

**EPIDEMIOLOGY OF VIRAL GASTROENTERITIS IN CHILDREN UNDER 5 YEARS
AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA**

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THE UNIVERSITY OF ZAMBIA

LUSAKA

1996

DEDICATION

This work is dedicated to my late father who was instrumental in my taking up of a medical career. He would have loved to see this work.

DECLARATION

252550

I hereby declare that this dissertation is my own work and that it has not been previously submitted for degree purposes here or in any other University.



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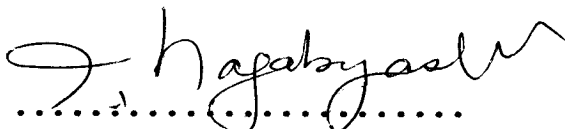
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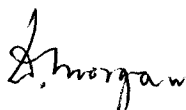
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SUMMARY

The clinical and epidemiological aspects of rotavirus diarrhoea was studied in hospitalized children under five years with acute gastroenteritis in Lusaka, Zambia for a period of one year. Two hundred and fifty six (24.1 %) of 1,064 children admitted to the study were shedding rotavirus. Rotavirus positive rate was higher in children less than one year (37.6 %) and also those less than six months. Rotavirus diarrhoea was seen throughout the year with higher rotavirus positive rate in the cool dry season. In rotavirus positive diarrhoea patients, more children had dehydration (82.4 %) than in rotavirus negative group (56.2 %). In malnourished children rotavirus positive rate was 12.1 % (9 / 71) which was lower than in those with normal nutritional status (29.0 %, 162 / 558). Case fatality rate in the rotavirus positive group was 6.4 % and mortality cases were only seen in children less than 2 years. Enteric adenovirus contributed much less (3.63 %, 6 / 164) to the viral aetiology of acute gastroenteritis and no fatality was noted in this group. This study failed to yield any Small Round Viruses in hospitalised children with acute gastroenteritis.

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LIST OF ABBREVIATIONS

A	- Adenine
CaCl	- Calcium Chloride
DTU	- Diarrhoea Training Unit
dsDNA	- Double stranded Deoxyribonucleic Acid
dsRNA	- Double stranded Ribonucleic Acid
E.M.	- Electron Microscope
ELISA	- Enzyme Linked Immunosorbent Assay
EPI - INFO	- Epidemiological Information Soft ware
HRV	- Human Rotavirus
IgA	- Immunoglobulin A
IgG	- Immunoglobulin G
IgM	- Immunoglobulin M
IEM	- Immuno-Electron Microscopy
LAT	- Latex Agglutination Test
mRNA	- Messenger Ribonucleic Acid
nm	- Nanometer
NS	- Non-structural Proteins
OD	- Optical Density
OPD	- Out Patient Department
PAGE	- Polyacrylamide Gel Electrophoresis
-PBS	- minus Phosphate Buffered Saline
PEM	- Protein Energy Malnutrition
RIA	- Radio Immuno Assay
RIA-BL	- Radio Immuno Assay - Blocking technique
ScIgA	- Secretory Immunoglobulin A
SRV	- Small Round Viruses
SRSV	- Small Round Structured Viruses
T	- Thymidine
UTH	- University Teaching Hospital
VP	- Viral Protein
WHO	- World Health Organization

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CHAPTER 1

BACKGROUND

Diarrhoea in humans has been documented since pre-Hippocratic times. Discoveries made in the past century in the fields of bacteriology and parasitology resulted in the elucidation of the aetiology of a portion of diarrhoeas. However, it soon became apparent that despite the bacteriological and parasitic discoveries, significant proportion of epidemic and infantile gastroenteritis could not be ascribed to any aetiological agent (13,51).

In 1945, the transmission of gastroenteritis illness to volunteers following administration by the respiratory route nebulised bacteria-free filtrates of faecal suspensions from gastroenteritis patients. By exclusion, it was assumed that many infectious gastroenterides were virus (13,51).

During the past two decades viral causes of gastroenteritis have been uncovered for the first time, and viruses have joined bacteria and parasites as recognized pathogens involved in medically important diarrhoeal disease. Worldwide, acute gastroenteritis and its associated dehydration afflicts 500 million children annually. In underdeveloped or developing countries, acute gastroenteritis, including viral gastroenteritis is the leading cause of death of children under five years (13,51). Five major categories of viral aetiological agents of human gastroenteritis have been defined: rotavirus, enteric adenovirus, and small round viruses namely norwalk virus, calicivirus and astrovirus (19). Rotavirus has been known as a major causative agent of acute diarrhoea among infants and young children the world over (13,19).

