SUBJECT

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

By

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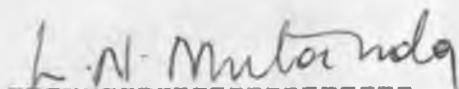A thesis submitted in fulfilment for the
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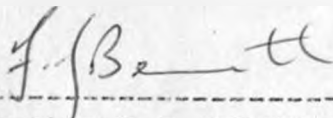
This thesis is my original work and has not been presented for a degree in any other University.

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Abstract

     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 aetiological 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. adeno-
virus 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 anti-
bodies. 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 entero-pathogens . Out of 288 children hospitalized for diarr-hoea 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 gastro-
     enteritis 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 rota-
     virus 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 entero-
pathogens.
(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 <u>Shigella</u>.

I.   INTRODUCTION

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

Leeuwenburg 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 sero-types. 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 immuno-
genically. 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 dia-
rrhoea (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 E.coli in infantile diarr-
hoea in Nairobi.

Invasive strains of 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 conjuctiva (Sereny, 1955). A third type of mechanism
of Escherichia coli, a cytotoxin similar to that produced
by Shigella relative to their diarrhoegenic potential has
been suggested by Canty and Blake (1977). Presently,
reports on the significance of enteropathogenic E.coli in
childhood diarrhoea are conflicting. Gangarosa and Merson
(1977) who have reviewed epidemiologic assessment of the
relevance of enteropathogenic serogroups of 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 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 E.coli
noted that although serotyping rarely identify invasive
or enterotoxin-producing organisms, clinical features of
infants with enteropathogenic 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 vulcaris 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 diarr-
hoea 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 adeno-viruses 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 volun-teers 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 Paver 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/in (nm) 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

antibodies (Mathan et. al, 1977). The external component
is species-specific (Matsuno et. al, 1977).

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, follcwed 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 gastroenteriłis varies with time and place.
In temperate climates the infection is most frequently seen
in winter (Editorial British Medical Journal, 13 August,
1977). Schoub 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 (Schoub 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 entero-viruses 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 (SA
11) has been readily propagated in cell culture
(Scpub 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 line

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. Rota-
viruses 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 Organi-
zation 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 greate 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 entero-
pathogenic 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 leuco-
cytes 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 of 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 meteo-
    rological 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 gastro-
enteritis 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 agricul-
tural 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. M A T E R I A L S A N D M E T H O D S

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 back-
ground. 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 res-
piratory 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 $-20^{\circ}C$ 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 0 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 $4^{\circ}C$, 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 3ml
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 phosphotung
- state. 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 1.5×10^5 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 4000r.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

methyline 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

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
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 mono-
layers 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, 0.025ml 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
thenreciprocal 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.   R E S U L T S

11.   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 <u>Shigella</u> was isolated more
in May, a rainy month, than in any other month, and
enteropathogenic <u>E.coli</u> were isolated more in June.
<u>Salmonella</u> 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 <u>Kisumu Provincial Hospital and Lumumba Health Centre</u>
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 <u>E.coli</u>, 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 <u>Mombasa Provincial Hospital and Old Town Health Centre</u>
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, <u>Shigella</u> 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 rota-
virus 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 anti-
bodies 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 practise 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 sero-type 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. <u>Shigella</u>
<u>flexneri</u> sero-type 4 was isolated from a stool
specimen.

6.1.2 <u>Duration of illness</u>

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 admittanc
The enteric pathogens isolated from these 30 children
were analysed. <u>Shigella</u> was the pathogen most
frequently isolated from such cases; 8 (27%)
isolates of this agent were made against only 2
enteropathogenic <u>E.coli</u> and 1 rotavirus. <u>Gardia</u>
<u>lamblia</u> were present in 3 specimens. The remaining
16 (53%) specimens were negative.

6.1.3 <u>Degree of dehydration</u>

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 in-
fants 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 diag-
nostic 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 anti-
bodies 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 Masai 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. D I S C U S S I O N

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 adeno-
viruses 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 child-
hood 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 rota-
virus 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 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 entero- viruses 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 sub-
clinical infections may boost pre-existing antibodies
which could explain the higher titres of anti-LT anti-
bodies in the older children of 20 positives (Figure 6).

Gurwith and Williams (1977) concluded that entero-
toxigenic 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 signi-
   ficantly 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,
the results suggest that the rate of rotavirus infection
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

biochemical disturbances (Editorial, British Medical
Journal, 17 December, 1977; Tripp et. al, 1977).

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 anti-
bodies 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,(Table 13). 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 con-current data based on larger samples are needed. Human milk and colostrum reportedly contain several factors considered to afford resistance against infection.

Svirsk-Gross (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 new-borns. 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 immuno-
fluorescent tests that human milk in the early puerperium
contains rotavirus antibodies that decline to undetactable
levels by five days after birth. Also Inglis et. al, (1978
found rotavirus antibody in colostrum and maternal serum.
More recently, Sinhon and Mata (1978) have found rota-
virus 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 bnly 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 disacchari- dases. 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

$37^{O}C$ than from cases under $37^{O}C$, 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 $37.2^{O}C$. The rest were afebrile. On the contrary Shepherd et. al, (1975) found fever ($>37.5^{O}C$) 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 E.coli from stools containing blood and mucus. Masembe (1977) working in Uganda found that the classical enteropathogenic 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 entero-
pathogenic 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 obser-

vation 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 rota-

virus 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.

