding

1 Bottle feeding

2 Mixed feeding

Severity of diarrhoea: 0 Mild

1 Moderate

2 Severe

Nutritional status 0 Normal

1 Marasmus

2 Kwashiorkor

3 Marasmic/kwashiorkor

Maternal HIV status 0 Negative
 1 Positive

Child HIV status 0 Negative
 1 Positive

Laboratory results:

1. Stool examination

a. Wet preparation 0 Pus cells
 1 Red blood cells
 2 Ova or cysts of parasites

b. Culture and ELISA

Salmonella 0 Negative
 1 Positive

Shigella 0 Negative
 1 Positive

E. coli 0 Negative
 1 Positive

Campylobacter 0 Negative
 1 Positive

Rotavirus 0 Negative
 1 Positive