These various viral agents were discovered by the method of electron microscopy (E.M.), using E.M. to examine stools or intestinal biopsies from these patients (3,4).

Limited studies in Malawi and Kenya have shown that rotavirus account for 41 % and 42 % to the aetiology of diarrhoea in unhospitalised children respectively (41,44). There are conflicting reports from UTH about the prevalence of rotavirus diarrhoea in hospitalized children. Reports from the Virology laboratory show that rotavirus diarrhoea account for 38.8 % of all diarrhoeal diseases in children (personal communication). Other studies from the Department of Paediatrics showed that rotavirus accounted for 19.08 % (51). However, the difference could be due to the different diagnostic methods used. A rotaclone antigen detection kit was used in the former and latex agglutination test was used in the latter. In Malawi, Adenovirus accounted for 4.2 %, Astroviruses 1.2 %, and Norwalk agent and small round viruses 0.6 % to the aetiology of childhood diarrhoea (44). Other viral agents of gastroenteritis have not been documented in Zambia, and these viruses include enteric adenovirus, and small round viruses.

Viral enteropathogens are particularly potent inducers of diarrhoea, vomiting and subsequent dehydration which contributes to high rates of mortality. The widespread provision of oral rehydration salts for the management of acute attacks of diarrhoea is now beginning to contribute to a reduction in the morbidity and mortality. However, a greater reduction in morbidity and mortality can be achieved by using selected vaccines (16).

In Zambia, diarrhoeal disease in the under 5 year old children is among the top five major causes of admission to hospital and also the major killer (51). The Government of Zambia is strongly committed to reducing the morbidity and mortality of acute diarrhoea through the National Control of Diarrhoea Diseases Programme.

HYPOTHESIS

1. Several viral agents of gastroenteritis exist in Zambia.
2. Rotavirus is a significant contributor to gastroenteritis in infants.

CHAPTER TWO
THE VIRAL AGENTS OF CHILDHOOD DIARRHOEA

PART 1: ROTAVIRUS

CLASSIFICATION AND MOLECULAR BIOLOGY

The International Committee for the Taxonomy of viruses has classified the rotaviruses as a separate genus within the family reoviridae of the RNA viruses (Table 1) (19,51).

TABLE 1: CLASSIFICATION OF THE FAMILY REOVIRIDAE (29)

GENUS	HOST	REPRESENTATIVE DISEASE
Rotavirus	Mammals	Gastroenteritis
Phytoreovirus	Plants	Dwarf Rice
Fijivirus	Plants	Dwarf Maize Rough
Cytoplasmic Polyhydrosis Virus	Insects	Polyhydrosis

Rotaviruses share common morphological and biochemical properties (Table 2).

TABLE 2: GENERAL CHARACTERISTICS OF ROTAVIRUSES (29)

STRUCTURE

- 70 nm icosahedral particles
- Double-layered protein capsid
- Nonenveloped (resistant to lipid solvents)
- Capsid contains all enzymes for mRNA production

GENOME

- 11 segments of dsRNA
- Purified RNA segment codes for at least one protein
- Each RNA segments from different viruses reassort at high frequency during dual infections of cells.

REPLICATION

- Cultivation facilitated by protease
- Cytoplasmic replication
- Inclusion body formation
- Unique morphogenesis involves transient enveloped particles
- Virus normally released by cell lysis

The most salient features are as follows (29,35);

- (a) Mature virus particles are approximately 70 nm in diameter and possess a double-layered icosahedral protein composed of an outer layer, an inner layer, and a core.
- (b) Particles contain an RNA-dependant RNA polymerase and other enzymes capable of producing capped RNA transcript.
- (c) Virus genome contains 11 segments of double stranded RNA (ds RNA).
- (d) The viruses are capable of genetic reassortment.
- (e) Virus replication occurs in the cytoplasm of infected cells

- (f) Virus cultivation in vitro is facilitated by treatment with proteolytic enzymes, which enhance infectivity by cleavage of an outer capsid polypeptide.
- (g) The viruses exhibit a unique morphogenic pathway - virus particles are formed by budding into the endoplasmic reticulum (ER), and enveloped particles are evident transiently at this stage of morphogenesis. Mature particles are non-enveloped and these virions are liberated from infected cells by cell lysis.