CONCLUSIONS AND SUMMARY

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 gastro-enteritis 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. Rota-
virus 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 rota-
virus 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

Number of children with severe childhood diarrhoea observed in hospitals in Nairobi City

Name of hospital and year observed	Total Number admitted	Number with diarrhoea	Percent with diarrhoea
Kenyatta (Feb. - Sept. 1971)	19,530	3,800	19.4
Aga Khan (1971-72)	2,808	656	23.3
Gertrude Garden (1975-77)	3,498	728	20.8
T o t a l	25,836	5,184	20.0

Table 2

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

Month	1975 Number admitted	1975 Number with diarrhoea %	1976 Number admitted	1976 Number with diarrhoea %	1977 Number admitted	1977 Number with diarrhoea %	Total admitted	Total with diarrhoea %
January	67	25(37.3)	99	43(43.4)	120	28(23.3)	286	96(33.5)
February	70	18(25.7)	78	24(30.7)	88	21(23.8)	236	63(26.7)
March	46	9(19.5)	138	23(16.6)	133	27(17.3)	317	55(17.3)
April	84	21(25.0)	128	21(16.4)	102	12(11.7)	314	54(17.2)
May	63	15(23.8)	80	11(13.7)	128	25(19.5)	271	51(18.8)
June	61	12(19.6)	112	21(18.7)	101	16(15.8)	274	49(17.9)
July	103	31(30.0)	84	18(21.4)	105	25(23.8)	292	74(25.3)
August	78	22(28.2)	109	24(22.0)	112	21(18.7)	299	67(22.4)
September	84	26(30.9)	104	22(21.1)	118	20(16.4)	306	68(22.2)
October	67	14(20.8)	86	8(9.3)	131	20(15.2)	284	42(14.8)
November	77	14(18.2)	111	31(27.9)	108	14(12.9)	296	59(19.9)
December	88	21(23.8)	112	11(9.8)	123	18(14.6)	323	50(15.5)
T o t a l	888	228(25.6)	1241	257(20.7)	1369	243(17.7)	3498	728(20.8)

Table 3

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

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

Table 4

Enteric pathogens isolated from 36 children with diarrhoea and 36 controls

Enteric pathogen	Children with diarrhoea	Control group	Significance
Rotavirus.............	14(38.9%)	2(5.6%)	(P⧸0.01)
Shigella spp........	6(16.7%)	0 -	(P⧸0.05)
Enteropathogenic E.coli (EEC)........	3(8.3%)	1(2.8%)	-
Enterotoxigenic E.coli..............	1(2.8%)	1(2.8%)	-
Salmonella spp.....	2(5.6%)	0 -	-
Enterovirus........	6(16.7%)	7(19.4%)	-

Table 5

Single and mixed infections in children with diarrhoea

Enteric pathogen		Number of times isolated
Rotavirus + EEC		2
Rotavirus + shigella		4
Rotavirus + salmonella		1
Rotavirus + enterovirus		3
E E C + toxigenic E.coli		1
Rotavirus only		4
Shigella only		2
E E C only		0
Salmonella only		1
Enterovirus only		3

Table 6

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

	Number of pairs of sera tested	Antibody		
		Number with signifi-cant rise in titre	No rise in titre	No anti-bodies in acute phase and second sera
Patients with gastroenteritis, rotavirus in faeces (IF test positive)	9	7	0	2
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

	Number of pairs of sera tested	Antibodies		
		Number with significant titre rise	No rise in titre	No antibodies in acute phase and second sera
Patients with diarrhoea	12	1	6	5
Control patients	12	1	9	2

Titres are shown in Figure 6 .

Table 8

Monthly distribution of enteric pathogens in Nairobi during 1975-1976

Month	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Total
Number examined	14	19	16	13	18	21	11	9	11	7	5	16	160
Agent:													
Rotavirus	1	8	7	4	14	10	5	5	4	2	2	4	66
Shigella	5	1	1	1	0	3	1	1	0	0	1	3	17
E.coli (EEC)	3	6	3	2	2	4	3	1	1	0	2	1	28
Salmonella	2	0	1	0	0	0	0	0	1	0	0	0	4

Table 9

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

Month	In-patients						Out-patients					
	Number examined	Rota-virus	Shige-lla	E.coli	Salmo-nella	Nega-tive	Number examined	Rota-virus	Shige-lla	E.coli	Salmo-nella	Nega-tive
Jan.	12	6	0	1	0	5	2	1	0	0	0	1
May	28	5	4	0	1	18	19	1	2	1	0	15
Oct.	37	11	1	6	1	18	6	1	0	0	0	5
Total	77	22	5	7	2	41	27	3	2	1	0	21

Table 10

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

Month	In-patients						Out-patients					
	Number examined	Rota-virus	Shige-lla	E.coli	Salmo-nella	Nega-tive	Number examined	Rota-virus	Shige-lla	E.coli	Salmo-nella	Nega-tive
Jan.	12	1	1	1	1	8	19	3	4	0	0	12
May	30	5	10	3	0	12	32	7	4	1	0	20
Nov.	9	1	0	0	0	8	14	1	1	1	0	11
Total	51	7	11	4	1	28	65	11	9	2	0	43

Table 11

Age distribution of rotavirus infections in Nairobi, Kisumu and Mombasa

Place	Age in months				
	0-2	3-5	6-8	9-11	12 and over
	%	%	%	%	%
Nairobi	$5/12$ (41.6)	$27/58$ (46.5)	$16/40$ (40.0)	$7/15$ (46.6)	$10/35$ (28.5)
Kisumu	$3/13$ (23.3)	$6/23$ (26.0)	$7/27$ (25.9)	$5/17$ (29.4)	$2/24$ (8.3)
Mombasa	$2/15$ (13.3)	$4/16$ (25.0)	$4/15$ (26.6)	$1/17$ (5.8)	$6/49$ (12.2)

In this and subsequent tables the numerator indicates
number positive and the denominator number tested.

Table 12

Age distribution of rotavirus infections in out and in-patients in Mombasa

Type of patient	Age in months				
	0-2	3-5	6-8	9-11	12 and over
In-patient	$2/_{14}$ (14.3)	$2/_6$ (33.3)	$2/_8$ (25.0)	$1/_9$ (11.1)	$0/_{15}$ -
Out-patient	$0/_1$ -	$2/_{10}$ (20.0)	$2/_7$ (28.5)	$0/_8$ -	$6/_{34}$ (17.6)

Table 13

Age distribution of shigella infections in Nairobi, Kisumu and Mombasa

Place	Age in months									
	0-2		3-5		6-8		9-11		12 and over	
		%		%		%		%		%
Nairobi	$^0/_{12}$	-	$^6/_{58}$	(10.3)	$^2/_{40}$	(5.0)	$^2/_{15}$	(13.3)	$^7/_{35}$	(20.0)
Kisumu	$^1/_{13}$	(7.6)	$^0/_{23}$	-	$^1/_{27}$	(3.7)	$^2/_{17}$	(11.7)	$^3/_{24}$	(8.3)
Mombasa	$^1/_{15}$	(6.6)	$^0/_{16}$	-	$^3/_{17}$	(17.6)	$^4/_{17}$	(23.5)	$^{10}/_{49}$	(20.4)

Table 14

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

Place	Age in months				
	0-2	3-5	6-8	9-11	12 and over
	%	%	%	%	%
Nairobi	$4/12$ (33.3)	$11/58$ (18.9)	$6/40$ (15.0)	$4/15$ (26.6)	$4/35$ (11.4)
Kisumu	$1/13$ (7.7)	$1/23$ (4.3)	$3/27$ (11.1)	$2/17$ (11.7)	$1/24$ (4.1)
Mombasa	*$4/15$ (26.6)	$0/16$ -	$0/17$ -	$2/17$ (11.7)	$0/49$ -

*All four were bottle-fed neonates.

