SUBJECT

EPIDEMIOLOGY OF ACUTE GASTROENTERITIS IN EARLY CHILDHOOD IN SELECTED URBAN AREAS OF KENYA

By

LABIUS NSANGWA MUTANDA M.H.Sc. (U.S.A.)

THIS THESIS HAS BEEN ACCEPTED FOR THE DEGREE OF D.P.H. 1978 AND A COPY MAY BE PLACED IN THE UNIVERSITY LIBRARY

A thesis submitted in fulfilment for the degree of DOCTOR OF PHILOSOPHY

in the

UNIVERSITY OF NAIROBI

This thesis is my original work and has not been presented for a degree in any other University.

LABIUS NSANGWA MUTANDA
(Candidate)

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

PROFESSOR F.J. BENNETT
(University Supervisor)

DR. D. METSELAAR
(University Supervisor)
So many people have been helpful in one way or another during these studies that it is not possible to thank them individually. In particular, I would like to thank Dr. D. Metselaar for having allowed me, in the first instance, to work in his laboratory as a visiting research worker and also later for having accepted to be one of my project advisors. I am also very grateful to the Director and Mr. W.t'Mannetje of the Medical Research Centre, Nairobi; the former provided me with free laboratory facilities and the latter with statistical expertise. I am very much indebted to both the former Director, Dr. J.N. Itotia, of the National Public Health Laboratories and the Director of the International Centre of Insect Physiology and Ecology, Nairobi, for their offers of Laboratory facilities as well. My thanks are due to Professor F.J. Bennett, Department of Community Health, Nairobi University for his invaluable advice and also to Dr. Audrey Glanert (WHO Short-term Consultant to the International Centre of Insect Physiology and Ecology) and Mr. M.D. Chimtawi of the same Centre for their help with the electron-microscopy of some faecal preparations. I acknowledge with thanks the services rendered to me during the period of data and/or specimen
collection by the following: Nursing Sister Anne Marie Christensen, Mombasa Old Town Health Centre; the Matron, F. Fenwick, Gertrude Garden Children's Hospital; Miss A.J. Tennant, Medical Records Officer, Aga Khan Hospital and the Paediatricians of Kenyatta National Hospital Observation Ward, and those of Kisumu and Mombasa Provincial Hospitals. I owe many thanks to Mrs. Mary Maina for typing this thesis and to my wife for her encouragement during the initial stages of this study. Many thanks are due to the former East African Community which funded most of the project and the Kenya Government which has continued financing the rest of this long-term research project.

TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Table of Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>i</td>
</tr>
<tr>
<td>Declaration</td>
<td>ii</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>iii</td>
</tr>
<tr>
<td>Table of contents</td>
<td>iv</td>
</tr>
<tr>
<td>List of tables</td>
<td>ix</td>
</tr>
<tr>
<td>List of figures</td>
<td>xi</td>
</tr>
<tr>
<td>Abstract</td>
<td>xii</td>
</tr>
</tbody>
</table>
# I. INTRODUCTION

Aim and objectives of the study 17

Definitions 19

## II. GEOGRAPHICAL BACKGROUND OF THE COUNTRY.

Position, size and relief 20

Climate 21

Population 22

## III. MATERIALS AND METHODS

1. Sources of data:
   1.1 Data on the magnitude of the problem of childhood gastroenteritis and on age-incidence of infants and children with diarrhoea. 23
   1.2 Feeding practice data 24
   1.3 Meteorological information 24

2. Population studied for aetiological agents and periods of study:
   2.1 Group 1: Hospitalized 25
   2.2 Group 2: Out-patients 26
   2.3 Group 3: Asymptomatic 26

3. Selection and examination of children 27
4. Collection and examination of specimens

4.1 Collection

4.2 Examination of stool specimens and sera

4.2.1. Stool culture for bacteria

4.2.2. Virology of stools

4.2.2.1 Demonstration of rotavirus by E.M. and by immunofluorescence.

4.2.2.2 Testing the specificity of the immunofluorescent test.

4.2.2.3 Isolation of enteroviruses.

4.2.3 Tests for antibodies in serum specimens

4.2.3.1 The neutralization test for antibodies against E.coli toxins.

4.3 Macroscopic and microscopic inspection of stools

4.3.1 Inspection of stools.

4.3.2 Microscopy of stools.

IV. RESULTS

1. Observations on the frequency of gastroenteritis

1.1. Observations in Nairobi

1.2. Age distribution of hospitalized children.
2. **Aetiology of acute childhood enteritis**

2.1. Results of the search for aetiological agents. 39

2.2. Seroconversion to rotavirus infection. 41

2.3. Seroconversion to E. coli (LT) toxin. 41

2.4. Seroconversion to adenovirus infection. 41

2.5. Further studies on evidence of infection in children by adenovirus and entero-toxigenic E. coli:

2.5.1. Adenovirus 42

2.5.2. Enterotoxigenic E. coli. 43

3. **Distribution over the period of observation of the aetiological agents.**

3.1. Kenyatta National Hospital 43

3.2. Kisumu Provincial Hospital and Lumumba Health Centre. 44

3.3. Mombasa Provincial Hospital and Old Town Health Centre. 44

3.4. Masailand. 45

3.5. Patterns of relative enteropathogen frequency in Nairobi, Kisumu and Mombasa 45

3.6. Distribution of the aetiological agents by age and sex:

3.6.1. Rotavirus incidence by age. 46

3.6.2. Shigella incidence by age. 46
3.6.3. *E. coli* incidence by age. 47

3.6.4. Distribution in Nairobi of the aetiological agents by sex. 47

4. Testing the hypothesis that the peak age incidence is inversely related to waning of rotavirus maternal antibodies: 47

5. The Possible contributory factor of bottle-feeding to the aetiological agents: 49

6. Some clinical and Laboratory characteristics relative to the aetiological agents: 50

   6.1. Clinical Features:

   6.1.1. Fatality of acute diarrhoea 50

   6.1.2. Duration of illness 51

   6.1.3. Degree of dehydration 51

   6.1.4. Body temperature 52

   6.2. Laboratory findings 52

   6.2.1. Macroscopy of stool appearance 52

   6.2.2. Microscopy of stools 53

7. Extent of rotavirus infection in animals 54

   7.1. Domestic animals 54

   7.2. Rodents 54

V. DISCUSSION 55

1. Incidence of early childhood gastroenteritis in hospitals in Nairobi. 55

2. Aetiology. 58
3. Distribution of Patterns of the aetiological agents in different geographical areas. 63
4. Prevalence of the aetiological agents by age and sex. 66
5. The possible contributory factor of bottle feeding in frequency of infections. 69
6. Some clinical characteristics relative to the aetiological agents. 72
7. Conclusions and Summary 78

VI. REFERENCES 112

List of Tables

Table 1  Number of children with severe childhood diarrhoea observed in hospitals in Nairobi City. 81
Table 2  Monthly distribution of diarrhoeal cases at Gertrude Garden Children's Hospital. 82
Table 3  Age distribution of infants and children admitted to various hospitals with severe diarrhoea. 83
Table 4  Enteric pathogens isolated from 36 children with diarrhoea and 36 controls. 84
Table 5  Single and mixed infections in children with diarrhoea. 85
Table 6 Results of tests for complement fixing antibodies against rotavirus in children with acute gastroenteritis and in controls.

Table 7 Seroconversion to \textit{E.coli} heat-labile enterotoxin.

Table 8 Monthly distribution of enteric pathogens in Nairobi during 1975-1976.

Table 9 Number of enteropathogens isolated from in and out-patients in Kisumu during January, May and October, 1977.

Table 10 Number of enteropathogens isolated from in and out-patients in Mombasa during January, May and November, 1977.

Table 11 Age distribution of rotavirus infections in Nairobi, Kisumu and Mombasa.

Table 12 Age distribution of rotavirus infections in out and in-patients in Mombasa.

Table 13 Age distribution of shigella infections in Nairobi, Kisumu and Mombasa.

Table 14 Age distribution of \textit{E.coli} infections in Nairobi, Kisumu and Mombasa.

Table 15 Relation of sex to infection with rotavirus, shigella and \textit{E.coli} in Nairobi.

Table 16 Levels of antibodies in cord and follow-up of children's sera.

Table 17 Child feeding practices in Nairobi, Kisumu and Mombasa.
Table 18 Enteropathogens isolated from breast, bottle and mixed fed infants in Nairobi.  
Table 19 Degree of dehydration and aetiological agents.  
Table 20 Relationship between temperature and enteric pathogens.  
Table 21 Stool appearance and enteropathogens.  
Table 22 Association between Erythrocytes and Leucocytes in faeces and enteropathogens.  
Table 23 Rotavirus C.F. antibodies in sera of animals.  

List of Figures  
Figure 1 Map of Kenya showing major geographical divisions.  
Figure 2 Monthly distribution of cases with diarrhoea admitted to Gertrude Children's Hospital compared with the total number of admissions.  
Figure 3 Incidence of diarrhoea in relation to rainfall.  
Figure 4 Comparative age distribution of infants and children hospitalized to POW for severe diarrhoea, measles and those seen
A study aimed at elucidation of the incidence and aetiology of early childhood acute gastroenteritis in Kenya was undertaken. Included among areas of study were the distribution of the aetiologica l agents in time, place and by age and sex, the role of animals, if any, as reservoirs of viral agents, the contributory factor of bottle-feeding to the aetiological agents and some clinical characteristics relative to the aetiological agents.
It was found that during some selected periods between 1971-1977, nearly 20% of admissions to Kenyatta National Hospital Paediatric Observation Ward, Gertrude Garden Children's Hospital and Aga Khan Children's Ward, all in Nairobi City, were due to diarrhoea. The number of cases admitted to Gertrude Garden Children's Hospital during different months of the year differed significantly, but with no apparent correlation with either the temperature or relative humidity or rainfall. January gave the highest percentage (33.5%) and October the lowest (14.8%).

The age distribution of infants and children admitted for diarrhoea to Kenyatta National, Gertrude Garden Children's, Kisumu Provincial and Siaya District hospitals indicated that the peak incidence was in the 3-5 months age-group. Comparison of the ages of infants and children admitted to one of the hospitals (Kenyatta National) for severe diarrhoea, measles and those seen at the Filter Clinic with mild diarrhoea, revealed that severe diarrhoea peaked in the 3-5 months age-group, measles infections in the 6-8 months and mild diarrhoea in the 12-14 months age-groups. The peak age-incidence of measles cases coincided with the waning of maternal antibodies previously reported by a collaborative study of the Ministry of Health of Kenya and the World Health Organization. This observation led to the investigation whether
the peak age incidence of early childhood gastroenteritis was also related to the waning of maternal antibodies against the major aetiological agent.

In the case-control study conducted at Kenyatta National Hospital, rotavirus was found to be the major aetiological agent of severe childhood diarrhoea; 14 (39%) of the 36 children with severe diarrhoea tested for viral excretion, by electron microscopy and the immunofluorescent test on infected tissue culture, had evidence of rotavirus infection as opposed to 2 (6%) in 36 controls. Six shigella species were isolated from the test group and none from the control. No significant difference was found between children with diarrhoea and controls with regard to frequency of isolation of *Escherichia coli* (both enteropathogenic and enterotoxigenic), *Salmonella* species and enteroviruses. Mixed infections occurred between rotaviruses and other enteropathogens. Of the 9 cases from which rotaviruses were isolated and paired sera tested, 7 seroconverted. Of the 14 serum pairs obtained from the control-group only 1, from whom a rotavirus had been isolated, had a four-fold Complement-fixation (C.F.) antibody titre rise. Only two children, from whom enterotoxigenic *E. coli* had been isolated, had four-fold titre rises against this agent. None of the 17 serum pairs obtained from children with
diarrhoea showed a significant titre rise to C.F. adenovirus group antigen.

In all the stools collected from a sample of 160 children with diarrhoea admitted to Kenyatta National Hospital over a one year period a probable aetiological agent was found in 72%. The overall isolation rate of all the enteropathogens looked for was 42(40%) out of 104 in Kisumu and 45(39%) out of 116 specimens in Mombasa. Rotavirus alone was isolated from 66(41%) of 160 specimens collected from children admitted to Kenyatta National Hospital. In Kisumu, rotavirus was isolated from stools of 22 (29%) out of 77 in-patients and 3(11%) out of 27 out-patients; whereas in Mombasa the virus was isolated from stools of 7(20%) out of 51 in-patients and 11(18%) out of 65 out-patients. Rotavirus was found in only 3 out of 139 asymptomatic Masai children. Rotavirus was isolated more frequently from infants than from children of 1 year and above. Infection with this virus was independent of the sex of the children tested.

Rotavirus antibodies were detected in 38(73%) out of a total of 52 sera collected from cord blood and neonates. Antibodies were found in only 7(11%) out of 61 sera obtained from the 2-5 months age-group. In the age-group 0-5 months there appeared to be an inverse relationship between patients with gastroenteritis
requiring admission to hospital and the pattern of waning of rotavirus maternal antibodies. After this age there was a gradual increase in the percentage of rotavirus antibodies in children tested and by the age of 3 years about 90% of the children had acquired antibodies. Rotavirus antibodies were found in 10(20%) out of 51 sera of adult cows and in 22(81%) out of 27 sera of Masai sheep. Antibodies were also detected in 2(2%) out of 24 sera of wild rats tested.

Like rotavirus Shigella was isolated from specimens collected in widely separated areas in the country, but probably due to an epidemic, it was isolated more often in Mombasa than in Nairobi or Kisumu. Infections were found to reach a peak in all the three places during the long rains (April-June) when rotavirus isolations in Nairobi and Kisumu were at their lowest rate. In Kisumu and Mombasa Shigella infection was found to be of rare occurrence in infants of 0-5 months; whereas in Nairobi Shigella species were isolated from 6(11%) of 56 infants aged 3-5 months. Shigella species were more frequently isolated from females than from males.

Enteropathogenic E. coli (EEC) was found most in Nairobi and least in Mombasa. Differences in bottle-feeding practices possibly contributed to these differences. Infection due to EEC was observed more in males than in females.
Salmonella was isolated infrequently in all the three places.

Attempts were made to assess the possible contributory factor of bottle-feeding to infection by enteropathogens. It was found that feeding practices among mothers in Kisumu and Mombasa were almost similar. Among those interviewed mixed feeding did not start until the second month after birth, and bottle feeding not until the third month. Mothers in Kisumu, moreover, breast-fed longer than those in Mombasa. In Nairobi both bottle and mixed feeding began during the first month and after the child reached 3 months of age most of the mothers had changed to mixed or bottle-feeding. A study of the distribution over the age-groups of enteropathogens isolated from breast-fed, bottle-fed and mixed-fed infants aged 0-5 months in Nairobi, failed to prove that bottle-feeding promoted the rate of E.coli infection. The results also failed to indicate whether bottle-feeding favours infection by rotavirus or Shigella.

Attempts were also made to assess the significance of some clinical characteristics related to enteropathogens. Out of 288 children hospitalized for diarrhoea only two cases were fatal. From both, Shigella was isolated. Among the three clinical features investigated, duration of illness was the only one found to be of some diagnostic help. Shigella was found to be
the agent most frequently isolated from diarrhoeas that had lasted for more than 1 week. Body temperature and degree of dehydration were found not to be of diagnostic value. Rice-water or white loose stools were fairly strongly associated with rotavirus infection, blood-stained and mucoid appearance with infection by *Shigella* species. Leucocytes and red blood cells in faeces were fairly strongly associated with *Shigella* and unlikely to be found in rotavirus infections.

When evaluating what knowledge has been obtained in pursuing the objectives of this study the following conclusions were drawn:

1. The magnitude of severe acute childhood gastroenteritis was 20% of admissions of children to hospital.

2. The highest percentage for admittance to hospital was in the 3-5 months age-group which coincided with the waning of maternal antibodies to rotavirus found to be the major aetiological agent.

3. The aetiological agents were widely distributed but the pattern and rate of infection was found to differ among places.

4. Antibodies to rotavirus were found in rats for the first time.

5. The results failed to show convincingly that
bottle-feeding promotes infection by enteropathogens.

(6) Rotavirus occurring alone was not isolated from diarrhoeal cases lasting more than 7 days.