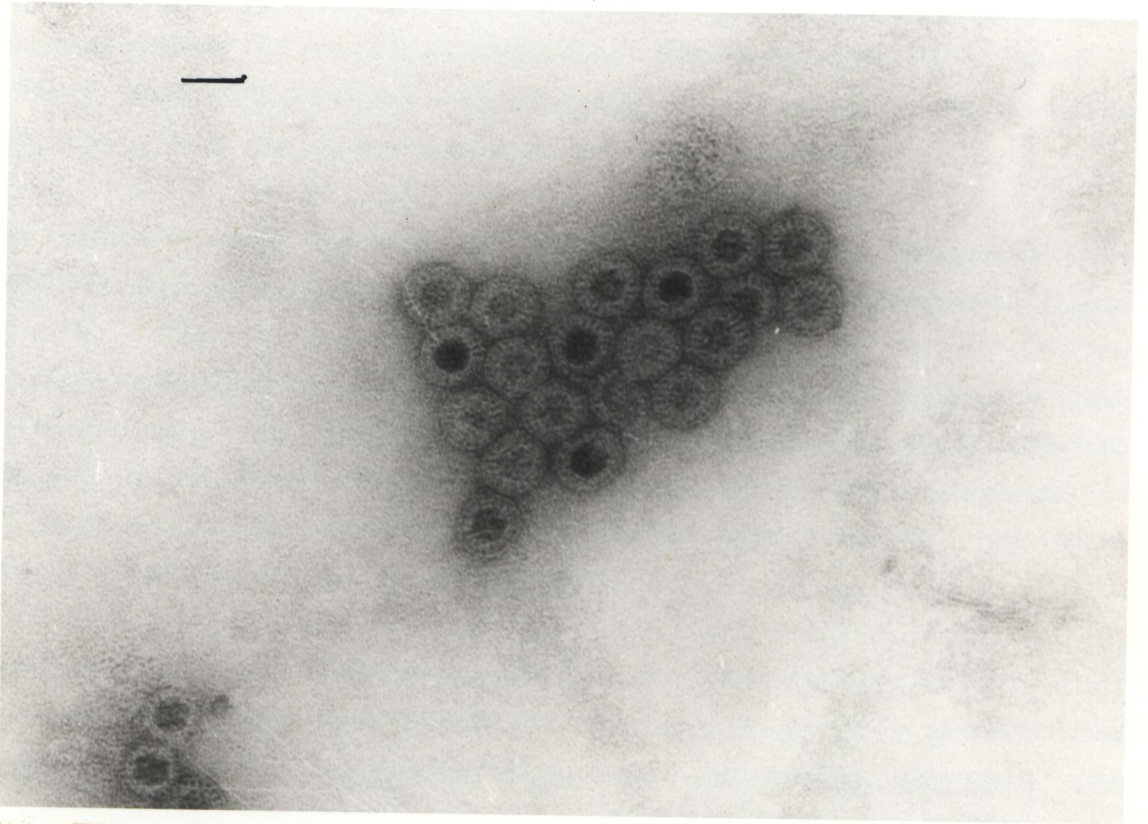
Rotaviruses are classified serologically by a scheme that allows for the presence of multiple serotypes within each group. A rotavirus group (or serogroup) includes viruses that share cross-reacting antigens detectable by a number of serological tests.

Rotaviruses comprise six distinct groups (A to F). Groups A, B, and C are those currently found in both humans and animals to date (29,35,63).

STRUCTURE

The morphologic appearance of rotavirus particles is distinctive, and four types of particles (double shelled, single shelled, filamentous, and core) are often observed by electron microscopy (EM). Mature particles are approximately 70 nm in diameter and they resemble a wheel, and possess a double layered icosahedral, protein capsid (Figure 1). The name rotavirus (from the Latin *rota*, meaning wheel) was suggested on the basis of this morphology (29).

Double shelled particles resemble a wheel with short spokes



Studies of nonprotease-treated virus particles had also revealed structural features of sixty spikes 4.5 - 6.0 nm in length with a knob at the distal end which extend from the smooth surface of the outer shell. The proteins in these have not yet been identified, but they may be composed of the haemagglutinin (VP4) known to be present on the outer capsid of particles (29).

CHEMISTRY

Infectivity depends on the presence of the outer capsid and treatment with calcium-chelating agents (eg. EDTA and EGTA) remove the outer capsid and result in a loss of infectivity.

Core particles can be produced by disruption of single-

Figure 1: Electron micrograph of single shelled Rotavirus thiocyanate Particles (X40000) (taken by the author; JEM 100-SX).

Double shelled particles resemble a wheel with short spokes and a well-defined smooth outer rim. Single shelled particles are often described as rough particles because their periphery show projecting trimeric subunits of the inner capsid. Cores are seen less frequently; they usually lack genomic RNA and are aggregated. The filamentous type is seen rarely.

Recently, it has been shown by freeze-drying techniques that rotavirus has 132 capsomeres arranged in a skew symmetry. They have also showed that the outer layer contain small holes that correspond one - to - one with holes in the inner capsid. Another distinctive feature of the virus structure is the presence of 132 large channels spanning both shells and linking the outer surface with the inner core (29,63).