Table 15

Relation of sex to infection with rotavirus, shigella and E.coli in Nairobi

Agent	Male (100% = 67)	Female (100% = 91)
Rotavirus	25 (37.3%)	40 (43.9%)
Shigella	4 (5.9%)	12 (13.1%)
E.coli	16 (23.8%)	11 (12.0%)

Titres in cord sera	4-6 weeks after birth					8-12 weeks after birth					17-23 weeks after birth				
	<3	3	6	≥ 12	Total	<3	3	6	≥ 12	Total	<3	3	6	≥ 12	Total
<3	1	0	1	0	2	1	0	0	0	1	2	2	0	0	4
3	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2
6	0	1	3	0	4	8	0	2	0	10	10	2	0	1	13
≥ 12	0	0	5	1	6	1	0	2	0	3	2	0	1	0	3
Total	1	1	9	1	12	10	0	4	0	14	15	4	2	1	22

Table 17

Child feed practices in Nairobi, Kisumu and Mombasa

Age in months	Nairobi			Kisumu			Mombasa		
	Breast feeding	Bottle feeding	Mixed feeding	Breast feeding	Bottle feeding	Mixed feeding	Breast feeding	Bottle feeding	Mixed feeding
	%	%	%	%	%	%	%	%	%
∠1	9(45.0)	4(20.2)	7(35.0)	2(100.0)	0 -	0 -	3(100.0)	0 -	0 -
1	5(20.8)	8(33.3)	11(45.8)	17(100.0)	0 -	0 -	26(100.0)	0 -	0 -
2	8(29.6)	5(18.5)	14(51.8)	18(85.7)	0 -	3(14.2)	9(75.0)	0 -	3(25.0)
3	4(17.3)	7(30.4)	12(52.1)	18(81.8)	0 -	4(18.1)	6(66.6)	1(11.1)	2(22.2)
4	0 -	1(8.3)	11(91.6)	8(40.0)	3(15.0)	9(45.0)	6(50.0)	1(8.3)	5(41.6)
5	1(7.1)	6(42.8)	7(20.0)	2(20.0)	2(20.0)	6(60.0)	9(75.0)	2(16.6)	1(8.3)
6	1(6.6)	1(6.6)	13(86.6)	10(55.5)	2(11.1)	6(33.3)	7(36.8)	3(15.7)	9(47.3)
7	0 -	2(16.6)	10(83.3)	5(45.4)	3(27.2)	3(27.2)	2(28.5)	3(42.8)	2(28.5)
8	0 -	5(71.4)	2(28.5)	3(30.0)	3(30.0)	4(40.0)	2(25.0)	0 -	6(75.0)
9	0 -	8(66.6)	4(33.3)	2(50.0)	0 -	2(50.0)	1(20.0)	2(40.0)	2(40.0)
10	0 -	2(40.0)	3(60.0)	5(83.3)	0 -	1(16.6)	1(33.3)	0 -	2(66.6)
11	0 -	0 -	1(100.0)	2(50.0)	0 -	2(50.0)	1(100.0)	0 -	0 -
12	0 -	6(37.5)	10(62.5)	8(80.0)	0 -	2(20.0)	1(12.5)	0 -	7(87.5)
Total	28(14.1)	57(28.6)	114(57.3)	100(64.5)	13(8.4)	42(27.1)	74(59.2)	12(9.6)	39(31.2)

Table 18

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

Enteric pathogen	Age in months					
	∠3 months (n=56)			3-5 months (n=89)		
	Breast fed	Bottle fed	Mixed fed	Breast fed	Bottle fed	Mixed fed
Rotavirus	6	7	5	12	7	12
E.coli	*1	4	4	5	3	3
Shigella	1	0	0	3	3	2
Negative	14	9	5	7	17	15
T o t a l	22	20	14	27	30	32

*Occurred together with a rotavirus

Table 19

Degree of dehydration and aetiological agents

Agent	Degree of dehydration[*]			
	Mild	Moderate	Severe	Total
Rotavirus	14	26	15	55
Shigella	5	5	3	13
E.coli (EEC)	7	8	5	20
Salmonella	0	2	1	3
Negative	6	23	13	42

[*]According to Jelliffe, 1973.

Table 20

Relationship between temperature
and enteric pathogens

Range of temperature	Number of enteric pathogens isolated				
	Rotavirus	Shigella	E.coli	Salmonella	Negative
Below 36.5°C	11	2	8	1	13
36.6 - 37°C	10	4	3	0	9
Over 37°C	16	3	6	2	12
T o t a l	37	9	17	3	34

Table 21

Stool appearance and enteropathogens

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

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

Enteric pathogen isolated	Number of stools containing leucocytes and RBC's	Number of stools containing leucocytes only	Number of stools without leucocytes and RBC's	Total number of stools
Rotavirus......................	0 -	65(73.0)	24(27.0)	89
Shigella spp...............	18(42.9)	13(31.0)	11(26.2)	42
E.coli (EEC)...............	0 -	10(30.3)	23(69.7)	33
Salmonella spp.............	0 -	2(28.6)	5(71.4)	7
Rotavirus + shigella......	1 (20.0)	3(60.0)	1(20.0)	5
Rotavirus + E E C	0 -	1(11.1)	8(88.9)	9
Shigella + E.E 6.........	0 -	1(100.0)	0 -	1
Rotavirus + salmonella....	0 -	0 -	1(100.0)	1
No pathogens	9 (5.1)	70(39.8)	97(55.1)	176

Table 23

Rotavirus C.F. antibodies in sera of animals

Animal species	Number tested	Number and percent positive
Adult cow	51	10 (19.8%)
Bovin foetus	9	0 -
Sheep	27	22 (81.4%)
Rattus rattus kijabe	109	2 (1.8%)
Mastomys natalensis	7	0 -
Otomys irroratus	5	0 -