(7) White-watery stools were strongly associated with rotavirus and mucoid blood-stained stools with Shigella.
Acute gastroenteritis constitutes a major cause of morbidity and mortality in children the world over, particularly in the developing countries. In these nations, the incidence may be as high as 115 episodes per 100 children per year for breast-fed infants under 5 months of age, and may reach 275 attacks per 100 children per year by weanling age. (Benenson, 1975). In India diarrhoeal diseases, excluding cholera, account for 1.4 million deaths in children annually (Paniker et. al, 1977). A study of the out-patients records in the Ethio-Swedish Paediatric Clinic in Addis Ababa showed that gastroenteritis was one of the leading complaints bringing children to the clinic (Habte and Debesai, 1964). In the experience of Erwa et. al, (1971) diarrhoea headed the list of all serious diseases prevalent among Sudanese children. In Uganda the disease is a major cause of death of infants and children (Bennett and Huckstep, 1975). Cobban, reported by Sen, Lapage and Glasset (1963) found that of the children attending the general outpatient's department of the University College Hospital, Ibadan, Nigeria, 23% were suffering from gastrointestinal infections. In Kenya, out of a total of 19,530 children admitted to Kenyatta National Hospital Observation Ward 3,800 (19.5%) had gastroenteritis (Kalya and Oduori, 1972). However, except for the later report data on the incidences of this disease in other hospitals within and outside Nairobi are not documented. Recently
Lee wenburg et al, (1978) have reported in a population of 4000 children under 5 years of age from a rural district (Machakos, Northern division) in Kenya, an average monthly incidence of diarrhoea of 21%, observed over a period of 1 year.

Observation on causation of childhood gastroenteritis from numerous studies points to an infectious origin. In the first place, the disease is primarily affecting small children. Incidence increases with age up to a period marked in developing countries by the end of the weaning period (Mata et. al, 1967).

In their letter to the Editor of the Lancet, Patterson and his co-workers (1975) stated that, like respiratory infections, acute gastroenteritis may be due to a multiplicity of viral organisms and these may differ in their relative occurrence in different age-groups and in different areas. Non-viral diarrhoea is frequently due to one of the classical enterobacteria such as Shigella, Salmonella and Escherichia coli (E.coli). After Bray (1945) had recovered strains of E.coli of the same biochemical and antigenic type from the majority of infants with summer diarrhoea, it was generally realised that community and hospital-acquired epidemic diarrhoea of infants often with high case-fatality rates was frequently associated with antigenically distinct serotypes. Diarrhoea may also be due to enterotoxigenic E.coli strains which do not belong to recognised pathogenic sero-
types and which are readily over-looked. The pathogenesis of diarrhoeal illness caused by these enterotoxigenic E. coli has been attributed to their ability to produce enterotoxins (Gorbach and Khurune, 1972). Evidence now suggests that transmissible plasmids determine the production of two types of toxins namely heat-labile (LT) enterotoxin and heat-stable (ST) enterotoxin. Some strains of E. coli produce both toxins. These toxins cause fluid to accumulate in the intestinal lumen (Smith and Gyles, 1970; Gyles et. al, 1974). E. coli LT toxin resembles cholera toxin antigenically and immunogenically. Both toxins stimulate adenyl cyclase activity in a variety of mammalian cultured cell systems (Formal et. al, 1973; Smith and Gyles, 1970 and Kantor, 1975). Several workers have incriminated these toxigenic strains in childhood diarrhoea (Gorbach and Khurune, 1972; Rudoy and Nelson, 1975; Guerrant et. al, 1975; Nalin et. al, 1975; Donta et. al, 1977 and Sebodo et. al, 1977). Data on the possible role of enterotoxigenic E. coli in acute infantile diarrhoea in African subjects are still scarce. Schoub et. al, (1975) reported enterotoxigenic E. coli in 3 out of 101 South African children with diarrhoea, and none from 93 controls. Among the 173 species of enterotoxigenic bacterial strains isolated from 354 stools obtained from Ethiopian infants and children with acute gastroenteritis 38% were enterotoxigenic E. coli (Wadstrom et. al, 1976). More recently Sack et. al, (1977) have found enterotoxigenic E. coli in 17 of the 27 adult
American Peace Corps Volunteers who, while in Kenya, developed diarrhoea and in 1 of the 12 healthy persons. Their findings, therefore, prompted the investigation of the relative role of these \textit{E. coli} in infantile diarrhoea in Nairobi.

Invasive strains of \textit{E. coli} that cause an illness similar to shigellosis (DuPont et al, 1971 and Formal et al, 1973) usually are able to penetrate the intestinal mucosa, cultured mammalian cells, and the guinea pig or rabbit conjunctiva (Sereny, 1955). A third type of mechanism of \textit{Escherichia coli}, a cytotoxin similar to that produced by \textit{Shigella} relative to their diarrhoeagenic potential has been suggested by Canty and Blake (1977). Presently, reports on the significance of enteropathogenic \textit{E. coli} in childhood diarrhoea are conflicting. Gangarosa and Merson (1977) who have reviewed epidemiologic assessment of the relevance of enteropathogenic serogroups of \textit{E. coli} in diarrhoea concluded that although serotyping can be a useful tool in the investigation of nursery out-breaks when the number of diarrhoea cases exceeds expected levels, diagnosis of diarrhoea caused by enteropathogenic \textit{E. coli} is unreliable when the evidence is based solely on serologic identification. On the other hand Gurwith et al, (1977a) who have carried out clinical and laboratory assessment of
the pathogenicity of serotyped enteropathogenic \textit{E. coli}
noted that although serotyping rarely identify invasive or enterotoxin-producing organisms, clinical features of infants with enteropathogenic \textit{E. coli}-associated gastro-enteritis suggest that these infants may represent a distinctive and clinically important group with gastro-enteritis of greater severity than in non-bacterial gastro-enteritis. This view was supported by experiments by Levine et. al, (1978). They fed three non-enterotoxigenic but enteropathogenic serotypes isolates from outbreaks of infantile diarrhoea to adult volunteers. Two strains caused diarrhoea.

Various subgroups of enterobacteriaceae have also at one time been incriminated in the causation of infantile diarrhoea (Wilson and Miles, 1955). The role of these various intestinal bacteria of doubtful pathogenic potential was thus again studied bacteriologically and clinically by Mohieldin et. al, (1966) in Cairo, Egypt in 100 cases of infantile diarrhoea and 50 controls. The organisms studied included species and subgroups of the Para Colon group, Proteus group, Enterobacter and Citrobacter groups as well as Alcaligenes, Pseudomonas aeruginosa, Aeromonas and Klebsiella organisms. The results led to the conclusion that although Proteus providenciae organisms might be involved in some diarrhoeal cases there was not sufficient proof of the pathogenicity of the other bacteria studied. Wadstrom et. al, (1976) found that these organisms can produce
enterogenic toxin similar to that of *E. coli*. Since their study was not controlled it was not known whether the toxin was the one responsible for the acute childhood gastro-enteritis. Recently, Schoub et al. (1977b) isolated 4 strains of *Klebsiella pneumoniae*, 2 of *Enterobacter cloacae*, 1 of *Proteus vulgaris* that produced heat-labile toxin. However, six of the patients were concomitantly infected with other enteric pathogens which made it difficult to decide on the role of the toxin in the causation of symptoms.

Studies designed to provide data on the role of different types of the classical enteropathogenic bacteria in infantile diarrhoeal diseases in African countries have been carried out by various investigators. In Uganda Buttner and Lado-Kenyi (1973) studied 1018 children under the age of four years; 272 (26.6%) of the children had diarrhoea. The 1018 children yielded only 34 (3.3%) *Shigella*, 54 (5.3%) enteropathogenic *E. coli* and 8 (0.8%) *Salmonella*. The prevalence of intestinal pathogens differed little between children with diarrhoea and children without diarrhoea. In Kenya, Wamola et al. (1974) undertook a hospital records study covering the period 1966-72 to elucidate the frequency of isolations of enteropathogens from patients with diarrhoea seen at Kenyatta National Hospital. Of a total of 4396 isolations, *Shigella* was found to account for 3060 (70%) compared with *Salmonella* types 769 (17.5%) and
enteropathogenic *E. coli* serotypes 567 (13%). Analysis of the results obtained in Nairobi by Kalya and Oduori (1972) from their study of diarrhoea in children at Kenyatta National Hospital Observation Ward, showed that 29 (16.1%) of the 180 children sampled had pathogenic bacteria: 17 with *E. coli*, 8 with *Shigella*, 4 with *Salmonella*. It can be clearly seen that at Kenyatta National Hospital, infections with these groups of pathogenic bacteria account for only a small proportion of the total cases of diarrhoea. Kalya and Oduori (1972) made a comment that perhaps the cause of gastroenteritis is viral or parenteral in origin.

Even in the most carefully conducted studies of acute gastroenteritis in different parts of the world, bacterial pathogens have reportedly been isolated in less than 30% of the children. It was, therefore, assumed that viruses might play some part in the aetiology, but until recently there was no clear-cut evidence to support this. Only in some of these earlier studies significantly higher virus isolation rates were found in children with diarrhoea in comparison with virus occurrence in non-diarrhoeal infants and children (Ramoz-Alverez and Olarle, 1964 and Goodwin et. al, 1967). Other studies like those of Yow et. al, (1963); Malherbe and Roux, (1963); Scott et. al, (1967) and Bell and Grist, (1967), failed to show significant differences in the percentage of enterovirus isolations in children with
diarrhoea and controls. More recently Abraham and Dammin (1977) in Egypt have carried out a study in which virus isolation was attempted from cases of diarrhoea and controls not suffering from diarrhoea. Their findings failed to incriminate enteroviruses and adenoviruses in the aetiology of acute diarrhoeal disease in the study. In a similar study equal rates of isolation of enteroviruses and adenoviruses from Taiwan children with diarrhoea and from healthy controls were also obtained by Echeverria et. al, (1977). Echovirus, poliovirus, adenovirus or herpesvirus was isolated from 16.4% of the 75 children with acute diarrhoea and from 19.8% of 131 healthy controls. Adenovirus-like particles were seen by electron microscopy in 9% of the 75 stools and only 1 was isolated in tissue culture.

After the failure of attempts to relate enteroviruses and adenoviruses with sporadic cases of diarrhoea, there has recently been great interest in rotaviruses as causes of infantile gastroenteritis. Although some evidence for the viral aetiology of cases of unexplained gastroenteritis was obtained from transmission studies performed in volunteers with bacteria-free filtrates of stool specimens collected from outbreaks of diarrhoea (Reimann et. al, (1945); Gordon et. al, (1947); Dolin et. al, (1971); it is only recently that Kapikian et. al, (1972) and Faver et. al, (1973) have managed to demonstrate by electron-microscopy a new virus, the Norwalk agent. However, the demonstration
of a reo-virus like agent by Bishop et. al, (1973) was of much more importance.

Flewett et. al, (1973) substantiated the findings of the latter virus by demonstrating the presence of the agent in the faeces of children with diarrhoea. Since these early communications were published the agent, now generally referred to as "rotavirus" has been reported from many countries all over the world (Editorial, British Medical Journal, 24 September, 1977). In addition to the names of countries in that editorial, rotavirus has now been shown to be present in many other countries, including Kenya. (Savadia et. al, 1976 and Mutanda, 1977).

Rotavirus, also known as "duovirus", "reovirus-like agent", "orbivirus", "infantile gastroenteritis virus" is morphologically identical with simian, calf, pig and foal rotaviruses and the virus of epizootic diarrhoea of infant mice. The virus particles occur in two forms, one about 65 nanometer\(^\text{(nm)}\) in diameter - the "complete" particle, having a sharply defined circular outline giving the appearance of the rim of a wheel. These are referred to as "smooth" particles. The smaller or "rough" particles, are viruses which have lost the surface rim and appear about 56-60 nm in diameter. This internal component represents the group specific antigen for the rotavirus group, and stimulates the production of heterologous

Disease by rotavirus is mostly restricted to children under 6 years of age, most of the children being under two years of age (Davidson et al, 1975; Bryden et al, 1975; Shepherd et al, 1975), but the age range of patients from whom rotavirus has been isolated varies from 2 days to 95 years. (Editorial British Medical Journal, 26 March, 1977).

The incubation period of clinical cases is generally between 48-72 hours. In about half of the cases the initial symptom is vomiting, followed within hours by diarrhoea. In nurseries for newborns rotavirus infections sometimes are endemic. Clinically the infections run on subclinical or mild course (Chrystie et al, 1978). Also in older hospitalized patients, diarrhoea usually is not severe and generally less than 10 stools per day are produced. However, some deaths have been reported (Middleton et al, 1974; Davidson et al, 1975). Most children recover within a week (Shepherd et al, 1975). Virus excretion is usually limited to a period not surpassing 8 days from the onset of symptoms.

The frequency with which rotavirus is isolated from patients with gastroenteritis varies with time and place. In temperate climates the infection is most frequently seen in winter (Editorial British Medical Journal, 13 August, 1977). Scoub et al, (1977a) also reported a higher incidence of rotavirus infection in winter in 14 (61%) of 23 white South African children and 49% in black infants with severe gastroenteritis (Scoub et al, 1977b) compared
with the incidence in black children of only 16% samples in summer and reported earlier (Schoub et. al, 1975). However, studies extending over a period of one year have not been reported from Africa.

In one study from Keneba, a rural village in Gambia, West Africa, Rowland and McCollum (1977) and Rowland and others (1978) did not detect rotaviruses by electron-microscopy in faeces of children collected between July and September 1975 when there was frequent diarrhoea. The high prevalence of diarrhoeal disease seen in their studied population was attributed to bacterial colonization of the upper bowel rather than rotaviruses. Their finding thus indicates that rotavirus need not be endemic and may be absent for some time from an area.

Mixed infections in humans between rotavirus and enteroviruses or bacteria have been reported by several investigators (Cruickshank et. al, 1975; Esparza et. al, 1977; Bryden et. al, 1975). In a detailed study of viral and bacterial enteropathogens in infants with diarrhoea in Glasgow, Madeley et. al, (1977) found that a number of different viruses (including rotavirus) and bacterial pathogens often occurred within a few days in different stool samples from the same infants with diarrhoea. Carr et. al, (1976) found that infants were more severely affected when enteropathic coliforms were also present, besides rotavirus. When several
different pathogens are present it is not clear yet which one causes the illness.

Rotavirus infections have also been found in calves, pronghorn antelope, rabbits, piglets, young monkeys, lambs, deer, foals and mice. Antibodies to the virus have been found in rabbits, guinea-pigs and goats. The human virus has been transmitted to piglets, calves and colostrum-deprived baby monkeys; the calf virus also has been transmitted to piglets, calves and colostrum-deprived baby monkeys (Reviews by Flewett, 1976a; Flewett and Woode, 1978).

The calf rotavirus has been isolated in, and with difficulty adapted to, tissue cultures of calf kidney cells (Woode et al., 1974). The simian rotavirus (SA11) has been readily propagated in cell culture (Scub et al., 1977c). However, the human virus has been more difficult to isolate in tissue culture. Infection in man has thus been diagnosed mainly by electron-microscopy of faeces after differential centrifugation. Only recently Banatvala et al., (1975) have shown that the virus would infect pig-kidney cells if the inoculum and cell monolayer were centrifuged together at 2000-4000 rev/min. Bryden et al., (1977) extended the application of that method to detect rotavirus in faeces, with cell cultures of human embryo kidney (HEK cells, calf kidney (CK) cells and a...
rhesus monkey kidney cells (LLC-MK2); the three cell cultures were compared for sensitivity in isolating virus. LLC-MK2 cells were found to be the most sensitive. Rotavirus were detected in 31 (37%) out of 84 specimens in these cells and in 35 (42%) by electron microscopy.

Several groups of workers extended the work on the Norwalk agent and reported the presence of virus particles 27 nm in diameter and of cubic symmetry (Kapikian et al., 1973) resembling "parvoviruses" in faeces of infants with and without gastroenteritis (Dolin et al., 1972; Paver et al., 1973; Flewett and Davies, 1976b; World Health Organization Weekly Epidemiological Record 23 April, 1976; Kjeldsberg, 1977 and Thornhill et al., 1977). Kapikian and his co-workers (reported in Elliot and Knight, 1977) associate mild diarrhoea that affects all age with this Norwalk and other small viruses (named astrovirus, Appleton and Higgins, 1975; Madeley and Cosgrove, 1975a; Lee and Kurtz, 1977) and caliciviruses (Madeley and Cosgrove, 1975b); and more severe sporadic infantile gastroenteritis which affects mainly infants and young children with rotavirus. More recently Cameron et al., (1978) observed shedding of two non-cultivable viruses in stools of newborn babies in Melbourne, Australia. Ten babies admitted to a nursery were found excreting rotavirus and seven of the ten babies also excreted detectable amounts of a 28nm virus-like particles morphologically resembling Norwalk and astrovirus.
Excretion of this 28-nm particle coincided with symptoms of diarrhoea in four babies, all of whom were also excreting rotavirus. The workers, therefore, found little evidence of an association between excretion of the 28-nm virus-like particle and development of diarrhoea and concluded by saying that the particle may be a common "commensal" infecting the gastro-intestinal tract of many newborn babies.

Also coronavirus-like particles have been seen in faecal samples from three outbreaks of non-bacterial gastroenteritis in humans (Caul and Clarke, 1975). Rowland and McCollum (1977) visualized by electron microscope, corona-like viruses in 5 of the 27 diarrhoeic specimens examined in Gambia, West Africa. On the other hand Sebodo et. al, (1977) working in Central Java made 6 coronavirus isolations from 16 stools obtained from children without diarrhoea and none from the 41 specimens of the diarrhoeic group. Thus the role of coronavirus or corona-like agents in childhood diarrhoea is not yet clearly established.