1. Lake Victoria basin
2. The Central Rift and associated highlands
3. The eastern plateau 'oreland
4. The coast
5a/b The semi-arid and arid northern and southern Kenya
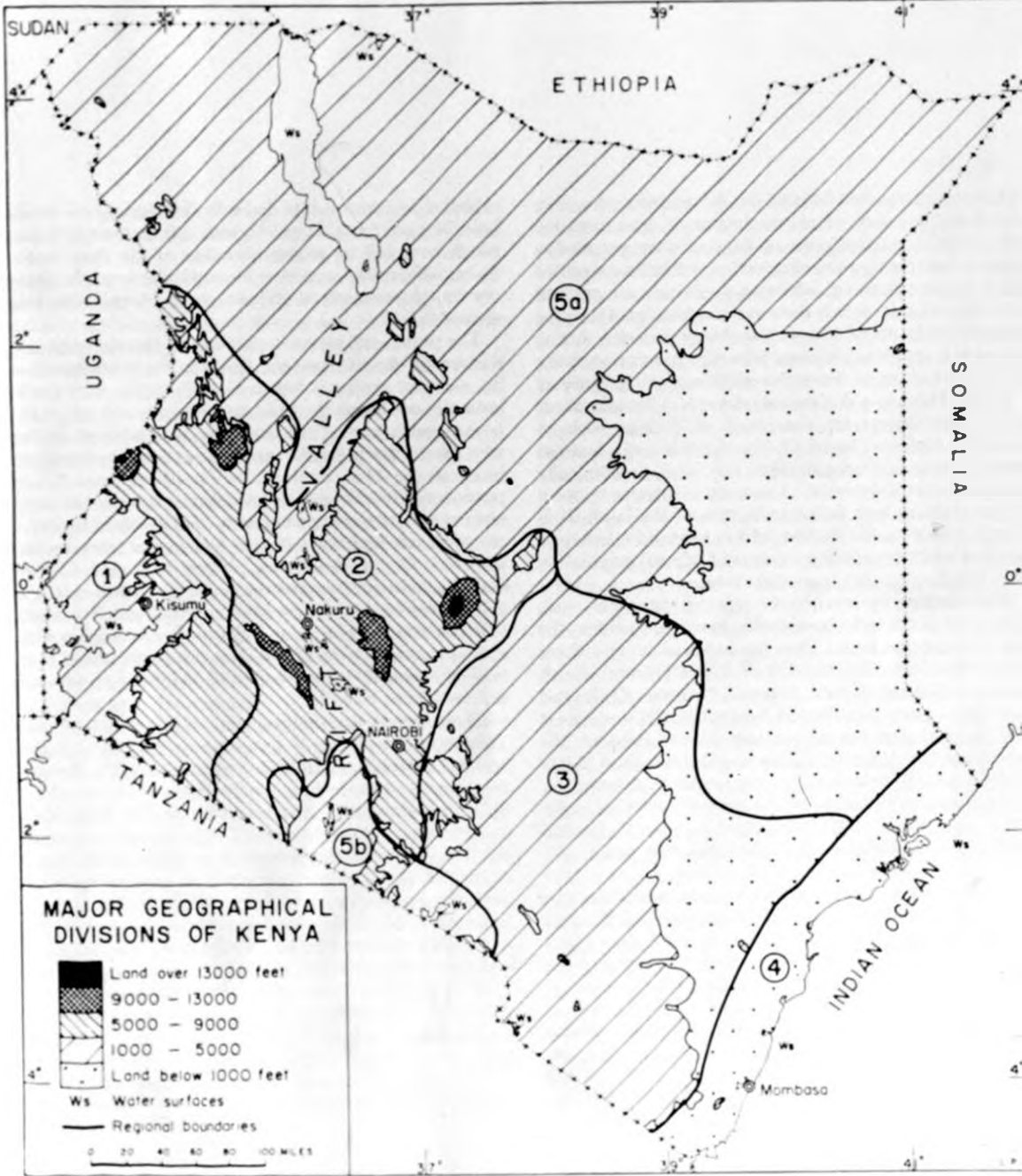
Figure 1. Map of Kenya showing major geographical divisions
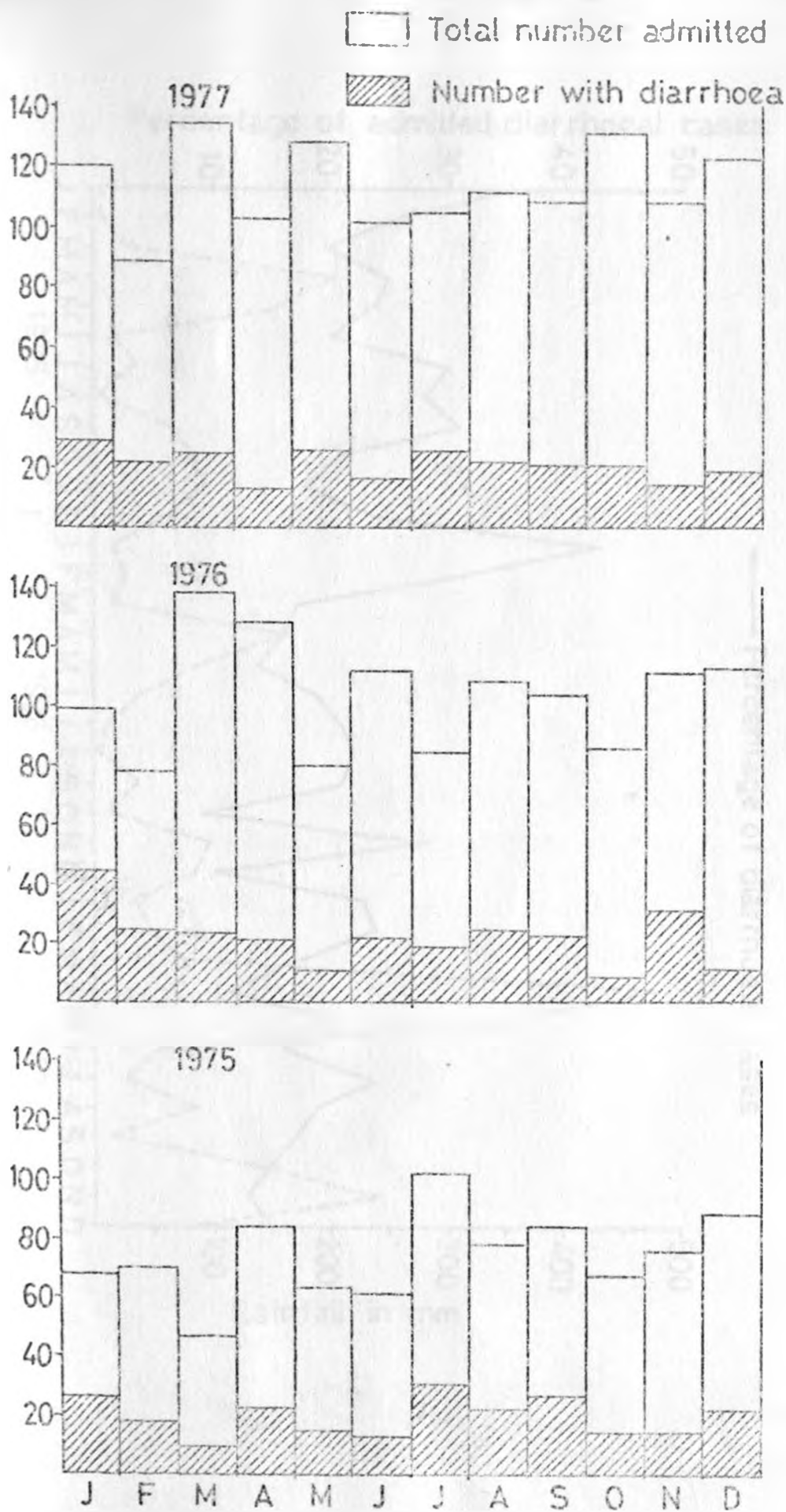
Figure 2   Monthly distribution of cases with diarrhoea admitted to Gertrude Children's Hospital compared with the total number of admissions.
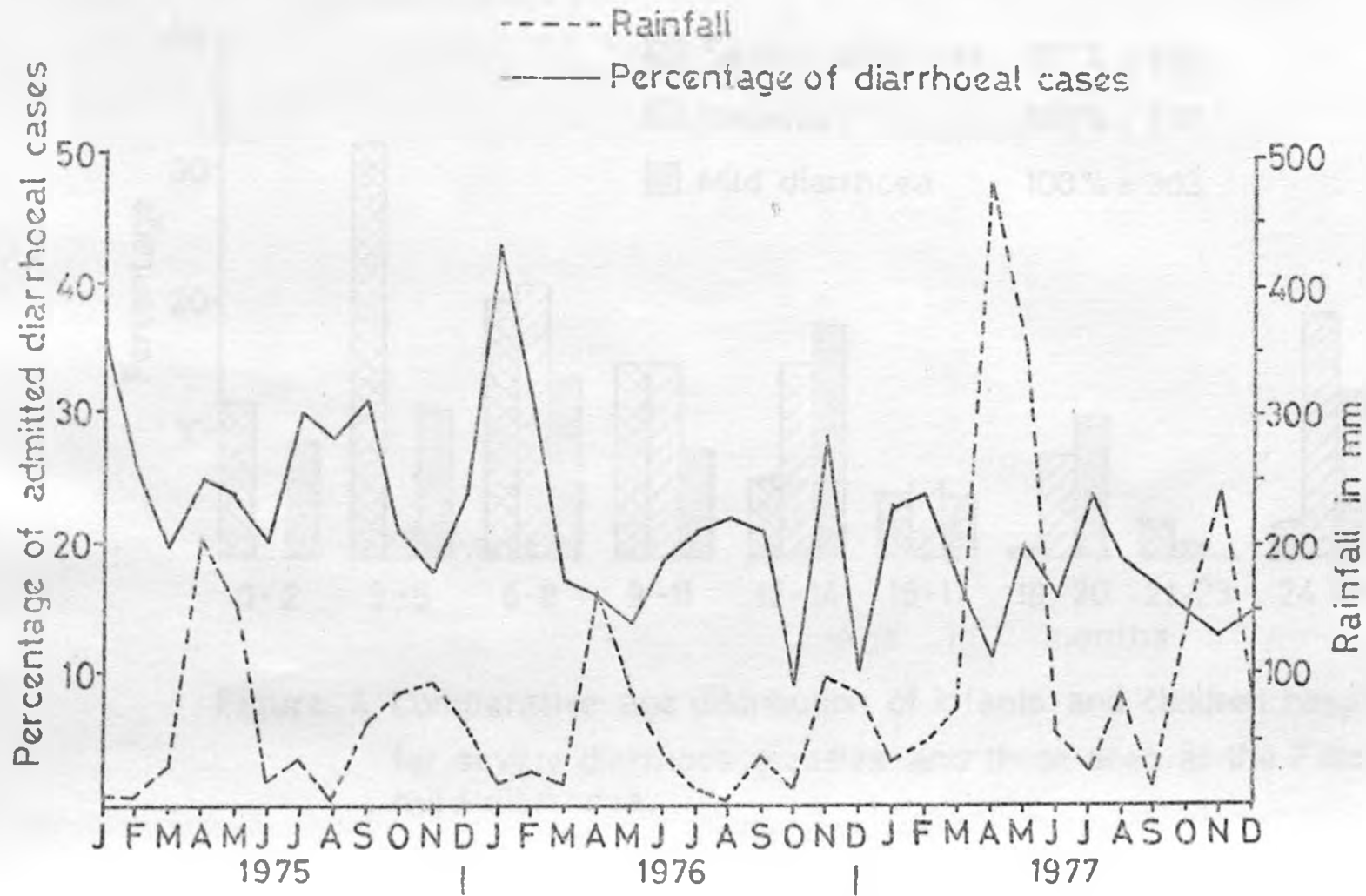
Figure 3.   Incidence of diarrhoea in relation to rainfall.
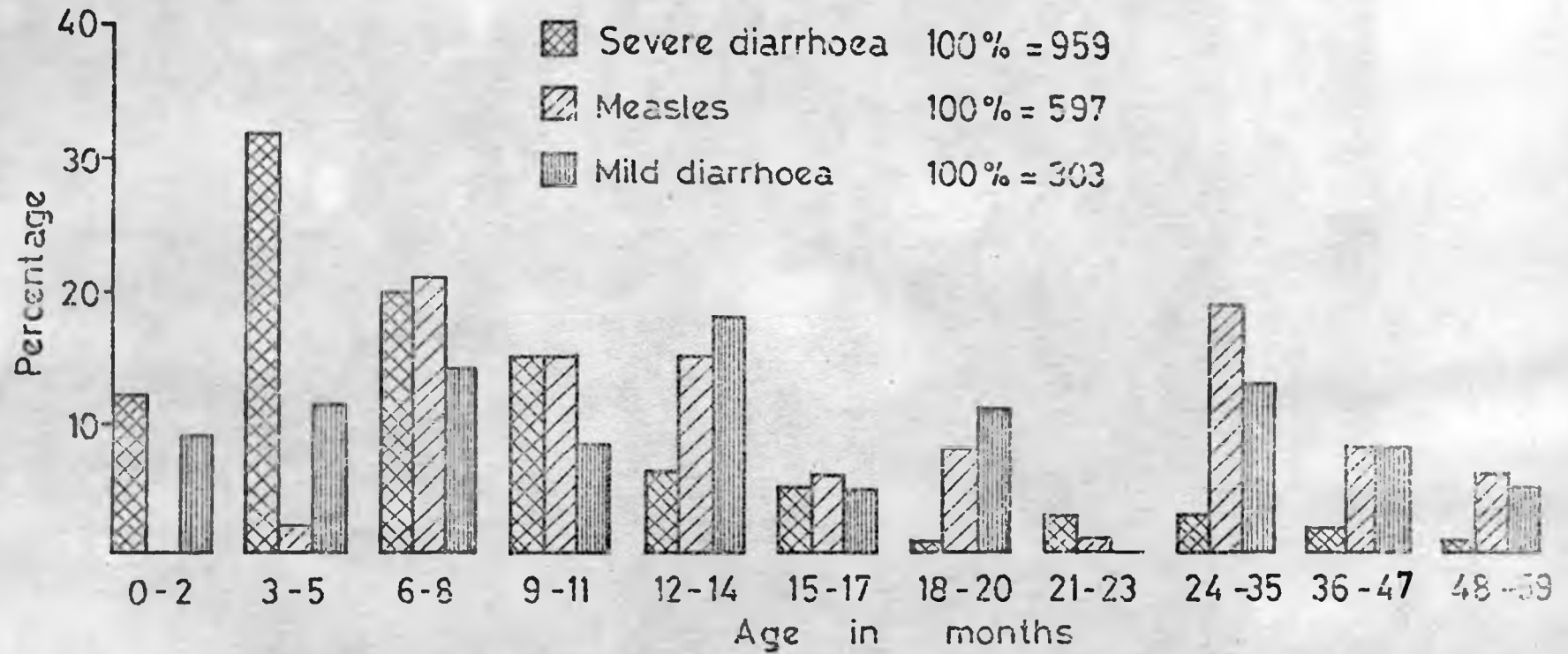