Diarrhoea which is regarded as non-infective can occur in malnutrition. In a study of 100 consecutive out-patients with gastroenteritis in South Africa, 47% of the severely ill children were underweight as against only 6% of those mildly ill (Truswell et. al, 1963). Also Gordon et. al, (1964) found that gastroenteritis was more severe in Guatemalan malnourished children than
in those of normal nutritional state.

The weaning period has been characterised as the most critical time in the life of the African child (Jelliffe, 1973). Welbourn (1963) and Gordon et. al, (1963) found the incidence of diarrhoeal disease to be greatest when children were being weaned and immediately thereafter. Field studies in India and Guatemala showed a peak incidence of childhood diarrhoeal disease during the weaning period, enhanced by a progressively developing malnutrition and greater exposure to environmental risks of infection (Mata et. al, 1967). More recently Rowland and McCollum (1977) have found the initial weaning food, often introduced around the age of four months, to be highly contaminated with bacteria. Weanling diarrhoea is thus thought to be produced by the ingestion of organisms in large numbers, especially through bottle-feeding. However, swabs taken simultaneously from rectums and feeding bottles of children suffering from diarrhoea in a study done by Habte and Debesai (1964) in Ethiopia showed no correlation. On the other hand, Mata et. al, (1967) found that enteropathogenic E. coli was essentially absent in cases of diarrhoeal disease and in healthy Guatemalan village infants who were breast-fed. Totterdell et. al, (1976) and Chrystie et. al, (1975) observed that bottle feeding in nurseries for the newborn favoured infection with rotaviruses. Data on the extent of bottle-feeding in Kenya and whether or not bottle-feeding
promotes *E. coli* infections are not documented.

Observations on the consistency and appearance of stools collected from diarrhoeal children in several countries have failed to provide a convincing answer to clues leading to a rapid tentative diagnosis of the causal agent of the diseases. Ramos-Alvarez and Olarte (1964) found that the presence of blood in stools was observed more frequently in children infected with *Shigella* alone or with *Shigella* and viruses than in children with *Salmonella* either alone or with viruses. In Sudan, Erwa et. al, (1971) isolated *E. coli* strains more frequently from yellow fluid faeces than from green fluid or semisolid faeces; no isolation of *E. coli* from mucus and blood-stained specimens was made. Somewhat similar results were those of Kalya and Oduori (1972) from Kenya. In their view, blood and mucus in the stools did not necessarily imply presence of bacteria. On the other hand, Wilson and Luder working in Uganda, (1957) found that the presence of pus was indicative of the presence of one of the classical entero-pathogenic bacteria. Masembe (1977) found blood in the stools of newborn in Mulago Hospital, Uganda to be associated with enteropathogenic strains of *E. coli* infection. Wamola et. al, (1974) in Kenya also observed that enteropathogenic *E. coli* infection in the under two years old often presented as diarrhoea sometimes with blood in it and that shigellosis was characterised by bloody diarrhoea
with mucus or pus. Reportedly, in viral enteritis leucocytes may not be present in the stools (Editorial, British Medical Journal 24 September, 1977). Ryder et al. (1976) also did not find leucocytes in faeces of rotavirus-infected children in Bangladesh. These reports on the consistency and appearance of diarrhoeic stools are not all of the same tenor. This, together with the fact that results obtained in one part of the world cannot be taken for granted to apply in another, has prompted me to conduct another survey in Kenya aimed at elucidating the relative importance of the appearance of stools and the presence or absence of faecal leucocytes and red blood cells as rapid tentative diagnostic indicators of either viral or bacterial causes of childhood gastroenteritis.

**Aim and Objectives of the Study**

The aim of the work that will be discussed in this thesis was to contribute to the knowledge of severe acute childhood gastroenteritis in Kenya. To reach the aim, the following objectives were pursued:

1. To gain an insight into the magnitude of the problem of severe acute childhood gastroenteritis by gathering data on admission for the disease to a number of hospitals and by relating these data to the total
number of admissions of infants and children to these hospitals.

2. To assess whether fluctuation in admissions to a hospital for acute childhood gastroenteritis observed over a number of years were associated with meteorological parameters.

3. To assess the age incidence of acute gastroenteritis in infants and young children admitted to hospitals and whether or not this age-related incidence differed from that of measles, a disease frequently associated with diarrhoea.

4. To assess as much as possible, in a case-control study which were the major aetiological agents of acute childhood gastroenteritis during the observation period, giving special attention to newly discovered pathogens which are presently identified by techniques that were developed recently and were new to Kenya.

5. To assess fluctuations in incidence of infection by the aetiological agents thus identified in different periods of the year and in different places.

6. To assess the distribution of the agents by age and sex.

7. To assess the extent of infection of rotavirus, one of the newly discovered enteropathogens in a number of animal species.

8. To assess practices of breast and bottle feeding
prevailing in a number of places in Kenya and the possible contributory factor of bottle feeding to the frequency of infections by the aetiological agents of acute childhood gastroenteritis.

9. To assess a possible specific relationship between one or more clinical characteristics of acute gastroenteritis and aetiological agents, with special attention to the newly discovered ones.

10. To assess a possible relationship between macroscopic and/or microscopic aspects of stool specimens in acute childhood gastroenteritis and aetiological agents, with special attention to the newly discovered ones.

Definitions

Childhood acute gastroenteritis is defined as a clinical syndrome characterized by anorexia, nausea or vomiting, diarrhoea of variable severity and abdominal discomfort or pain. In the text gastroenteritis is used interchangeably with diarrhoea to denote, according to the observation of the mothers, three or more loose stools per 24 hours in children over one year of age and five or more watery stools per 24 hours in children under one year of age. Febrile diarrhoea is diarrhoea accompanied by fever.

The degree of dehydration is recorded as mild, moderate and severe, by the criteria of the five clinical signs stipulated by Jelliffe (in Medical care in Developing
countries, edited by King, 1973). Severe diarrhoea is diarrhoea leading to admission to hospital.

Children are categorized as neonates, infants, and young children. Neonates are babies under four weeks old, infants are children less than 1 year old and young children are those between 1 and 5 years of age. Faecal leucocytes are defined as white blood cells to include polymorphs and macrophages.

II. GEOGRAPHICAL BACKGROUND OF THE COUNTRY

Position, size and relief

The Republic of Kenya stands almost exactly astride the Equator. It stretches from the western shores of the Indian Ocean to Mount Elgon and Lake Victoria in the West. Its total area is 583,000 square kilometres of which some 14,000 square kilometres are under water bodies (Ominde, 1974). The rest of Kenya's landscape is an environment of great topographic diversity. Like much of Eastern and Southern Africa, it is essentially an upland plateau that has been raised relatively higher than much of Equatorial Africa by a series of broad continental-type uplifts. The altitudinal statistics of the country are: 38% of the country lies below 500 metres, 26% between 1,000-2,500 metres and 1.5% above 2,500 metres; 34.5% of the country therefore lies between 500-1,000 metres (Ominde, 1974). By relief units
as individual environments, Kenya is divided into six distinct relief regions (Figure 1). These are the Lake basin, the Central Rift and associated highlands, the Eastern plateau foreland, the Coast, the Semi-arid and arid Northern and Southern Kenya.

Climate

Because of its varied terrain, different parts of Kenya experience an extremely varied climate both in rain and temperature as well as humidity. Lake Victoria is the source of the high rainfall of the West Kenya Highlands, and higher areas receive more of this essentially afternoon rainfall than the lower parts. The rains in other parts of the country show a close association with the convergence zone where the northern airstream meets the south-easterly airstream. Kenya has a definite rainfall deficiency which is far out of proportion for its latitude or mean height above sea level. The rainfalls in Kenya are unreliable both in amount and time of arrival. Rains to be expected, say, in March may easily arrive in January in one year and in May the next.

Relief as well as the sea have effects on the pattern of the temperature as far as attitude and seasons are concerned. Over much of the higher parts of Kenya, relief has reduced the temperature so that it is only in the hot semi-arid north, parts of the Rift Valley, Lake Basin, and the Coast that heat is a real source of discomfort to man.
Population

The August 1969 census gave the country's population as 10.9 million people who were thought to be increasing at an average rate of 3.3% per annum. There is a close relation between population distribution and land of good agricultural quality. The dry north, east and south are only sparsely peopled. Also the rural economy influences the diet available to the local inhabitants.

The age and sex structure from the 1969 census data indicate that the broad characteristics of age and sex are consistent with those of developing countries of the world experiencing high fertility. The age and sex pyramid thus shows a large base of children and a low proportion of old people. The infant mortality rate in 1969 was estimated at 126 per thousand live births for males and 122 for females (Ominde, 1974).
III. MATERIALS AND METHODS

1. Sources of data

1.1. Data on the magnitude of the problem of childhood gastroenteritis and on age incidence of infants and children with diarrhoea admitted to hospitals.

Information on the incidence of childhood diarrhoea in Nairobi was obtained from admission records of Kenyatta National, Aga Khan and Gertrude Garden Children's hospitals. Ages of infants and children admitted with diarrhoea and/or measles were obtained from admission records of Kenyatta National, Gertrude Garden, Kisumu and Siaya hospitals; correct ages were not available on the admission records of Aga Khan hospital. Also annual reports of the Ministry of Health do not give separate figures on early childhood (pre-school age) gastroenteritis.

Kenyatta National Hospital is the central referral and the main teaching hospital in the country. The set up of the children's clinic is such that new cases are seen at a Filter Clinic, and those with mild or moderate complaints are treated there and then; those with severe complaints are admitted to the Paediatric Observation Ward (POW), and only the very few with severe complications are admitted to hospital wards. Usually acute diarrhoeal cases with minor complications stay for 1-3 days in the POW where facilities for re-hydration are available. The children who are either seen or admitted to Kenyatta National Hospital Observation Ward are mostly African children of low-income parents.
Gertrude Children's and Aga Khan hospitals are privately owned. The children patients come from either middle or high income families. These hospitals cater for all races within and outside Nairobi City.

Nyanza Provincial Hospital in Kisumu is the largest hospital within that Province. It admits mostly African children of families of low income from urban and rural areas.

Siaya Hospital is the District's largest hospital serving purely a rural African community. It is situated right in the centre of Luoland.

1.2. Feeding practice data

Mothers bringing children to Kenyatta National Hospital Paediatric Demonstration Clinic for educational instructions were interviewed on their feeding practices. All mothers were Africans of varied social groups. They came from within and around Nairobi City. Also information on the type of feeding of infants was obtained from mothers in Kisumu and Mombasa, through interviews, while they were attending Maternal and Child Health clinics. The information gathered included the ages of the children; whether breast or bottle-fed or mixed fed, and at what age the different types of feeding were stopped or started.

1.3. Meteorological information

Temperature, rainfall and relative humidity were chosen
for meteorological parameters. Figures were kindly supplied by the Nairobi Central Station of the Kenya Meteorological Department.

2. Population studied for aetiological agents and periods of study.

Three groups of populations were studied. These were:

1) Hospitalized patients
2) Out-patients and
3) Masai children at home.

All the three groups consisted of children aged below 6 years.

2.1 Group 1: This group included infants and children hospitalized for diarrhoea as the main complaint and who had received no antibacterial drugs other than penicillin. The admitting institutions were Kenyatta National Hospital Paediatric Observation Ward, and the paediatric wards of Mombasa and Kisumu Pro­vincial hospitals.

At Kenyatta National Hospital, patients were systematically sampled during May 1975 through April 1976. Due to difficulties encountered in retaining infants and children not suffering from diarrhoea, only 36 children with diarrhoea were matched with controls with respect to age, sex and social background. The controls had been hospitalized for respiratory diseases. The patients admitted to
Mombasa Provincial hospital were sampled during 10th-21st January; April 14th-May 3rd and October 28th to November 11th 1977. Those admitted to Kisumu Hospital were sampled during 31st January to 12th February; 14th-25th May and 5th-19th October 1977. Mombasa and Kisumu were chosen because of the climatic differences of the two places and with Nairobi. Mombasa and Kisumu Provincial hospitals are, respectively, the largest hospitals in the Coast and Nyanza provinces. The two hospitals admit mostly Africans of varied tribes.

2.2 Group 2: Included in this group were out-patient infants and children aged below 6 years attending old Town Health Centre in Mombasa and Lumumba Health Centre in Kisumu because of diarrhoea. They were sampled during the same periods as those in the respective hospitals.

2.3 Group 3: Included in this group were Masai sampled at home irrespective of whether or not they had diarrhoea. Stool samples were taken between August 24th and 31st when it was dry and from November 28th to 8th December when it was raining. Samples were taken from 39 children during the first visit and from 100 children in the second visit. It was not possible to persuade Masai mothers to allow that blood be taken from their children.

The Masai are nomads and live in enclosures known as
"Manyatta". In a small Manyatta one can easily find 10 children under 6 years old. In the big Manyatta it is normal to find up to 45 children under six. At night the Cattle and domestic animals stay in the same enclosures as the people.

This tribe was chosen firstly because a preliminary serological study had indicated a high rate of rotavirus antibodies in the sheep sera obtained from there, and secondly because as a consequence of the living conditions, a high degree of contamination with enteropathogenic agents was expected.

3. Selection and examination of children

At Kenyatta National Hospital, only one patient namely the first one admitted during the morning was chosen, everyday, five days per week and samples taken. When available on the same day or the following morning, a matched control was also selected from patients admitted for respiratory diseases. In Mombasa and Kisumu, samples were taken from all children with diarrhoea hospitalized and/or seen at the out-patients clinics and health centres. It was difficult to get controls. At the out-patient clinics, patients were first examined by clinical officers and when seriously ill referred to paediatric wards where clinical histories were taken by the ward doctors. At the health centres, the clinical officers did all the medical examinations (these officers are known as medical assistants in other countries). The clinical history collected on the hospitalized
and out-patients included sex and age, duration of illness, feeding habits and previous treatment for the present illness. The degree of dehydration, temperature and frequency of motions and vomiting were also noted down by the staff. I copied the notes from admission charts.

4. Collection and Examination of specimens.

4.1. Collection: In Nairobi, stool and acute phase blood samples were taken from some patients with diarrhoea and their matched controls on the first day of admission. From some, second samples of blood were taken 14-21 days after admission by venepuncture. Also for assessment of prevalence of rotavirus and adenovirus antibody blood was taken from infants and children admitted for various complaints.

In Mombasa and Kisumu only stool specimens were obtained and only from patients with diarrhoea. After collection, specimens were frozen at -20°C until transported to Nairobi in a box packed with ice bricks.

Blood of neonates was taken from the umbilical cord immediately after delivery at Pumwani Maternity Hospital, Nairobi. Second blood samples were taken from the children by finger prick, collecting 0.25ml. of blood into tubes containing 0.25ml. of phosphate buffered saline (PBS) solution. The tubes were centrifuged to give a 1:3 serum dilution.

Bovine and sheep samples were collected near Nairobi
from slaughter animals. Samples of blood were also taken from rats, trapped around a Nairobi housing estate. After separation from the clots, all sera were kept at -20°C until tested.

4.2 Examination of stool specimens and sera:

4.2.1 Stool culture for bacteria: Each stool was cultured by inoculation of MacConkey and desoxycholate citrate (DCS) agar plates. A portion equivalent to 1 gram was suspended into selenite F enrichment broth medium. The cultures were incubated aerobically at 37°C overnight. The remainders of the samples were stored into a -70°C freezer until processed for isolation of viruses. After overnight incubation, the broth was subcultured on MacConkey and DCS agar plates. Ten lactose-fermenting colonies were picked off MacConkey agar plates and one per segment subcultured on a blood agar plate divided into 10 segments. The next day, attempts were made to type the isolates with the following enteropathogenic E. coli O group antisera purchased from the Wellcome Company, London: the Polyvalent sera 2, 3 and 4 and monovalent sera of the following types - 026:K60, 044:K74, 055:K59, 086:K61, 0111:K58, 0112:K66, 0114:K90, 0119:K69, 0124:K72, 0125:K70, 0126:K71, 0127:K63, 0128:K67 and 0142:K86. Procurement of typing antisera to newly recognized pathogenic serotypes was not possible.
The same colonies as used for typing were pooled and transferred to plain agar slants. These were later mailed to Dr. D.A. Sack, Johns Hopkins University, U.S.A. where the adrenal-cell assay, described by Sack and Sack (1975) was used to determine heat labile enterotoxin (LT) production by the organisms. Pale non-lactose fermenting colonies were picked off DCS agar plates and subcultured onto triple sugar-iron agar (TSI) slants as well as put into Christensen's urea medium. Identification of suspect organisms as Salmonella or Shigella was done by conventional methods, with use of commercial antisera (Wellcome Company, London). Later the identification was confirmed by biochemical tests as described by Edwards and Ewing (1972).