Figure 4 Comparative age distribution of infants and children hospitalized to POW for severe diarrhoea, measles and those seen at the Filter clinic with mild diarrhoea.
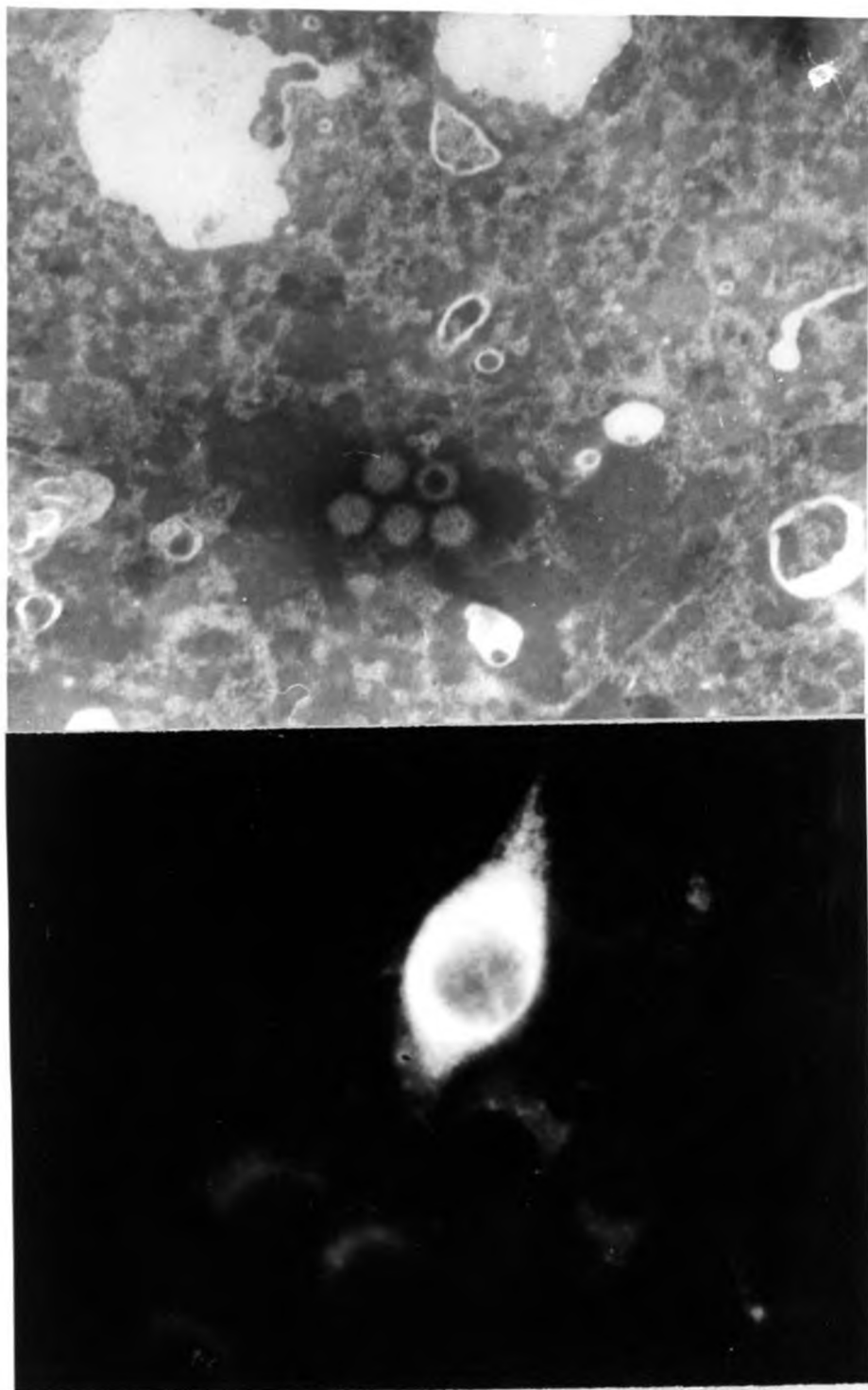
Figure 5. Top: A micrograph of negatively stained rotavirus
particles in a faecal extract (X45,000).

Bottom: Rotavirus-infected LLC-MK$_2$ cell showing
typical cytoplasmic fluorescence (X250).
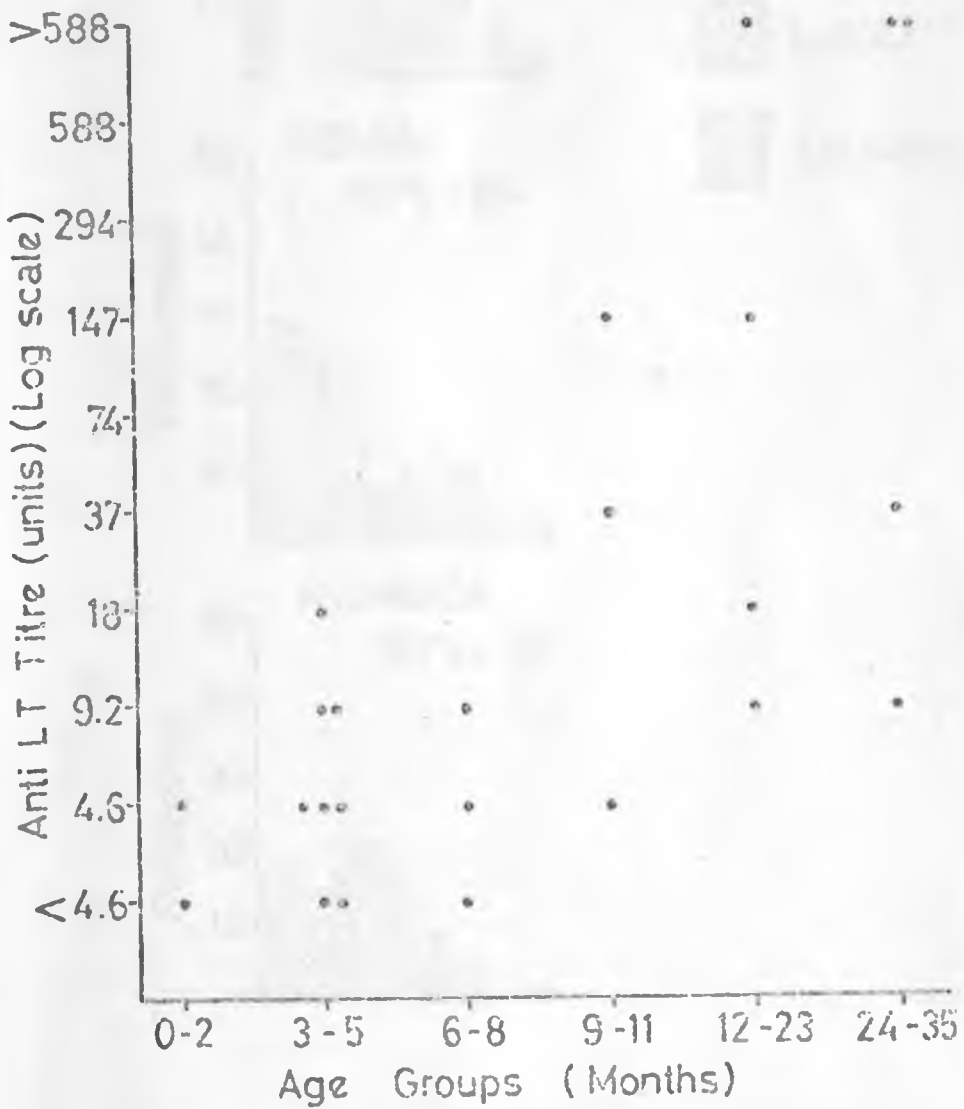
Figure 6. E.Coli L.T antibody titres in 24 serum pairs from children with and without diarrhoea.
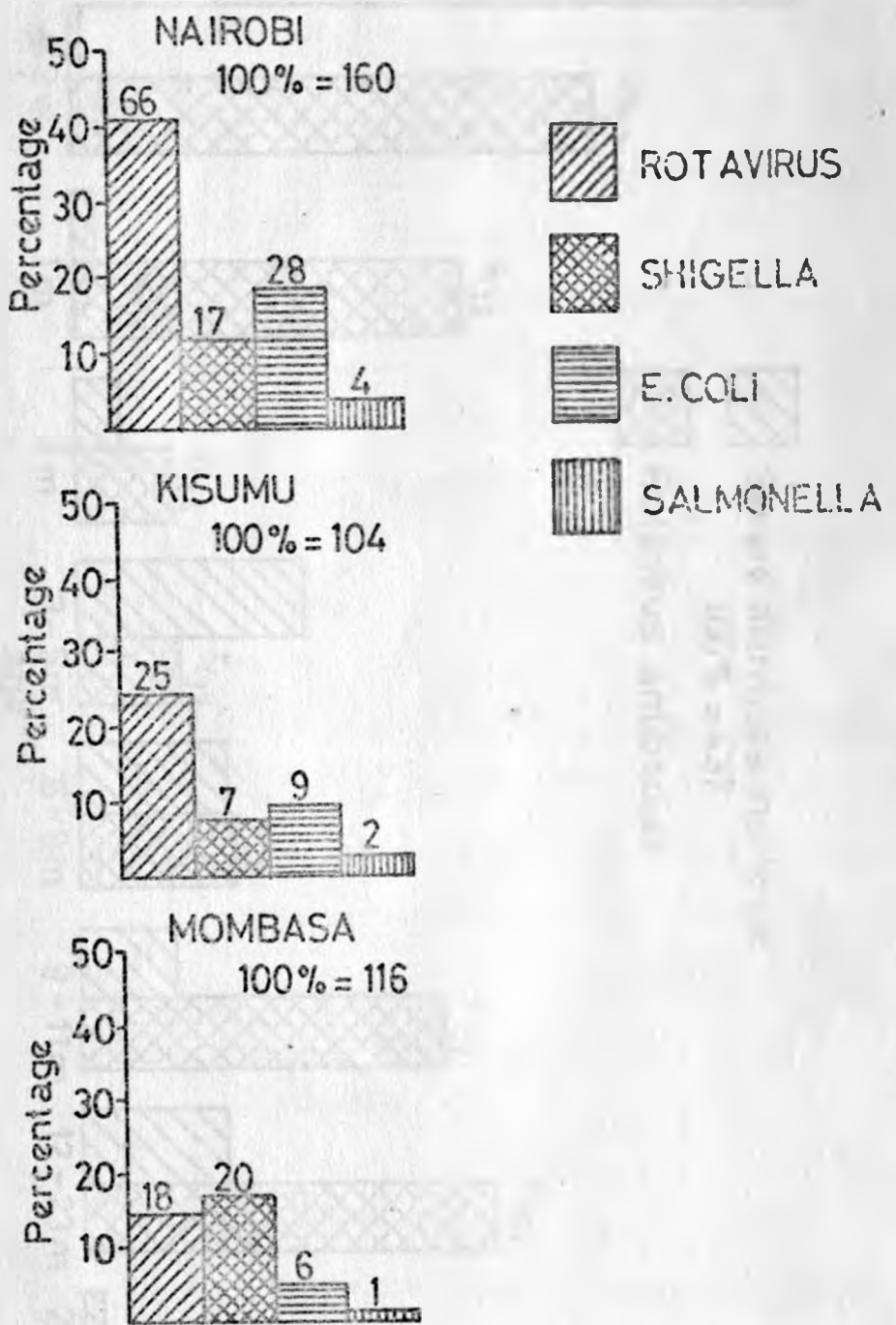
Figure 7. Patterns of enteropathogens in Nairobi, Kisumu and Mombasa (Figures on top of histograms indicate number of isolates)