4.2.2. Virology of stools

4.2.2.1 Demonstration of rotavirus by electron microscopy:

The method described by Bishop et. al, (1974) was first used. Stool specimens were thawed and 5 g. was thoroughly mixed by hand with 10 ml distilled water. 10 ml. trifluoretrichloroethane ('Arktone', I.C.I.) was then added and the sample was homogenised by shaking with hand for ten minutes. The homogenate was centrifuged at 10,000 g. for 30 min. at 4°C. The supernatant was removed, the volume measured, and 8% polyethylene glycol 600 (Union Carbide) was added.
The mixture was stored overnight at 4°C, then centrifuged at 4,000 g for 30 min. and the supernatant discarded. The deposit was resuspended in 2 ml of distilled water. This suspension was then layered on to 3 ml of 45% sucrose in 0.002M tris buffer, PH 7.0 and centrifuged at 100,000 g for 150 min. The supernatant was discarded and the deposit was suspended in 3 drops of distilled water. A drop of this suspension was allowed to dry on a formvar coated microscope grid, washed three times in distilled water and then negatively stained with 2% potassium phosphotungstate. Grids were examined in an 201 Philips electron microscope (EM) at 45 KV. So far this method has not been routinely used in Kenya. Later on a tissue culture immunofluorescence technique as described by Bryden et al., (1977) was employed for the rest of the whole study. Also specimens that had been examined by EM were retested using this technique. Monolayer cultures of LLC-MK2 cells (obtained from Flow Laboratories, England) were grown on round coverslips 13 mm in diameter held on the bottom of flat-bottomed screw-capped blood collection tubes (Pathlab Supplies Ltd.). Each tube was seeded with 1.5 x 10^5 cells, in 1 ml of Eagle's minimum essential medium (MEM) containing 10% (v/v) of foetal calf serum (FCS), 1% of a non-essential amino acids solution, 200 units of penicillin, 200 units of streptomycin, 120 units of
neomycin and 100 units of mycostatin per millilitre.

When confluent monolayers had formed they were re-fed with 1 ml of Eagle's maintenance medium containing only 2% (v/v) of FCS. The tubes were then inoculated with 0.2 ml amounts of the test material consisting of faecal supernates which were prepared by making approximately 20% stool suspensions in Eagle's MEM maintenance medium and centrifuging the suspension at 4000 r.p.m. for 30 min.

Each specimen was done in duplicate. The tissue culture tubes were centrifuged at 4300 r.p.m. for 45 min. to facilitate infection of the cells and then incubated at 37°C for 18 hours. The coverslips were removed, washed in PBS three times, fixed in acetone for 10 min. at room temperature and allowed to dry. The infected cells were then treated with 1-2 drops of a 1 in 10 dilution of specific anti-rotavirus convalescent human serum kindly supplied by Dr. A.S. Bryden, Birmingham Regional Hospital, England, incubated at 37°C for 45 min., washed in PBS three times, stained for 45 min at 37°C with fluorescein-conjugated rabbit anti-human IgG serum, washed again three times in PBS, counterstained with a 1 in 10,000 methylene blue solution and mounted in buffered glycerol on microscope slides for examination. This method is new to Kenya. The controls included rotavirus infected cells (positive control) plus human anti-rotavirus reference serum kindly supplied by Dr. N. Zygraich, Recherche et
33

Industrie Therapeutique (RIT), Belgium, followed by conjugate; uninfected cell (negative control) plus immune serum followed by the conjugate; and uninfected cells plus PBS instead of immune serum and then followed by the conjugate for aspecific fluorescence of cells.

4.2.2.2 Testing the specificity of the immunofluorescent test

Twelve stool specimens from children with diarrhoea and an equal number of specimens from matched controls which had been examined by electron microscopy were re-examined using the technique of tissue culture - immunofluorescence. Five specimens that had been found containing rotaviruses by EM were also positive in the immunofluorescent test. The rest of the specimens were negative in both systems. Further to confirm the specificity of the immunofluorescent test, rotavirus was grown on coverslips for 18 hours as described above. The coverslips were washed with PBS and fixed in acetone as above. Some coverslips were treated with a human anti-rotavirus immune serum and others with serum containing no rotavirus antibodies. Both the anti-rotavirus and the negative serum were reference human sera obtained from Dr. N. Zygraich, (RIT), Belgium. Other Coverslips were treated with one of the three local human sera, respectively, containing antibodies to poliovirus 1, echovirus II and coxsackievirus B-5, but negative for rotavirus C.F. and neutralizing antibodies.
Treatment of all the coverslips was followed by the addition of the immunofluorescein - labelled conjugate.

The results were in accordance with specificity of the calf rotavirus complement-fixing antigen, and a negative immunofluorescent test.

Another check was afforded by sending 2 stool specimens found positive and another 2 found negative to Dr. A.S. Bryden, Birmingham Hospital, England without telling him my findings. The results agreed.

4.2.2.3. Isolation of Enteroviruses: Centrifuged faecal suspensions that had been prepared as mentioned above and frozen at -70°C were thawed. Test tubes with monolayers of Baboon-kidney and human foetal fibroblast cells were each inoculated with 0.2 ml of the suspension. The tubes were incubated at 37°C on a roller drum and examined microscopically for cytopathic effect (CPE) every other day for 14 days. Those showing CPE were harvested and stored at -70°C. Typing of enteroviruses was not attempted.

4.2.3. Tests for antibodies in serum specimens:
The complement-fixation test (CF) was used for detecting against rotavirus and adenovirus. The microtitre C.F. technique was used, employing 0.025 ml heat-inactivated (56°C for 30 min) serum, 2 units of antigen and 2 units of complement, each in quantities of 0.025 ml. Mixing of the reagents in the cups slightly was followed by overnight incubation at 4°C and then addition of 0.025 ml of a 1.5%
suspension of sensitized sheep red blood cells and mixing
the reagents thoroughly by tapping.

Calf rotavirus complement-fixing antigen, and a negative
control preparation were purchased from Dr. N. Zygraich,
(RIT). Adenovirus complement fixing group antigen and
antiserum were purchased from the Central Public Health
Laboratory at Colindale, England. The diluent used for
the tests was diethyl barbital acid-sodium barbital
buffer (DBB).

Three controls were set-up for every test. The first one
was to check serum anticomplementary activity. For this,
0.025 ml of complement was added to each of 0.025 ml of
serum dilutions followed by 0.025 ml of DBB and incubated
overnight at 4°C. The second control was meant to check
a possible non-specific reaction of the test serum. For
this 0.025 ml of the negative preparation control of the
antigen (diluted as the positive antigen) was added to
dilutions of test sera plus 0.025 ml complement and
followed by overnight incubation. The third control was
to check the haemolytic system. To 0.025 ml sensitized
sheep red blood cells was added 0.025 ml DBB and 0.025 ml
complement. A control titration of the test dilution of
2 units of complement was included also. After overnight
incubation at 4°C, the plates were transferred to an
incubator and kept at 37°C for 30 min.; subsequently,
sensitized erythrocytes were added. One volume of only
sensitized erythrocytes plus 3 volume of DBB was also
included. The microplates were tapped several times and
incubated at 37°C for 30 min., tapped again and reincubated for another 30 min. The antibody titre of the serum was then reciprocal of the last dilution where no haemolysis was observed.

4.2.3.1 The neutralization test for antibodies against E.coli toxins:

24 serum pairs previously obtained from 12 patients with diarrhoea and 12 controls not suffering from diarrhoea were kindly tested by Dr. D.A. Sack, Baltimore City Hospitals and the Johns Hopkins University, the U.S.A. for E.coli heat-labile enterotoxin-neutralization. Tests for heat-stable toxin were not done. The tests are cumbersome and require animals.

4.3. Macroscopic and microscopic inspection of stools:

4.3.1 Inspection of stools

The appearance of stools was described as being brown, yellow, green, white, colourless or blood-stained and mucoid. The consistency was described as formed, soft or loose and watery or fluid. Formed stools were those not taking the shape of the container; soft or loose were those as soft as tooth-paste or as jelly respectively; watery were those in a fluid form and blood stained and mucoid were those containing blood and mucus respectively.

4.3.2. Microscopy of stools

The presence of leucocytes or erythrocytes or combined
together in stools was recorded as ♞(10-19) ♞ ♞ (20-39) cells per high power field, ♞�� (40-99) cells per high power field and ♞��� 100 and more cells per high power field.

IV. RESULTS

1. Observations on the Frequency of Gastroenteritis:

1.1 Observations in Nairobi

Table 1 shows the number of infants and children hospitalized for various complaints. Nearly 20% of the admissions were due to diarrhoea. There was no significant difference between hospitals.

The monthly distribution of all admissions of children under 6 years old to Gertrude Garden Children's Hospital and the number per month of those admitted for diarrhoea during 1975-1977 are shown in Figure 2. From the total number of admissions, diarrhoeal cases admitted during different months of the year differed significantly. In Table 2 it can be seen that January gives the highest mean percentage (33.5%) and October the lowest (14.8%). The difference is statistically significant (P < 0.01). When these monthly percentages of diarrhoeal cases and some meteorological parameters available from weather observations during the period of study are compared
severe diarrhoea, measles and those seen at the Filter clinic with mild diarrhoea were compared (Figure 4), it was found that the frequency distributions of each of the three categories of diseases differed from one another. Whereas severe diarrhoea admissions reached a peak in the 3-5 months age-group, that of measles was observed in the 6-8 months group. The peak of mild diarrhoea was in the 12-14 months group. (Note the change of age-groups after 23 months).

Of interest was the observed measles peak age-incidence, because it coincided with the period of waning of maternal antibodies, and a sharp rise in measles incidence observed by a collaborative study-team of the Ministry of Health, Kenya and the World Health Organization (Bull. Wld. Hlth. Orgn. Vol. 55, p-21, 1977).

From the above observations, it was hypothesized that there could also be an inverse relationship between the peak age incidence of infantile diarrhoeal diseases requiring hospitalization and the practical disappearance of maternal antibodies to the major aetiological agent of childhood gastroenteritis.

2. Aetiology of Acute Childhood Enteritis

2.1 Results of the Search for aetiological agents.

Enteric pathogens isolated from 36 stools obtained from children with severe acute diarrhoea and from 36 matched controls are shown in Table 4. Before the immunofluorescent test was employed, 12 stools from
children with diarrhoea and an equal number from the matched controls were examined by using both electron-microscope and tissue culture-immunofluorescent technique as described above. Micrographs of rotavirus are shown in Figure 5. Five diarrhoeic stools were found positive for rotavirus particles and the rest negative in both systems. The immunofluorescent test was then adopted for the rest of the study, with omission of electron microscopy.

The frequency in isolation or detection of rotavirus was significantly higher (P < 0.01) in children with severe acute diarrhoea than in the control group. Also Shigella isolation was higher in children with diarrhoea (P < 0.05) than in the controls. For *E.coli, Salmonella* and enteroviruses, the differences were not significant.

In Table 5 the frequency of a combination of two infections and single infections in children with diarrhoea are shown. Mixed infections occurred between rotaviruses and other enteropathogens, but as the figures are small no attempt was made to test the significance of their frequency.

The enterotoxigenic *E.coli* isolated from a child with diarrhoea occurred together with an enteropathogenic classical *E.coli*, serotype 026/B6. Of interest was the finding that the stool of the control child admitted for respiratory disease from whom an enterotoxigenic *E.coli* was isolated was watery, indicating diarrhoea possibly of mild nature combined with the respiratory infection.
2.2. **Seroconversion to rotavirus infection**

Paired sera, acute and convalescent, collected from 22 infants and children with diarrhoea and 14 pairs from the control group were tested for rotavirus antibodies. The results are shown in Table 6. Of the 9 cases from which rotaviruses were isolated 7 sero-converted.

Two children seroconverted without rotavirus in their faeces being detected.

Of the 14 serum pairs obtained from the control-group, only 1 had a four-fold C.F. antibody titre rise.

Rotavirus was isolated from the stool of this child.

2.3. **Seroconversion to E.coli (LT) toxin**

The anti-LT titre changes in 12 serum pairs obtained from the children with diarrhoea and 12 pairs from the control group are shown in Table 7. Only two children, a patient and a control from whom entero-toxigenic E.coli strains were isolated had a four-fold titre rise. When the titres of 20 (out of a total of 24 serum pairs) containing LT antibodies were plotted against the ages of the children from whom they were taken, a correlation was evident (Figure 6). High levels of titres were found more often in older children.

2.4 **Seroconversion to adenovirus infection**

The causal relation of adenoviruses to infantile diarrhoea was studied serologically, because previous
reports had indicated the possibility of unsuccessful
cultivation of some of these viruses in tissue culture
(Echeverria et. al, 1977; Madeley at. al, 1977).
However, all human adenoviruses possess a group
complement-fixing antigen, and this antigen was thus
employed to test 17 pairs of sera obtained from infants
and children with diarrhoea. Sera were tested at a
1 in 8 initial serum dilution. None of the 17 serum
pairs showed a significant titre rise; one had a two-
fold titre rise only. Antibody was not detected in
the rest of the 33 sera. A reference positive serum
control reacted well in the test. All the 17 serum
pairs were retested by Dr. Chiba, then a short-term
World Health Organization Consultant to the East African
Virus Research Institute, Entebbe, Uganda, and the
results agreed.

2.5 Further studies on evidence of infection in Children by
Adenovirus and Enterotoxigenic E.coli:

2.5.1. Adenovirus: Before adenovirus could be ruled out
as a significant aetiological agent of early
childhood diarrhoea, a serum-survey was undertaken.
Blood that had been collected from 146 infants and
young children aged under 6 years and admitted to
Kenyatta Hospital for varied illnesses, were
screened for C.F. adenovirus antibodies. Only 9
(6%) sera had antibodies detectable at a 1 in 8
serum dilution. The ages of these children with
antibodies ranged between 10 and 35 months.
2.5.2 Enterotoxigenic E. coli:
To obtain more information on whether or not the rate of isolation of enterotoxigenic E. coli could be influenced by weather seasons, another 22 E. coli strains isolated from 22 diarrhoeic stools after a period of 3 months from the time of collection of the first samples were mailed to Dr. D.A. Sack. Only one strain was found to produce heat-labile enterotoxin. The child from whom this E. coli was isolated was harbouring rotavirus as well.

3. Distribution over the period of observation of the Aetiological Agents:
3.1 Kenyatta National Hospital
Results of attempted isolation of enteropathogens from infants and young children admitted to Kenyatta National Hospital with acute diarrhoea during a 12 months period are given per calendar month in Table 8. Rotavirus was the most frequently isolated pathogen in the group studied. E. coli was second in frequency and Shigella occupied the third position. Only 4 isolations of Salmonella were made out of 160 stool specimens. Rotavirus was isolated throughout the period when the study was done. The number of isolations was found to be highest in the month of September and lowest in the month of May. Although the number of specimens was too small to draw firm conclusions,
it is worthy of note that *Shigella* was isolated more in May, a rainy month, than in any other month, and enteropathogenic *E. coli* were isolated more in June. *Salmonella* was isolated less frequently than the rest. Due to small figures no attempt was made to find a correlation between individual enteropathogens and the weather seasons.

3.2 Kisumu Provincial Hospital and Lumumba Health Centre

The number of enteropathogens isolated in Kisumu from in and out patients with diarrhoea during the months of January, May and October, 1977 are given in Table 9.

Rotavirus was the most frequently isolated enteropathogen followed by *E. coli*, and was significantly more detected in hospitalized children than in the out-patients. Forty-two (40%) out of 104 specimens yielded a possible pathogen; 47% in-patients and 22% out-patients yielded a possible pathogen.

3.3 Mombasa Provincial Hospital and Old Town Health Centre

Table 10 shows the number of enteropathogens isolated in Mombasa during the months of January, May and November, 1977. Different from the results in Nairobi and Kisumu, *Shigella* was most commonly isolated, followed by rotavirus. There was no significant difference in the rate of isolation of these agents between in-patients and out-patients. Forty-five (39%)
out of 116 specimens yielded a possible pathogen; 45% in-patients and 34% out-patients yielded a possible pathogen.

3.4 Masailand:

In Masai children only prevalence of rotavirus was sought; 3 children out of 139 all in the 0-5 years age-group were found shedding rotavirus in their faeces. Of interest was the finding that the three children were from the same Manyatta and had been sampled on the same day during the second visit in November 1977. Diarrhoea was not among the complaints noted down for these children by our medical team at the time of stool collection.

3.5 Patterns of relative enteropathogen frequency in Nairobi, Kisumu and Mombasa:

Figure 7 depicts patterns of relative enteropathogen frequency in Nairobi, Kisumu and Mombasa. In Nairobi and Kisumu rotavirus was the most frequently isolated enteropathogen followed by enteropathogenic E. coli. In Mombasa, however, Shigella was the most commonly isolated organism. Salmonella species was encountered infrequently in all the three areas. Out of 380 specimens from patients examined in the three areas, 202 (53%) yielded a possible pathogen.

3.6 Distribution of the aetiological agents isolated by age and sex:

Ages were obtained on 160, 104 and 112 infants and
children with diarrhoea in Nairobi, Kisumu and Mombasa respectively. Distribution of enteropathogens by sex was determined from 168 infants and children admitted to POW, Kenyatta National Hospital with diarrhoea.

3.6.1 Rotavirus incidence by age

Rotavirus infection in children of various age groups in Nairobi, Kisumu and Mombasa is shown in Table 11. In Mombasa the percentages peaked in the 3-8 months age-group. In Nairobi and Kisumu the rate of isolation was almost similar in trend in the age groups 0-8 months. Amongst the children examined there were relatively fewer patients of 1 year of age and above in whom rotavirus infections were detected.

Table 12 shows that in Mombasa isolation rates for rotavirus were highest in infants of 3-8 months of age in both hospitalized and out-patients. However, in the age-group above 1 year old rotavirus was found in only out-patients (6 out of 34), and none of the 15 in-patients. In Kisumu only 3 rotaviruses were isolated from out-patients (Table 9).

3.6.2 Shigella incidence by age

Age distributions of Shigella infection are shown in Table 13. In Kisumu and Mombasa, Shigella infection was rarely isolated from infants of 0-2 and 3-5 months old. Only 2 infants out of 67 had Shigella bacilli. In Nairobi, Shigella was not isolated from the 12
of the 0-2 months age-group, but it was isolated from 6 out of 58 infants in the age-group 3-5 months.

3.6.3 E. coli incidence by age

In Table 14 the age distribution of enteropathogenic E. coli infection among the children examined is shown. Except in neonates E. coli was rarely found in the Mombasa children examined. Also in Kisumu E. coli was rare (2 isolates out of 36 specimens) in the 0-5 months old infants compared to Nairobi (14 isolates out of 70 specimens). In Nairobi, in 3 out of 12 (25%) of the studied infants under 3 months, E. coli was isolated.

3.6.4 Distribution in Nairobi of the aetiological agents by sex

The distribution according to sex of the 108 patients with diarrhoea from whom enteropathogens were isolated and in whom sex was recorded is shown in Table 15. Rotavirus infection seemed to be independent of sex in the children sampled. E. coli was isolated significantly more from males than from females. The reverse was true for Shigella.

4. Testing the hypothesis that the peak age incidence of rotavirus infections is inversely related to waning of maternal antibodies:

After the case versus control study had produced strong evidence that the major aetiological agent of acute gastro-enteritis among the patients examined was a rota-
virus, sera randomly collected from neonates and children of different age groups were tested for rotavirus C.F. antibodies to test the hypothesis that there is an inverse relationship between the peak age incidence of childhood diarrhoea observed and waning of maternal antibodies to this major causal agent.

The patterns of antibodies in percentages in the different age groups are depicted in Figure 8; 38(73%) of the 52 sera from neonates under two weeks of age had antibodies, probably of maternal origin. Antibodies were found in only 7(11%) out of 61 sera collected from the 2-5 months age groups. In the 3-5 months age-group this low percentage coincides with the highest percentage of detection of rotavirus infections. Thereafter, there was a gradual rise, and by the age of 3 years, 62(86%) out of 72 of the tested children had acquired antibodies.

The regular decline of the titres of paired sera shown in Table 16 supports the assumption that the antibodies detected in the youngest age-groups are mainly of maternal origin. Only 1 serum (figures lying above darkened lines) out of 12 taken 4-6 weeks after birth had an antibody titre of 1:6 while the cord blood was negative. None of the sera collected 8-12 weeks after birth showed conversion or a titre rise. In the age-group 17-23 months, 2 infants converted and two showed antibody titres slightly higher than that
observed in the cord sera. Figure 8 gives support to the hypothesis that the incidence of childhood diarrhoea in the age bracket 0-5 months is inversely related to the prevalence of maternal antibodies against rotavirus.

5. The Possible Contributory Factor of Bottle-feeding to the Aetiological Agents:

The low figures for enteropathogenic *E. coli* isolation in the 0-5 months age-group in Kisumu and Mombasa apart from that for neonates (Table 14) and the high figures in Nairobi in infants of this age-group raised a question as to whether bottle-feeding could be a contributory factor to the frequency of infection.

Data on feeding practices in Nairobi, Kisumu and Mombasa are shown in Table 17. Of interest is the finding that feeding practices among those interviewed in Kisumu and Mombasa are almost similar. Among those questioned in the latter two towns mixed feeding did not start until the third month after birth, and bottle feeding not until the fourth month. However, the mothers interviewed in Kisumu practised breast-feeding longer than those - mostly of Arab-African ethnic origin - in Mombasa.

On the other hand in Nairobi both bottle and mixed feeding began during the first month, and after 3 months virtually all the mothers had changed to mixed or bottle-feeding. It is tempting to relate the higher isolation rate of *E. coli* in Nairobi with the earlier bottle feeding practice.
To find support for this possible relation of bottle-feeding and isolation of enteropathogenic *E. coli* an estimation of the distribution of enteropathogens isolated from breast-fed, bottle-fed and mixed-fed infants 0-5 months old in Nairobi was undertaken. Table 18 shows the enteropathogens isolated from such individual groups. There was only a slight indication that bottle-feeding favoured the rate of *E. coli* infection in the under 3 months age-group, but not in the older age-group. The only *E. coli* isolated from the former age-group occurred together with a rotavirus. However, because of the small numbers no conclusion regarding the occurrence of *E. coli* in children of different feeding patterns can be drawn. There was no difference in the rate of isolation of rotavirus and *Shigella* species between breast and mixed or bottle-fed infants in the age-groups studied.

6. Some Clinical and Laboratory Characteristics Relative to the Aetiological Agents:

6.1. Clinical Features

6.1.1. Fatality of Acute diarrhoea

Only 2 out of 288 of the hospitalized patients with diarrhoea that provided specimens died. One fatal case was one of twin children. From both children strains of *Shigella flexneri* serotype 2 were isolated. The fatal case was 13 months old, and had been admitted to Kenyatta Hospital. The other patient who died was a
neonate admitted to Mombasa Hospital. *Shigella flexneri* sero-type 4 was isolated from a stool specimen.

### 6.1.2 Duration of illness

Due to shortage of hospital beds children admitted to the Paediatric Observation Wards for acute diarrhoeal diseases are discharged as soon as they show signs of improvement; usually at Kenyatta National Hospital this is one or two days after admission.

Children admitted early in the disease could, therefore not be followed till symptoms had disappeared. However, 30 out of the 288 were admitted with a history of more than 7 days of illness before admission. The enteric pathogens isolated from these 30 children were analysed. *Shigella* was the pathogen most frequently isolated from such cases; 8 (27%) isolates of this agent were made against only 2 enteropathogenic *E. coli* and 1 rotavirus. *Gardia lamblia* were present in 3 specimens. The remaining 16 (53%) specimens were negative.

### 6.1.3 Degree of dehydration

The degree of dehydration in and the agents isolated from 133 infants admitted to Kenyatta National Hospital are given in Table 19. Statistically there was no significant relationship between the aetiological agent and the degree of dehydration.
Although rotaviruses were detected more frequently from moderately dehydrated cases than from either mild or severe ones, the picture did not differ significantly from that of the cases from whom no pathogens were isolated. Analysis of the data thus failed to establish a relation between the degree of dehydration of infants and children admitted with severe diarrhoea and any specific aetiological agent.

6.1.4 Body Temperature

The relationship between the aetiological agent and body temperature taken in the axilla on the day of admission by the ward nurses were analysed. It can be seen from Table 20 that the range of body temperatures and the occurrence of an agent were independent.

6.2 Laboratory Findings:

6.2.1 Macroscopy of stool appearance

The relationship between the appearance of diarrhoeic stools and enteric pathogens isolated from them is shown in Table 21. Occurrence of Shigella was by far most common in blood-stained and mucoid stools. More than half of white-watery stools contained rotaviruses. The 1 Shigella and 3 E.coli strains isolated from white-watery or loose stools occurred together
with rotaviruses. No rotavirus was found in blood-stained and mucoid stools. Rotavirus was also fairly frequently associated with green watery-mucoid and yellow watery stools. Of all rotavirus isolations 47 (52%) came from yellow watery, 16 (18%) from white watery or loose and 13 (14%) from green watery and mucoid stools. 10 (33%) of 30 E. coli were also isolated from yellow watery stools. Rotavirus appeared preferentially to produce watery stools of several colours, but when white and watery, there was a good chance of isolating rotavirus. However, yellow watery stools were produced more frequently, and a larger number of rotaviruses were isolated from these stools. Striking is that in 24 of 29 (83%) of colourless stools no agent was apparent.

6.2.2 Microscopy of stools
The usefulness of looking for the presence of leucocytes (polymorphs and macrophages) and red blood cells (RBC's) in faeces as a rapid diagnostic tool was evaluated (Table 22). None of 89 rotavirus isolates and none of 33 E. coli serotypes came from a stool containing both leucocytes and RBC's. The presence of both
leucocytes and RBC's was found to be strongly indicative of the presence of *Shigella*.

7. **Extent of Rotavirus infection in Animals:**

An attempt was made to find out whether and to what extent cattle, sheep and rodents are infected with viruses of the *rotavirus* group.

7.1 **Domestic Animals**

The results of tests for C.F. rotavirus antibodies against calf disease rotavirus antigen (CDV) in sera of cattle, sheep and rodents are shown in Table 23: 19.8% of sera from farm cows had antibodies to CDV detectable at 1 in 4 or greater serum dilutions. As was expected, none of the 9 bovine foetal sera had antibodies. A high rate of antibodies (81.4%) was found in sheep sera collected in the Maasai tribe area. The results of tests for C.F. rotavirus antibodies against calf disease rotavirus antigen (CDV) in sera of cattle, sheep and rodents are shown in Table 23: 19.8% of sera from farm cows had antibodies to CDV detectable at 1 in 4 or greater serum dilutions. As was expected, none of the 9 bovine foetal sera had antibodies. A high rate of antibodies (81.4%) was found in sheep sera collected in the Maasai tribe area.

7.2 **Rodents**

Only 2 rodents out of 121 had rotavirus C.F. antibodies detectable at 1 in 4 or greater serum dilutions using CDV as the antigen. The two rodents belonged to the same species (Table 23).
V. DISCUSSION

In the foregoing, certain aspects of the epidemiology of acute early childhood gastroenteritis in Kenya have been studied; the incidence of the disease in patients admitted to some hospitals and their age distribution, the aetiological agents found in patients with diarrhoea and their relative frequency and distribution according to age and sex.

Antibodies to rotavirus or a related virus were looked for in patients, controls, cattle, sheep and rodents. Antibodies against E. coli toxin and adeno­viruses were determined also. A possible role of bottle feeding in frequency of infection with enteropathogens was also investigated. Three clinical characteristics relative to the aetiological agents were studied as well as the appearance and consistency of stools.

1. Incidence of Early Childhood Gastroenteritis in Hospitals in Nairobi

Nearly 20% of admissions to Kenyatta National Hospital Paediatric Observation Ward, Gertrude Garden and Aga Khan Children's Wards between 1971 to 1977 were due to diarrhoea (Table 1). This figure is comparable to that obtained in Ethiopia in 1961 by Habte et. al, (1964), and in Nigeria by Cobban cited by Sen et. al, (1963).
Somewhat lower figures (13.9%) were obtained by Cook (1967) in Uganda for the years 1964 to 1967.

The mean monthly percentages over 3 years of diarrhoeal cases seen at Gertrude Children's Hospital (Table 2; Figure 2) differed significantly, but with no apparent correlation with either temperature, relative humidity or rainfall. Voyer et al. (1975) observed that in Dakar Senegal it was the hot humid periods during the course of the year which coincided with an increase in cases of weaning brash (weaning-time diarrhoea). Benenson (1975) wrote in his book that the highest incidence of childhood diarrhoea in developing countries tends to parallel hot-dry periods. In South Africa (Schoub et al., 1977 a, b.) and in Taiwan (Echeverria et al., 1977), the peak incidences of childhood gastroenteritis are experienced during summer months. On the contrary Cook (1967) found the peak incidence in Mbarara, Uganda to occur during the rainy months and so did Rowland et al., (1978) in Gambia. These reports, although conflicting, indicate that climatic, meteorological factors are not the only ones that influence the periodicity of the incidence of gastroenteritis in children. Possibly the pattern of the aetiological agents also plays a role in the periodicity.

Diarrhoea leading to admission to the four hospitals indicated in Table 3 was found not to be equally distributed
over the age groups, but the figures reached a peak in
the 3-5 months age-group. This finding contrasts with
previous reports from elsewhere which have documented
the peak age-incidence in hospital cases and/or out-
patients in developing countries to be in the 6-12
months age-group (Gordon et. al, 1963; Cook 1967;
Voyer et.al, 1975 and Rowland et. al, 1977.)

On the other hand in my investigations, mild diarr-
hoea was found to reach its peak in infants aged
between 12 and 14 months, and the rate did not decline
sharply until 20 months after birth. Interestingly, the
pattern of mild diarrhoea in children of different
age-groups is somehow similar to that observed by Kumar
et. al, (1977) of acute gastroenteritis associated with
carbohydrate intolerance in well-nourished Indian infants.
It may well be that apart from enteropathogens' other
factors like carbohydrate intolerance play a role in
setting the age-incidence patterns of mild diarrhoea in
Kenya.

When comparing the peak age-incidences for hospital
admissions for severe diarrhoea and measles infections
(Figure 4) it was found that each reached its highest
frequency in somewhat different age-groups of children.
While the age-group for admissions for acute diarrhoea
was between 3 and 5 months, that of measles was found
to be between 6 and 8 months, almost the same period
when measles maternal antibodies were found to have disappeared (WHO and Kenya Ministry of Health Collaborative Study 1977). This finding led to the investigation whether incidence of severe childhood gastroenteritis is associated with the waning of maternal antibodies to the major aetiological agent. Evidence was found that this is likely to be true (Results, para 4).

2. Aetiology:

In the case-control study, conducted at Kenyatta National Hospital and reported in this thesis (Table 4), rotavirus was found to be the major aetiological agent of childhood diarrhoea; 14(39%) of the 36 children tested for viral excretion, by electron microscopy and the immunofluorescent test on infected tissue culture cells had evidence of rotavirus infection as opposed to 2(6%) in 36 controls. A number of specimens obtained from patients not matched by controls was examined also (Results, para 3.1). In all the 160 stools collected from children with diarrhoea admitted to Kenyatta National Hospital over a one year period (Table 8) rotavirus or in combination was isolated from 66 (41%) specimens and enteropathogens, including rotavirus, from 115 (72%) samples. In Kisumu (Table 9) rotavirus was isolated from 22 (29%) out of 77
in-patients and from 3(11%) out of 27 out-patients; whereas in Mombasa (Table 10) the virus was isolated from 7(14%) out of 51 in-patients and from 11(18%) out of 65 out-patients. Overall 42(40%) out of 104 specimens from Kisumu and 45(39%) out of 116 specimens from Mombasa were positive for the enteropathogens looked for, either singly or in combination.

In Africa there have been few detailed surveys with which to compare these results. Cruickshank et. al, (1975) found that in Rhodesia 20(40% out of 50 cases of gastroenteritis were shedding rotavirus in their faeces. Schoub et. al, (1975 and 1977b) from South Africa found on two occasions isolation rates of 16% and 40%, respectively.

Exceptionally, Rowland and his group (1978) failed to demonstrate rotavirus in faeces collected from children in Keneba, a rural area in Gambia, West Africa during the period of July till September, 1975. The isolation rates of rotavirus in these three reports and in my study have thus varied from country to country and from area to area within the same country. Factors influencing prevalence of infections by this virus in a community, therefore, warrant future research.

The serological evidence (Results, para 2.2) presented in Table 6 provides further support for the aetiological role of rotavirus in the induction of acute gastroen-
teritis in infants and young children. There was significant association between identification of rotavirus in stool and sero-conversion to the agent. Only two patients out of 9 who were found shedding rotavirus did not sero-convert. On the other hand in two cases out of 13 from whom rotavirus was not isolated, the patients had antibody rises in their convalescent sera. Possibly the faeces contained degenerate particles which could not replicate in tissue culture or very few particles below the threshold of detection. Failure for some children to sero-convert to rotavirus infection was reported by Echeverria et. al, (1977). Also Morishima et. al, (1976) who conducted experimental studies found that IgG antibody response in rotavirus infection may not occur or may be delayed in some cases. They suggested that the invasion of rotavirus could be localised at intestinal epithelium, so that only a very small amount of viral antigen is recognised by immunocompetent cells.