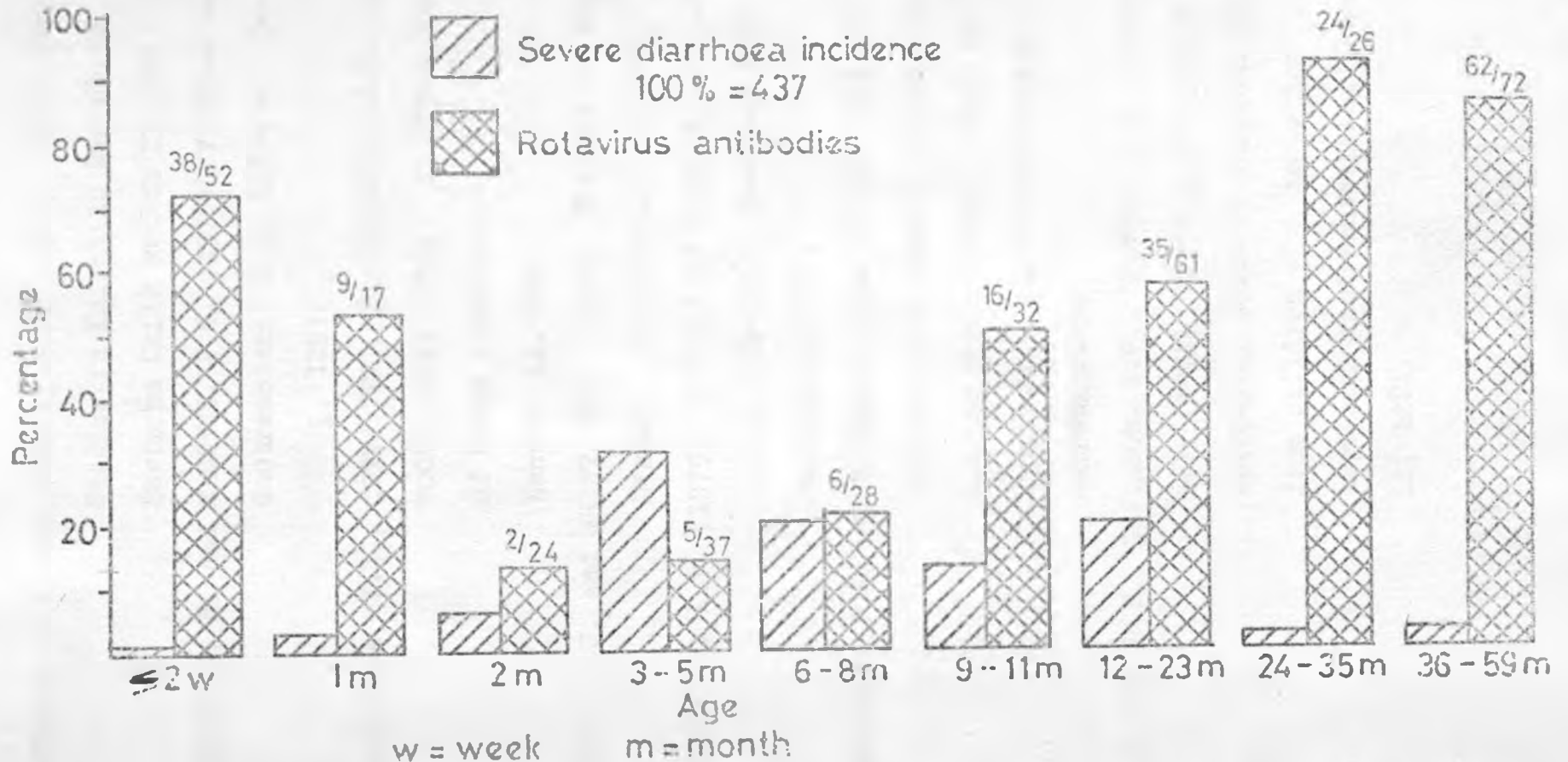
Figure 8 Incidence by age in children with severe diarrhoea and percentage of
rotavirus maternal and acquired antibodies. Figures on top of histograms
indicate number of seropostive over total number tested.

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