Enteroviruses were isolated with no greater frequency from children with diarrhoea than from the controls (Table 4). Neither was there convincing evidence from serology (Results, para 2.4) to show that adenoviruses played a causal role in childhood diarrhoea in the tested group. The only child who sero-converted to adenovirus infection happened to be shedding rotavirus in the faeces as well. This finding supports suggestions from earlier investigation
that as a rule, these viruses play at most a minor role in infantile gastroenteritis. Gramblett and others (1971) who have reviewed many studies found that these viruses were almost equally found among children with diarrhoea and controls. Recent studies of Rowland et. al, (1978) in Gambia also failed to associate enteroviruses and adenoviruses with acute diarrhoea.

On the other hand it could be that adenovirus infections may have occurred in my study infants and children without detectable C.F. antibodies. Fox et. al, (1969) found that only a proportion of infections with adenoviruses, especially those in young children, was followed by sero-conversion.

Among the classical enteropathogenic bacteria, only Shigella played a significant role. Six Shigella organisms were isolated from the test group and none from the controls (Table 4), comparable with results of other studies, (Kalya and Oduori, 1972). Salmonella was isolated only twice. Although the difference was not statistically significant enteropathogenic E.coli strains were more isolated from children with diarrhoea than from the controls (Table 4).

Enterotoxigenic E.coli was isolated from one patient and one control (Table 4). The isolate from the patient with diarrhoea occurred together with a pathogenic E.coli
026 strain. Interestingly, the control child from whom enterotoxigenic *E. coli* was isolated had a loose stool, presumably indicating diarrhoea of mild nature together with the respiratory disease for which the patient was admitted.

The prevalence of antibodies to heat-labile *E. coli* toxin (LT) in a proportion of the children's sera (Table 7) suggest that some children do definitely get exposed to LT which presumably results in diarrhoea usually not requiring admission to hospital. Moreover, quite frequently enterotoxigenic *E. coli* causes subclinical infections only, certainly in adults (Pickering et al., 1977) presumably also in children. Some of these subclinical infections may boost pre-existing antibodies which could explain the higher titres of anti-LT antibodies in the older children of 20 positives (Figure 6).

Gurwith and Williams (1977) concluded that enterotoxigenic and entero-invasive *E. coli* were rarely causally related to the childhood gastroenteritis, observed by the authors over a few years in children in Winnipeg, Manitoba, Canada. Enterotoxigenic *E. coli* occurred in 3 out of 276 children investigated and no enteroinvasive *E. coli* was detected.

On the other hand, Evans et al., (1977) have implicated *E. coli* strains that either produced LT or ST
childhood diarrhoea in Mexico City; 11 LT producing and 18 ST E. coli out of 62 cases of diarrhoea were isolated. Schoub et. al, (1977b) isolated 9 (24%) enterotoxigenic E. coli from 37 stools of infants with severe gastroenteritis in Pretoria, South Africa. These conflicting reports thus warrant more work to clarify the relative importance of enterotoxigenic E. coli in diarrhoeal diseases of infants and young children. Their importance may vary with time and place.

Mixed infections were found to occur fairly frequently (Table 5). Rotavirus occurred with any of the classical entero-bacteria and viruses. This is in agreement with previous findings elsewhere (Gruickshank et. al, 1975; Bryden et. al, 1975; Esparza et. al, 1977).

3. Distribution Patterns of the aetiological agents in different Geographical Areas:
Although the well known aetiological agents of childhood diarrhoea were found in all three towns (Tables 8, 9 and 10) the highest incidence was observed in Nairobi City (Figure 7). The isolation rate of rotavirus, Shigella and E. coli was significantly higher in Nairobi than in Kisumu, a smaller town. Shigella organisms were however, less frequently isolated in Nairobi than in Mombasa, also
smaller than Nairobi. The difference in patterns of enteropathogens between Nairobi and Mombasa could be explained by the out-breaks of Shigella infections that have been reported from the Coast since 1976 (Ministry of Health radio news reports). Factors leading to the present high incidence of shigellosis in that part of Kenya call for a detailed investigation.

As could be expected, Shigella organisms were isolated from widely separated areas in the country, but, probably due to the epidemic, they were isolated more often in Mombasa than in Nairobi or Kisumu. Interestingly, Shigella infection was found to reach a peak in all the three places during the long rains (April–June) when rotavirus in Nairobi and Kisumu was at its lowest rate.

Enteropathogenic E. coli was found most in Nairobi, and least in Mombasa (Figure 7). Studies are needed to establish whether difference in bottle-feeding practices contributes to the differences found, but the assumption was not proved. Salmonella organisms were found rarely in all three towns. Similar findings were reported for Nairobi by Kalya and Oduori (1972).

Rotavirus was found in only three faecal samples out of 139 taken from asymptomatic Masai children as detected by the indirect immunofluorescent tests on infected tissue culture. Remarkably, the three children were...
living within the same enclosure, possibly indicating circulation of the virus in that enclosure. Because no history of diarrhoea was taken and the specimens were taken during a one time visit without follow-up it is difficult to draw a conclusion on the meaning of this finding.

In temperate countries rotavirus occurs more frequently during the cold months than in summer (Editorial Brit. Med. J. Sept. 24, 1977). Scoub et al., have also reported from South Africa a higher incidence in winter, 61% in white children (1977b) compared with the incidence of 16% in black children in summer (Scoub et al., 1975). In Nairobi, Kenya, since seasons are not stable, only studies extending over several years will indicate whether periodicity in rotavirus prevalence is related to seasons.

Rotavirus antibodies were found in the three animal species tested (Table 23). Of interest was the findings of antibodies in two out of 121 rats. Whether infection in rats is due to the virus of epizootic diarrhoea of infant mice or due to other rotavirus types needs further investigation.

The high rate of antibodies in Masai sheep sera is also interesting and indicates yet another avenue for future research in the role of rotavirus in animal diarrhoeal diseases.
4. **Prevalence of the Aetiological Agents by Age and Sex:**

Rotavirus was found to infect mostly infants (Table 11). Infections in Nairobi and Kisumu did not differ very much in the age-groups 0-2, 3-5, 6-8 and 9-11 months. In Mombasa, however, the infection peaked in the 3-8 months age-group. These results are somewhat at variance with the findings of other workers. In the U.S.A., Kapikian et. al, (1976), found that the 6-12 and 13-24 months age-groups had the highest rates of rotavirus infection as indicated by virus shedding and serology.

The significantly smaller number of rotavirus in the less than 6 months age-group was attributed to lesser exposure to individuals shedding rotavirus or to the presence of antibody of maternal origin. In Australia the peak incidence of rotavirus infection was found by Davidson et. al, (1975) to be between 6-12 months. Also Tufresson and Johnson (1976) found that the highest rate of rotavirus infection in Swedish children was in the 6-18 months age-group. In England, Bryden et. al, (1975) reported the highest incidence in the 6 months to 3 years age group.

Orstavik et. al, (1977) found that in Norway most of the hospitalized patients due to rotavirus infection were between 6 months and 3 years. On the other hand Gurwith et. al, (1977) found that in Manitoba,
Canada, diarrhoea leading to hospitalization was most common in children younger than 6 months, and rotavirus was most frequently encountered in that age-group in hospitalized children. More recently Schnagl et. al, (1978) have found a greater proportion of hospitalized Aboriginal children under 6 months than the non-Aboriginal ones to experience rotavirus and the latter to experience infection between 6 and 12 months of age. Therefore, the age of highest incidence of rotavirus infection may well vary with either the Socio-economic status of parents or behaviouristic differences between ethnic groups.

Although the number of hospitalized and out-patient children with diarrhoea studied in Mombasa was small, in Kenya, by Motalear and others (personal communication), in the U.S.A, by Rayikian et. al, (1975) and Blacklow et. al, in children over 1 year old was higher in out-patients than in those hospitalized (Table 12). Thus comparative studies of this nature on larger samples is called for to confirm this difference. Factors leading to severe childhood gastroenteritis in the youngest age-groups requiring admission to hospital other than absence of rotavirus antibodies need to be established. The age of the child is reportedly an important determinant of the severity of infant diarrhoea. Babies aged less than 6 months were found much more likely to suffer serious or fatal illness. Presently there is no vaccine against

The results of a sero-survey (Figure 8) suggested that infection started to appear in numbers at the age of disappearance of rotavirus maternal antibodies. Only 7 (11%) out of 61 sera collected from the 3-5 months age-groups had antibodies, compared to 9(53%) out of 17 infants aged between 5 and 8 weeks. After the age of 6 months the number of children acquiring antibodies started rising, and by the age of 3 years about 90% of the tested children had antibodies. The pattern of acquisition of rotavirus antibody in the study population is comparable to that observed in Machakos, a rural area in Kenya, by Metselaar and others (personal communication), in the U.S.A. by Kapikian et. al, (1975) and Blacklow and co-workers (1977) and in Norway by Orstavik et. al, (1976). The gradual fall of antibodies and the failure to find antibody titre rises in most of paired sera of infants under 3 months old (Table 16) shows clearly that antibody being detected in this group is considerably of maternal origin.

I have found support for the hypothesis that in Kenya, the peak age-incidence of severe childhood gastro-enteritis leading to admission to hospital is inversely related to disappearance of maternal antibodies to rotavirus, rather than to introduction of bottle-feeding or weaning. Presently there is no vaccine against...
rotaviruses but should it shortly become available the optimum age for vaccination in Kenya may well be between 2 and 3 months.

The age distributions of the children from whom Shigella was isolated in Kisumu, Nairobi and Mombasa were generally the same. Shigella organisms were rarely isolated from infants less than 3 months. In Nairobi, the highest isolation rate was in children over 1 year old while in Mombasa the isolation rates in the 6-11 and 12-months and over age-groups did not differ much. Except in bottle-fed neonates in whom out-breaks of diarrhoea were evident from the laboratory records, infections due to enteropathogenic E.coli in Mombasa were quite rare. (Table 14).

The rate of rotavirus isolations from males and females did not differ very much. (Table 15.) However, E.coli was isolated more frequently from males than from females, and the reverse was true for isolations of Shigella. The meaning of these figures is, however, questionable.

5. The possible Contributory Factor of Bottle Feeding in Frequency of Infections:

Data on the feeding practices of mothers in Kenya had not been documented. Table 17 shows that only 14% of the mothers questioned in Nairobi completely breast-
fed their babies as opposed to 64% in Kisumu and 59% in Mombasa. The somewhat different age-composition of the three groups does not detract from the large differences. Assuming that those questioned are representative of their respective areas, the feeding practices in Mombasa were almost similar to those in Kisumu except that mothers in Kisumu tended to breast-feed over a longer period after child-birth. Mixed-feeding in the latter two places does not begin until the third month and bottle-feeding not until the fourth month of life.

The lower rate of enteropathogenic *E. coli* infection in breast-fed infants under 3 months old than in those bottle-fed (Table 18), and the low rate of *E. coli* infection in Kisumu and Mombasa (except in neonates who were bottle-fed) in the age-group 0-2 months, (Table 14) seems to suggest that breast-feeding could have effect on the frequency of gastroenteritis in infants under 3 months. However, more concurrent data based on larger samples are needed. Human milk and colostrum reportedly contain several factors considered to afford resistance against infection.

Svirs-Kross (1958) and Tassovats et. al.,(1961) were able to stop *E. coli* serotype 0111/B4 epidemics in their nurseries by using human milk to feed their newborns. Recently Larguia et. al, (1977) found that an epidemic of diarrhoea due to enteropathogenic *E. coli* in a suite for prematures could not be brought under control until all the prematures were fed 5cc/kg. a day
of fresh human colostrum. However, the investigators were unable to detect any effect on the colonization rate of the children in the suite by enteropathogenic *E. coli* when colostrum was employed. Their studies thus suggested that protection by human milk may not involve a bactericidal or bacteriological mechanism.

Reports have also been documented on a more frequent isolation of rotaviruses from bottle-fed babies than from those breast-fed (Chrystie et al., 1975 and Totterdell et al., 1976). Thouless and co-workers (1977) have shown by neutralization and immunofluorescent tests that human milk in the early puerperium contains rotavirus antibodies that decline to undetectable levels by five days after birth. Also Inglis et al., (1978) found rotavirus antibody in colostrum and maternal serum. More recently, Simhon and Mata (1978) have found rotavirus antibody in human colostrum.

The results in Table 18 failed to show that bottle feeding involved higher risks for *E. coli* infection neither was a promoting effect on *Shigella* or rotavirus infections in the 0-5 months age-groups found. Although small, the figures are in accordance with the theory of Schoub et al., (1978) that, while breast-feeding is associated with a significantly low attack rate of gastroenteritis this protective effect disappears when artificial feeds are introduced into the diet in
combination with breast-feeding. Their experimental evidence from mice, together with their clinical observations strongly suggested that specific-anti-rotavirus immune factors are not present in human breast milk. The observed association of bottle-feeding with gastroenteritis thus appears to be the result of increased opportunity for growth of coliforms. Indeed, Rowland and his co-workers (1978) who have assessed the role of local weaning foods in weaning diarrhoea in Gambia found the food to be having heavy bacterial contamination.

6. Some Clinical Characteristics Relative to the Aetiological Agents:

In the 288 cases observed in this study fatal diarrhoea occurred only twice. Both cases were associated with Shigella. Gordon et. al, (1964) categorized severity of gastroenteritis on the basis of stool consistency. If blood and mucus were absent from stools, diarrhoeas were designated as mild or moderate. According to that classification only Shigella caused severe diarrhoea in my study series. The other organisms isolated from mucoid and blood-stained stools, occurred together with Shigella. Shephard et. al, (1975) who did not use the classification of Gordon et. al, (1964) also found that only 1 out of 30 children harbouring
rotavirus had severe diarrhoea, but apart from rotavirus the child was infected also by two different types of bacterial pathogens.

A recent study by Keusch and Jacewicz (1977) has revealed that a bacterial toxin may play a role in infections due to *Shigella flexneri*, *sonnei* as well as *dysenteriae* along with bacterial invasion. The pathogenesis of rotavirus on the other hand seems to be different from that of *Shigella*. Flewett and Wood (1978) in their review reported that infection by rotavirus destroys the fimbriated intestinal epithelial cells bordering the lumen which synthesise disaccharidases. Lack of these enzymes causes lactose and other disaccharides to remain in the lumen of the bowel. Only monosaccharides are absorbed, and because absorption of xylose is also impaired, this causes an osmotic drain, attracting body fluid into the bowel lumen.

Because of early discharge of patients, duration of illness in relation to the aetiological agents could not be assessed. However, Gurwith et. al, (1977) observed 1185 hospitalized patients till recovery and found that the average duration of diarrhoea in patients with enteropathogenic *E.coli* infection, rotavirus, gastroenteritis and *Shigella flexneri* infections was, respectively, 4.6, 3.6 and 5.1 days. Therefore, *Shigella flexneri* lasted longest. In agreement with
that observation is my finding that from the 30 patients of my series whose illness had lasted for more than one week when examined for the first time, *Shigella* was isolated in 8 (27%) ; enteropathogenic *E. coli* in 2 (16.6%) and rotavirus in only 1 (3.3%). In Japan only two infants out of 110 with diarrhoea continuing for two weeks shed rotavirus particles in the faeces on day 11 and 12 of illness, respectively. (Konno et. al, 1977). Only Flewett et. al, (1975) have exceptionally recorded one patient found to be harbouring rotavirus after 23 days of illness. Otherwise, rotavirus excretion is greatest during the third and fourth day of illness and is rarely detectable after the eighth day (Editorial Lancet, 1975). Ryder et. al, (1976) found that rotavirus diarrhoea lasted five to six days.

The degree of dehydration was found to make no practical value in diagnosing rotavirus infections (Table 19). There was no difference in the degree of dehydration between children infected with rotavirus and those infected with the classical enteropathogens. Similar findings were reported by Echeverria et. al, (1977) in infants and children with diarrhoea seen in the emergency rooms of three metropolitan hospitals in Taipei, Taiwan.

Body temperature was not a clue leading to a diagnosis of causative agent. Although rotavirus was isolated more often from cases with temperatures over
than from cases under \( 37^\circ \text{C} \), the difference was not significant. My observations are in accord with those of Flewett et. al, (1975) in their study of an epidemic of rotavirus enteritis in a long stay children's ward in England. They found that only 1 child out of 6 had a temperature of \( 37.2^\circ \text{C} \). The rest were afebrile. On the contrary Shepherd et. al, (1975) found fever (>\( 37.5^\circ \text{C} \)) in 19 out of 30 hospitalized cases infected with rotavirus.

The results of macroscopy and microscopy of stools (Tables 21 and 22) show that the observer's eye and the light microscope are still useful and inexpensive aids in rapid diagnosis of childhood diarrhoeas. Inspection of the stools is imperative. The rice-water or white loose stools (in the absence of a cholera epidemic) are to a certain extent characteristic of rotavirus infection. In Japan, rotavirus particles were found in all 5 infants admitted between December, 1974 and February, 1975 who were excreting white stools, lacking bile pigment (Yamashita et. al, 1975).

There have been reports of isolating enteropathogenic \textit{E. coli} from stools containing blood and mucus. Masembe (1977) working in Uganda found that the classical enteropathogenic \textit{E. coli} serotypes isolated from newborns came from stools tinged with blood. Also Wamola et.al, (1973) have reported that in diarrhoea, stool specimens
were sometimes stained with blood in cases of enteropathogenic E.coli infections in children under two years old. DuPont et al., (1971) observed that only in its most severe form the clinical picture produced by the invasive E.coli was dysentery-like and included blood, mucus and inflammatory cells in the diarrhoeal stool. On the other hand Erwa et al., (1971) in their study of 150 cases of childhood diarrhoea, isolated enteropathogenic E.coli more frequently from yellow fluid faeces than from green fluid or semisolid faeces; no isolation was made from mucus and blood-stained specimens. In my specimens the enteropathogenic E.coli isolated from blood-stained faeces occurred together with Shigella and both bacterial species and rotaviruses were isolated with almost equal frequency from yellow stools. Yellow colour was thus a general appearance of stools containing either Shigella, E.coli, Salmonella or rotavirus, with the best chance for rotavirus being isolated from it (Table 21). The high percentage of negative results from colourless watery stools (Table 21) is intriguing. One could speculate whether this type of stools could be associated with viral agents still not isolated or with carbohydrate intolerance.

The results of microscopic examination were in line with world-wide experience that microscopy could be of diagnostic value. In my series leucocytes and RBC's
were mostly associated with Shigella infections. Not a single Salmonella or E. coli was isolated from such stools. Only 1 rotavirus was isolated from a stool specimen containing RBC's, but even this isolate occurred together with a Shigella species.

Sixty-five (73%) out of 89 rotavirus isolates were made from stools containing leucocytes. This observation contrasts with that of Ryder et. al, (1976) working in Bangladesh, who did not find leucocytes in faeces of rotavirus-infected children. Possibly, the presence of leucocytes in stools of cases of rotavirus infection varies with either the physical state of the host or the agent itself. Different serotypes of human rotavirus have been recently reported by Zissis and Lambert (1978), Rodriguez et. al, (1978), Fonteyne et. al, (1978) and Thouless et. al, (1978). Whether or not these serotypes differ in their capability of destruction of the brush-bordering intestinal epithelial cells is not known.

My observations warrant the conclusion that rotavirus is frequently, but not exclusively, isolated from white-watery and yellow watery or loose stools and is very rarely isolated from stools containing both leucocytes and RBC's.
Finally when evaluating what knowledge has been obtained in pursuing the objectives of this study, the following conclusions can be drawn:

(1) Some insight into the magnitude of the problem of severe acute childhood gastroenteritis has been obtained; 20 percent of admissions of children to hospitals in Nairobi were due to gastroenteritis or diarrhoea.

(2) It was found that the highest percentage for admittance to a number of hospitals in Kenya of infants and children suffering from severe diarrhoea was in the 3-5 months age-group. This peak age-incidence was found to coincide with the waning of maternal antibodies to rotavirus, a major aetiological agent.

(3) An insight into the relative importance of aetiological agents of acute childhood gastroenteritis at the time of the surveys was obtained. In a case-control study isolations of enteropathogens were made in the following order of importance:

Rotavirus was significantly more isolated than any other enteropathogen. This was the first
time in Kenya that the role of rotavirus in childhood acute gastroenteritis was elucidated by applying techniques also new to this part of the world. Shigella was second in frequency and was isolated only from patients. There was no significant difference in isolation rate of Salmonella, enteropathogenic and enterotoxigenic E.coli and enteroviruses between children with diarrhoea and those without.

(4) The aetiological agents were widely distributed in the country, but the pattern and rate of infection was found to differ among places. Rotavirus was the most frequently isolated pathogen in Nairobi and in Kisumu whereas in Mombasa, Shigella was found to be the most commonly isolated organism.

(5) Antibodies to rotavirus were found in cows, sheep and rats. This is the first time to report rotavirus antibodies in rats.

(6) It was assessed that differences in breast and bottle-feeding practices prevailed between Kisumu or Mombasa and Nairobi. There was only a slight indication that bottle-feeding promotes infection
by *E. coli* in infants below 3 months of age but not in older ones, and had no effect on infection caused by either *Shigella* or rotavirus.

(7) Rotavirus occurring alone was not isolated from diarrhoeal cases that had lasted for more than 7 days. *Shigella* was the agent most commonly isolated from such cases.

(8) A weak relation was found between colour of faecal specimens and the presence of rotavirus and also between the consistency of stools and the presence of *Shigella*. White-watery stools were strongly associated with the presence of rotavirus and blood leucocytes and RBC's with *Shigella*.

When considering the aim of the study it may be concluded that the results have contributed to the knowledge of severe acute childhood gastroenteritis in Kenya.
### Table 1

<table>
<thead>
<tr>
<th>Name of hospital and year observed</th>
<th>Total Number admitted</th>
<th>Number with diarrhoea</th>
<th>Percent with diarrhoea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenyatta (Feb. - Sept. 1971)</td>
<td>19,530</td>
<td>3,800</td>
<td>19.4</td>
</tr>
<tr>
<td>Aga Khan (1971-72)</td>
<td>2,808</td>
<td>656</td>
<td>23.3</td>
</tr>
<tr>
<td>Gertrude Garden (1975-77)</td>
<td>3,498</td>
<td>728</td>
<td>20.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>25,836</strong></td>
<td><strong>5,184</strong></td>
<td><strong>20.0</strong></td>
</tr>
</tbody>
</table>
### Table 2

Monthly distribution of diarrhoeal cases at Gertrude Garden Children's Hospital

<table>
<thead>
<tr>
<th>Month</th>
<th>1975</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number admitted</td>
<td>Number with diarrhoea</td>
<td>%</td>
<td>Number admitted</td>
<td>Number with diarrhoea</td>
<td>%</td>
<td>Number admitted</td>
<td>Number with diarrhoea</td>
</tr>
<tr>
<td>January</td>
<td>67</td>
<td>25(37.3)</td>
<td></td>
<td>99</td>
<td>43(43.4)</td>
<td></td>
<td>120</td>
<td>28(23.3)</td>
</tr>
<tr>
<td>February</td>
<td>70</td>
<td>18(25.7)</td>
<td></td>
<td>78</td>
<td>24(30.7)</td>
<td></td>
<td>88</td>
<td>21(23.8)</td>
</tr>
<tr>
<td>March</td>
<td>46</td>
<td>9(19.5)</td>
<td></td>
<td>138</td>
<td>23(16.6)</td>
<td></td>
<td>133</td>
<td>27(17.3)</td>
</tr>
<tr>
<td>April</td>
<td>84</td>
<td>21(25.0)</td>
<td></td>
<td>128</td>
<td>21(16.4)</td>
<td></td>
<td>102</td>
<td>12(11.7)</td>
</tr>
<tr>
<td>May</td>
<td>63</td>
<td>15(23.8)</td>
<td></td>
<td>80</td>
<td>11(13.7)</td>
<td></td>
<td>128</td>
<td>25(19.5)</td>
</tr>
<tr>
<td>June</td>
<td>61</td>
<td>12(19.6)</td>
<td></td>
<td>112</td>
<td>21(18.7)</td>
<td></td>
<td>101</td>
<td>16(15.8)</td>
</tr>
<tr>
<td>July</td>
<td>103</td>
<td>31(30.0)</td>
<td></td>
<td>84</td>
<td>18(21.4)</td>
<td></td>
<td>105</td>
<td>25(23.8)</td>
</tr>
<tr>
<td>August</td>
<td>78</td>
<td>22(28.2)</td>
<td></td>
<td>109</td>
<td>24(22.0)</td>
<td></td>
<td>112</td>
<td>21(18.7)</td>
</tr>
<tr>
<td>September</td>
<td>84</td>
<td>26(30.9)</td>
<td></td>
<td>104</td>
<td>22(21.1)</td>
<td></td>
<td>118</td>
<td>20(16.4)</td>
</tr>
<tr>
<td>October</td>
<td>67</td>
<td>14(20.8)</td>
<td></td>
<td>86</td>
<td>8( 9.3)</td>
<td></td>
<td>131</td>
<td>20(15.2)</td>
</tr>
<tr>
<td>November</td>
<td>77</td>
<td>14(18.2)</td>
<td></td>
<td>111</td>
<td>31(27.9)</td>
<td></td>
<td>108</td>
<td>14(12.9)</td>
</tr>
<tr>
<td>December</td>
<td>88</td>
<td>21(23.8)</td>
<td></td>
<td>112</td>
<td>11( 9.8)</td>
<td></td>
<td>123</td>
<td>18(14.6)</td>
</tr>
<tr>
<td>Total</td>
<td>888</td>
<td>228(25.6)</td>
<td></td>
<td>1241</td>
<td>257(20.7)</td>
<td></td>
<td>1369</td>
<td>243(17.7)</td>
</tr>
</tbody>
</table>
### Table 3

**Age distribution of infants and children admitted to various hospitals with severe diarrhoea.**

<table>
<thead>
<tr>
<th>Age in months</th>
<th>Name of hospital</th>
<th>Kenyatta</th>
<th>Gertrude Garden</th>
<th>Kisumu</th>
<th>Siaya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>0-2</td>
<td></td>
<td>106(11.5)</td>
<td>29(5.0)</td>
<td>32(7.1)</td>
<td>10(4.8)</td>
</tr>
<tr>
<td>3-5</td>
<td></td>
<td>309(32.2)</td>
<td>93(16.2)</td>
<td>139(31.1)</td>
<td>39(19.0)</td>
</tr>
<tr>
<td>6-8</td>
<td></td>
<td>190(19.8)</td>
<td>91(15.8)</td>
<td>119(26.6)</td>
<td>37(18.0)</td>
</tr>
<tr>
<td>9-11</td>
<td></td>
<td>146(15.2)</td>
<td>83(14.4)</td>
<td>52(11.6)</td>
<td>27(13.1)</td>
</tr>
<tr>
<td>12-14</td>
<td></td>
<td>60(6.2)</td>
<td>69(12.2)</td>
<td>52(11.6)</td>
<td>26(12.6)</td>
</tr>
<tr>
<td>15-17</td>
<td></td>
<td>51(5.3)</td>
<td>34(5.9)</td>
<td>13(2.9)</td>
<td>7(3.4)</td>
</tr>
<tr>
<td>18-20</td>
<td></td>
<td>14(1.4)</td>
<td>39(6.7)</td>
<td>19(4.2)</td>
<td>13(6.3)</td>
</tr>
<tr>
<td>21-23</td>
<td></td>
<td>29(3.0)</td>
<td>12(2.9)</td>
<td>1(0.2)</td>
<td>1(0.4)</td>
</tr>
<tr>
<td>24-35</td>
<td></td>
<td>29(3.0)</td>
<td>60(10.4)</td>
<td>8(1.7)</td>
<td>19(9.2)</td>
</tr>
<tr>
<td>36-47</td>
<td></td>
<td>16(1.6)</td>
<td>36(6.2)</td>
<td>6(1.3)</td>
<td>13(6.3)</td>
</tr>
<tr>
<td>48-59</td>
<td></td>
<td>9(0.9)</td>
<td>28(4.8)</td>
<td>5(1.1)</td>
<td>13(6.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>959</td>
<td>574</td>
<td>446</td>
<td>205</td>
</tr>
</tbody>
</table>
### Table 4

**Enteric pathogens isolated from 36 children with diarrhoea and 36 controls**

<table>
<thead>
<tr>
<th>Enteric pathogen</th>
<th>Number of cases isolated</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Children with diarrhoea</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>14 (38.9%)</td>
<td>(P &lt; 0.01)</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>6 (16.7%)</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>Enteropathogenic E. coli (BBC)</td>
<td>3 (8.3%)</td>
<td></td>
</tr>
<tr>
<td>Enterotoxigenic E. coli</td>
<td>1 (2.8%)</td>
<td></td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>2 (5.6%)</td>
<td></td>
</tr>
<tr>
<td>Enterovirus</td>
<td>6 (16.7%)</td>
<td></td>
</tr>
<tr>
<td><strong>Control group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>2 (5.6%)</td>
<td></td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Enteropathogenic E. coli (BBC)</td>
<td>1 (2.8%)</td>
<td></td>
</tr>
<tr>
<td>Enterotoxigenic E. coli</td>
<td>1 (2.8%)</td>
<td></td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Enterovirus</td>
<td>7 (19.4%)</td>
<td></td>
</tr>
</tbody>
</table>
Table 5

Single and mixed infections in children with diarrhoea

<table>
<thead>
<tr>
<th>Enteric pathogen</th>
<th>Number of times isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus + EEC</td>
<td>2</td>
</tr>
<tr>
<td>Rotavirus + shigella</td>
<td>4</td>
</tr>
<tr>
<td>Rotavirus + salmonella</td>
<td>1</td>
</tr>
<tr>
<td>Rotavirus + enterovirus</td>
<td>3</td>
</tr>
<tr>
<td>E E C + toxigenic E.coli</td>
<td>1</td>
</tr>
<tr>
<td>Rotavirus only</td>
<td>4</td>
</tr>
<tr>
<td>Shigella only</td>
<td>2</td>
</tr>
<tr>
<td>E E C only</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella only</td>
<td>1</td>
</tr>
<tr>
<td>Enterovirus only</td>
<td>3</td>
</tr>
</tbody>
</table>

The stool specimen of this patient was positive for rotavirus.
Table 6

Results of tests for complement fixing antibodies against rotavirus in children with acute gastroenteritis and in controls

<table>
<thead>
<tr>
<th>Patients with gastroenteritis, rotavirus in faeces (IF test positive)</th>
<th>Number of pairs of sera tested</th>
<th>Antibody</th>
<th>Number with significant rise in titre</th>
<th>No rise in titre</th>
<th>No antibodies in acute phase and second sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

| Patients with gastroenteritis, no rotavirus detected in faeces (IF test negative) | 13 | 2 | 0 | 11 |

| Control patients | 14 | 1* | 1 | 12 |

*The stool specimen of this patient was positive for rotavirus.*
### Table 7

**Seroconversion to E. coli heat-labile enterotoxin**

<table>
<thead>
<tr>
<th></th>
<th>Number of pairs of sera tested</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number with significant titre rise</td>
</tr>
<tr>
<td>Patients with diarrhoea</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Control patients</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

Titres are shown in Figure 6.
Table 8

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number examined</td>
<td>14</td>
<td>19</td>
<td>16</td>
<td>13</td>
<td>18</td>
<td>21</td>
<td>11</td>
<td>9</td>
<td>11</td>
<td>7</td>
<td>5</td>
<td>16</td>
<td>160</td>
</tr>
<tr>
<td>Agent: Rotavirus</td>
<td>1</td>
<td>8</td>
<td>7</td>
<td>4</td>
<td>14</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>66</td>
</tr>
<tr>
<td>Shigella</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>E.coli (EEC)</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Salmonella</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>
## Table 9

**Number of enteropathogens isolated from in and out-patients in Kisumu during January, May, and October 1977.**

<table>
<thead>
<tr>
<th>Month</th>
<th>Number examined</th>
<th>In-patients</th>
<th>Rotavirus</th>
<th>Shigella</th>
<th>E.coli</th>
<th>Salmonella Negative</th>
<th>Out-patients</th>
<th>Rotavirus</th>
<th>Shigella</th>
<th>E.coli</th>
<th>Salmonella Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan.</td>
<td>12</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>28</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>18</td>
<td>19</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Oct.</td>
<td>37</td>
<td>11</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>18</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>22</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td>41</td>
<td>27</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 10

Number of enteropathogens isolated from in and out-patients in Mombasa during January, May and November 1977

<table>
<thead>
<tr>
<th>Month</th>
<th>In-patients</th>
<th></th>
<th></th>
<th></th>
<th>Out-patients</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Rota-</td>
<td>Shige-</td>
<td>E.coli</td>
<td>Salmonella</td>
<td>Negative</td>
<td>Number</td>
<td>Rota-</td>
</tr>
<tr>
<td></td>
<td>examined</td>
<td>virus</td>
<td>lla</td>
<td></td>
<td></td>
<td></td>
<td>examined</td>
<td>virus</td>
</tr>
<tr>
<td>Jan.</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>May</td>
<td>30</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>12</td>
<td>32</td>
<td>7</td>
</tr>
<tr>
<td>Nov.</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>7</td>
<td>11</td>
<td>4</td>
<td>1</td>
<td>28</td>
<td>65</td>
<td>11</td>
</tr>
</tbody>
</table>
### Table 11

**Age distribution of rotavirus infections in Nairobi, Kisumu and Mombasa**

<table>
<thead>
<tr>
<th>Place</th>
<th>0-2</th>
<th>3-5</th>
<th>6-8</th>
<th>9-11</th>
<th>12 and over</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nairobi</td>
<td>5/12 (41.6)</td>
<td>27/58 (46.5)</td>
<td>16/40 (40.0)</td>
<td>7/15 (46.6)</td>
<td>10/35 (28.5)</td>
</tr>
<tr>
<td>Kisumu</td>
<td>3/13 (23.3)</td>
<td>6/23 (26.0)</td>
<td>7/27 (25.9)</td>
<td>5/17 (29.4)</td>
<td>2/24 (8.3)</td>
</tr>
<tr>
<td>Mombasa</td>
<td>2/15 (13.3)</td>
<td>4/16 (25.0)</td>
<td>4/15 (26.6)</td>
<td>1/17 (5.8)</td>
<td>6/49 (12.2)</td>
</tr>
</tbody>
</table>

In this and subsequent tables the numerator indicates number positive and the denominator number tested.
<table>
<thead>
<tr>
<th>Type of patient</th>
<th>0-2</th>
<th>3-5</th>
<th>6-8</th>
<th>9-11</th>
<th>12 and over</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-patient</td>
<td>2/14 (14.3)</td>
<td>2/6 (33.3)</td>
<td>2/8 (25.0)</td>
<td>1/9 (11.1)</td>
<td>0/15 -</td>
</tr>
<tr>
<td>Out-patient</td>
<td>0/1 -</td>
<td>2/10 (20.0)</td>
<td>2/7 (28.5)</td>
<td>0/8 -</td>
<td>6/34 (17.6)</td>
</tr>
</tbody>
</table>
Table 13

Age distribution of shigella infections in Nairobi, Kisumu and Mombasa

<table>
<thead>
<tr>
<th>Place</th>
<th>Age in months</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2</td>
<td>3-5</td>
<td>6-8</td>
<td>9-11</td>
<td>12 and over</td>
<td></td>
</tr>
<tr>
<td>Nairobi</td>
<td>0/12 (0%)</td>
<td>6/58 (10.3)</td>
<td>2/40 (5.0)</td>
<td>2/15 (13.3)</td>
<td>7/35 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Kisumu</td>
<td>1/13 (7.6)</td>
<td>0/23</td>
<td>1/27 (3.7)</td>
<td>2/17 (11.7)</td>
<td>3/24 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Mombasa</td>
<td>1/15 (6.6)</td>
<td>0/16</td>
<td>3/17 (17.6)</td>
<td>4/17 (23.5)</td>
<td>10/49 (20.4)</td>
<td></td>
</tr>
</tbody>
</table>
### Table 14

**Age distribution of E. coli infections in Nairobi, Kisumu, and Mombasa**

<table>
<thead>
<tr>
<th>Place</th>
<th>0-2</th>
<th>3-5</th>
<th>6-8</th>
<th>9-11</th>
<th>12 and over</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nairobi</strong></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td><strong>Kisumu</strong></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td><strong>Mombasa</strong></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
</tbody>
</table>

*All four were bottle-fed neonates.*
<table>
<thead>
<tr>
<th>Agent</th>
<th>Male (100% = 67)</th>
<th>Female (100% = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>25 (37.3%)</td>
<td>40 (43.9%)</td>
</tr>
<tr>
<td>Shigella</td>
<td>4 (5.9%)</td>
<td>12 (13.1%)</td>
</tr>
<tr>
<td>E.coli</td>
<td>16 (23.8%)</td>
<td>11 (12.0%)</td>
</tr>
<tr>
<td>Titres in cord sera</td>
<td>4-6 weeks after birth</td>
<td>8-12 weeks after birth</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td></td>
<td>/3  3  6  ≥12  Total</td>
<td>/3  3  6  ≥12  Total</td>
</tr>
<tr>
<td>/3</td>
<td>1  0  1  0  2</td>
<td>1  0  0  0  1</td>
</tr>
<tr>
<td>3</td>
<td>0  0  0  0  0</td>
<td>0  0  0  0  0</td>
</tr>
<tr>
<td>6</td>
<td>0  1  3  0  4</td>
<td>8  0  2  0  10</td>
</tr>
<tr>
<td>≥12</td>
<td>0  0  5  1  6</td>
<td>1  0  2  0  3</td>
</tr>
<tr>
<td>Total</td>
<td>1  1  9  1  12</td>
<td>10  0  4  0  14</td>
</tr>
<tr>
<td>Age in months</td>
<td>Nairobi</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>0-1</td>
<td>9(45.0)</td>
<td>4(20.2)</td>
</tr>
<tr>
<td>1</td>
<td>5(20.8)</td>
<td>8(33.3)</td>
</tr>
<tr>
<td>2</td>
<td>8(29.6)</td>
<td>5(18.5)</td>
</tr>
<tr>
<td>3</td>
<td>4(17.3)</td>
<td>7(30.4)</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1(8.3)</td>
</tr>
<tr>
<td>5</td>
<td>1(7.1)</td>
<td>6(42.8)</td>
</tr>
<tr>
<td>6</td>
<td>1(6.6)</td>
<td>1(6.6)</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>2(16.6)</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>5(71.4)</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>8(66.6)</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>2(40.0)</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>6(37.5)</td>
</tr>
<tr>
<td>Total</td>
<td>28(14.1)</td>
<td>57(28.6)</td>
</tr>
</tbody>
</table>
Table 18

Enteropathogens isolated from Breast, Bottle and Mixed fed infants in Nairobi

<table>
<thead>
<tr>
<th>Enteric Pathogen</th>
<th>Age in months</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 months (n=56)</td>
<td>3-5 months (n=89)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Breast fed</td>
<td>Bottle fed</td>
<td>Mixed fed</td>
<td>Breast fed</td>
<td>Bottle fed</td>
<td>Mixed fed</td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td>7</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Shigella</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>17</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>20</td>
<td>14</td>
<td>27</td>
<td>30</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

*Occurred together with a rotavirus
Table 19

**Degree of dehydration and etiological agents**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>14</td>
<td>26</td>
<td>15</td>
<td>55</td>
</tr>
<tr>
<td>Shigella</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>E. coli (EED)</td>
<td>7</td>
<td>8</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Salmonella</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>23</td>
<td>13</td>
<td>42</td>
</tr>
</tbody>
</table>

*According to Jelliffe, 1973.*
Table 20

Relationship between temperature and enteric pathogens

<table>
<thead>
<tr>
<th>Range of temperature</th>
<th>Rotavirus</th>
<th>Shigella</th>
<th>E.coli</th>
<th>Salmonella</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 36.5°C</td>
<td>11</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>36.6 - 37°C</td>
<td>10</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Over 37°C</td>
<td>16</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>37</strong></td>
<td><strong>9</strong></td>
<td><strong>17</strong></td>
<td><strong>3</strong></td>
<td><strong>34</strong></td>
</tr>
</tbody>
</table>
Table 21

**Stool appearance and enteropathogens**

<table>
<thead>
<tr>
<th>Stool appearance</th>
<th>Rotavirus</th>
<th>Shigella</th>
<th>Salmonella</th>
<th>E.coli</th>
<th>Negative</th>
<th>Total number of stools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formed</td>
<td>3(6%)</td>
<td>3(6%)</td>
<td>1(2%)</td>
<td>4(8%)</td>
<td>38(78%)</td>
<td>49</td>
</tr>
<tr>
<td>Yellow paste</td>
<td>3(13%)</td>
<td>0 -</td>
<td>1(4%)</td>
<td>5(21%)</td>
<td>14(61%)</td>
<td>23</td>
</tr>
<tr>
<td>Yellow watery</td>
<td>47(43%)</td>
<td>6(5%)</td>
<td>2(2%)</td>
<td>10(9%)</td>
<td>45(41%)</td>
<td>110</td>
</tr>
<tr>
<td>Yellow mucoid</td>
<td>1(17%)</td>
<td>1(17%)</td>
<td>1(17%)</td>
<td>0 -</td>
<td>3(50%)</td>
<td>6</td>
</tr>
<tr>
<td>Brown watery</td>
<td>3(21%)</td>
<td>4(29%)</td>
<td>0 -</td>
<td>0 -</td>
<td>7(50%)</td>
<td>14</td>
</tr>
<tr>
<td>Green watery</td>
<td>13(39%)</td>
<td>3(9%)</td>
<td>1(3%)</td>
<td>2(6%)</td>
<td>14(42%)</td>
<td>33</td>
</tr>
<tr>
<td>Green paste</td>
<td>3(20%)</td>
<td>2(13%)</td>
<td>0 -</td>
<td>2(13%)</td>
<td>8(53%)</td>
<td>15</td>
</tr>
<tr>
<td>White watery or loose</td>
<td>16(64%)</td>
<td>1(4%)</td>
<td>0 -</td>
<td>3(12%)</td>
<td>9(36%)</td>
<td>25</td>
</tr>
<tr>
<td>Colourless watery</td>
<td>2(7%)</td>
<td>1(3%)</td>
<td>0 -</td>
<td>2(7%)</td>
<td>24(83%)</td>
<td>29</td>
</tr>
<tr>
<td>Blood stained and mucoid</td>
<td>0 -</td>
<td>16(73%)</td>
<td>0 -</td>
<td>2(9%)</td>
<td>6(27%)</td>
<td>22</td>
</tr>
</tbody>
</table>

The 3 E.coli and 1 Shigella isolated from white watery or loose stools occurred together with rotaviruses, and the 2 E.coli isolated from blood-stained and/or mucoid stools occurred together with Shigella.
Table 22

Association between Erythrocytes and Leucocytes in faeces and enteropathogens

<table>
<thead>
<tr>
<th>Enteric pathogen isolated</th>
<th>Number of stools containing leucocytes and RBC's</th>
<th>Number of stools containing leucocytes only</th>
<th>Number of stools without leucocytes and RBC's</th>
<th>Total number of stools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>0</td>
<td>65(73.0)</td>
<td>24(27.0)</td>
<td>89</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>18(42.9)</td>
<td>13(31.0)</td>
<td>11(26.2)</td>
<td>42</td>
</tr>
<tr>
<td>E. coli (EEC)</td>
<td>0</td>
<td>10(30.3)</td>
<td>23(69.7)</td>
<td>33</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>0</td>
<td>2(28.6)</td>
<td>5(71.4)</td>
<td>7</td>
</tr>
<tr>
<td>Rotavirus + shigella</td>
<td>1(20.0)</td>
<td>3(60.0)</td>
<td>1(20.0)</td>
<td>5</td>
</tr>
<tr>
<td>Rotavirus + E E C</td>
<td>0</td>
<td>1(11.1)</td>
<td>8(88.9)</td>
<td>9</td>
</tr>
<tr>
<td>Shigella + E E C</td>
<td>0</td>
<td>1(100.0)</td>
<td>0 -</td>
<td>1</td>
</tr>
<tr>
<td>Rotavirus + salmonella</td>
<td>0</td>
<td>0 -</td>
<td>1(100.0)</td>
<td>1</td>
</tr>
<tr>
<td>No pathogens</td>
<td>9 (5.1)</td>
<td>70(39.8)</td>
<td>97(55.1)</td>
<td>176</td>
</tr>
<tr>
<td>Animal species</td>
<td>Number tested</td>
<td>Number and percent positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------</td>
<td>-----------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult cow</td>
<td>51</td>
<td>10 (19.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovin foetus</td>
<td>9</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>27</td>
<td>22 (81.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rattus rattus</td>
<td>109</td>
<td>2 (1.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kijabe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastomys natalensis</td>
<td>7</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Otomys irroratus</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. Lake Victoria basin
2. The Central Rift and associated highlands
3. The eastern plateau "Ireland"
4. The coast
5a/b The semi-arid and arid northern and southern Kenya

Figure 1. Map of Kenya showing major geographical divisions
Figure 2  Monthly distribution of cases with diarrhoea admitted to Gertrude Children's Hospital compared with the total number of admissions.
Figure 3. Incidence of diarrhoea in relation to rainfall.
Figure 4: Comparative age distribution of infants and children hospitalized to PCW for severe diarrhoea, measles and those seen at the Filter clinic with mild diarrhoea.
Figure 5. Top: A micrograph of negatively stained rotavirus particles in a faecal extract (X45,000).

Bottom: Rotavirus-infected LLC-MK2 cell showing typical cytoplasmic fluorescence (X250).
Figure 6. E.Coli L.T antibody titres in 24 serum pairs from children with and without diarrhoea.
Figure 7. Patterns of enteropathogens in Nairobi, Kisumu and Mombasa (Figures on top of histograms indicate number of isolates)
Figure 8  Incidence by age in children with severe diarrhoea and percentage of rotavirus maternal and acquired antibodies. Figures on top of histograms indicate number of seropositive over total number tested.
VI - REFERENCES:


14. CAMERON, D.J.S., BISHOP, R.F. VEESTRA, A.A. BARNES, G.L. 
   HOLMES, I.H. and RUCK, B.J. (1978) 
   Pattern of Shedding of Two Non-cultivable 
   Viruses in Stools of Newborn Babies. 

15. CANTY, R.J. and BLAKE, R.K. (1977) Diarrhoea due to 
   Escherichia coli in the rabbit: A novel 

   The clinical features of infantile 
   gastroenteritis due to rotavirus. Scand. 
   J. Infect. Dis. 8, 241.

   Propagated from Patient with non-bacterial 

18. CHRYSTIE, I.L., TOTTERDELL, B., BAKER, M.J., SCOPES, J.W. 
   and BANATVALA, J.E. (1975) Rotavirus 
   Infections in a Maternity Unit. Lancet ii, 
   79.

   Asymptomatic Endemic Rotavirus Infections 
   in the Newborn. Lancet i, 1176.

20. COLLABORATIVE STUDY, MINISTRY OF HEALTH, KENYA and THE WHO 
   (1977) Measles Immunity in the first year 
   after birth and the optimum age for 
   Hlth. Org. 55, 21.

   1964-1967. Ministry of Health Uganda 
   Government (Report).


27. DuPONT, H.L. FORMAL, J.B., HORNICK, R.B. SHYDER, M.J.,

28. ECHEVERRIA, P., Ho, M.T., BLACKLOW, N.R. QUINMAN, G.,
    PORTNOY, B. OLSON, J.G., CONKLIN, R.,
    DuPONT, H.L. and CROSS, J.H. (1977)
    Relative Importance of Viruses and Bacteria in the Etiology of Pediatric

    J., 1, 344.

    August, 2, 465.

    J., 2, 784.

    Med. J. 2, 1562.

33. EDITORIAL (1975) Rotavirus of Man and Animals. Lancet
    1, 257.

34. EDWARDS, P.R. and EWING, W.H. (1972) Identification of
    Co. Minneapolis Minnessota.

35. ELLIOT, K. and KNIGHT, J. (1976) Acute Diarrhoea in
    Childhood Ciba Foundation Symposium, P-273
    No: 42 Amsterdam: Elsevier.


50. GORDON, J.E., INGRAHAM, H.S. and ZORUS, R.P. (1947)
Transmission of epidemic gastroenteritis to human volunteers by oral administration of faecal filtrates.
J. Exp. Med. 86, 409.

51. GORDON, J.E., CHITKARA, I.D. and WYON, J.B. (1963)


J. Infect. Dis. 135, 736.


60. **Jelliffe, D. (1973)** Diarrhoea in Childhood, in Medical Care in Developing Countries, edited by King, Oxford University Press, Nairobi, Chap. 15.


associated with acute infectious non-bacterial gastroenteritis.
J. Virology 10, 1075.


Viruses and Gastroenteritis.
Lancet 1, 451.


Enterotoxigenic *Escherichia coli*
Diarrhoea of Travellers. A
Prospective Study of American
Peace Corps Volunteers. Johns
Hopkins Med. J. 141, 63.


107. SCHNAGL, R.D., HOLMES, I.H. and MACKAY - SCOLLAY, E.M.

108. SCHOUB, B.D., KOOKHOP, H.J., LEGATSAS, G., PROZESKY,
O.W., FREIMAN, I., HARTMAN, E.,
and KASSEL, H. (1975) Viruses in
black infants. Lancet 1, 1093.

109. SCHOUB, B.D., ROBINS - BROWE, R.M., LEGATSAS, G.,
STILL, C.S., MILLOTIS, M., KOOKHOP,
H.J. and PROZESKY, O.W. (1977^3^)
Rotavirus and Winter Gastroenteritis
in White South African Infants.

110. SCHOUB, B.D., GREEN, A.S., LEGATSAS, G., PROZESKY, O.W.,
HAY, I.T., PRINSLOO, J.G. and
A microbiological investigation of acute summer gastroenteritis in black South African infants.
J. Hyg. (Camb.) 78, 377.


Serotypes of Human Rotavirus. Lancet 1, 39.


Gastroenteritis: A continuing problem of child health in Britain. Lancet 1, 233.