The function of these channels is not yet known, but it is possible that they are involved in importing the metabolites required for RNA transcription and exporting nascent RNA transcripts for subsequent viral replication process (29).

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CHEMISTRY

Infectivity depends on the presence of the outer capsid and treatment with calcium-chelating agents (eg.EDTA and EGTA) remove the outer capsid and result in a loss of infectivity.

Core particles can be produced by disruption of single-shelled particles with chaotropic agents such as thiocyanate or high concentration of calcium chloride (29).

Double- and single-shelled particles can be separated by centrifugation in a gradient of caesium chloride or sucrose, where the particles possess distinct densities and sedimentation values (29).

Rotavirus infectivity and particle integrity are resistant to fluorocarbon extraction and exposure to ether, chloroform or deoxycholate reflecting the absence of an envelope on mature particles. Chloroform treatment reduces infectivity slightly and destroys haemagglutination activity. Sodium dodecyl sulphate (0.1 %) inactivates infectivity but exposure to nonionic detergents can enhance infectivity, presumably by disrupting aggregates (29).

Rotavirus infectivity is relatively stable to inactivation. Infectivity is stable within pH range 3-9 for at least 15 min, but liability to acid (pH < 3.0) has been suggested to affect vaccine takes. Bovine and human samples have retained infectivity for months at 4⁰C or even 20⁰ C when stabilised by 1.5 ml calcium chloride. Infectivity is relatively stable even at 45 - 50⁰ C. Infectivity and haemagglutination activity are destroyed by repeated freezing and thawing (29).

Viruses can be inactivated by disinfectants such as phenols, formalin, chlorine, and beta-propiolactone. 95 % ethanol is perhaps the most effective disinfectant as it exerts its effect by removing the outer capsid (13,29).

GENOME STRUCTURE

The viral genome of 11 segments of ds RNA is contained within the virus core capsid. Deproteinised rotavirus dsRNAs are not infectious, reflecting the fact that virus particles contain their own RNA-dependent RNA polymerase to transcribe the individual RNA segments into active mRNAs. Isolated rotavirus RNA segments in solution indicate that packaging of these RNA

segments into the rotavirus capsid requires intimate protein-RNA interactions, and these proteins are largely unknown. The structural proteins present in core particles (VPI, VP2, and VP3) are obvious candidates, but nonstructural protein may also play a role (29).

The nucleotide sequence of 10 of the 11 RNA is now known. The general features of rotavirus gene structure are as follows (Figure 2);

- i) Genes lack a polyadenylation signal at 3' end.
- ii) They are A & T rich (58 - 67 %)
- iii) They contain conserved consensus sequences of their 5' and 3' ends which ends with guanidine and cytidine respectively - they are noncoding sequences.
- iv) An open-reading frame which codes for protein product and ends with the stop codon is found between these noncoding terminals.
- v) The length of the 3' and 5' noncoding sequences vary for different genes.

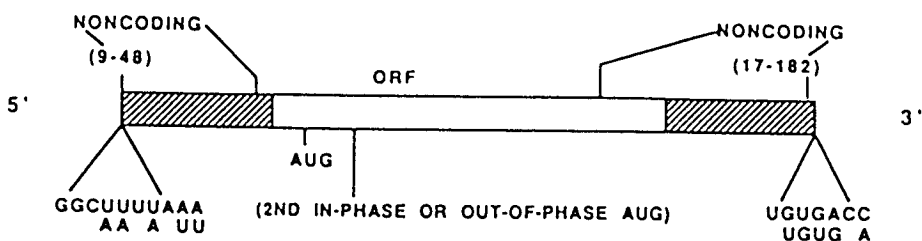


Figure 2: General features of rotavirus gene structure (29).

The strong conservation of terminal sequences in genome segments suggests that they contain signals important for transcription, replication, or assembly of the viral genome segments.

Each RNA segment appears to code for a single viral protein and the molecular weights of these proteins range from 20,000 to 125,000 daltons. The RNA segments are between 0.2×10^6 to

2.2×10^6 daltons in molecular weight. The largest of the RNA segments are numbered in order of decreasing molecular weight.

These segments are readily separated from one another by means of polyacrylamide gel electrophoresis (PAGE). Although different virus strains can sometimes exhibit similar electrophoretotypes, PAGE is often useful in distinguishing individual strains of rotaviruses (29,63).

In most cases the electrophoretic pattern of the genome of group A viruses is comprised of four high-molecular weight dsRNA segments (segments 1-4), five middle sized segments (segments 7-9) and two smaller segments (segment 10-11), (Figure 3).

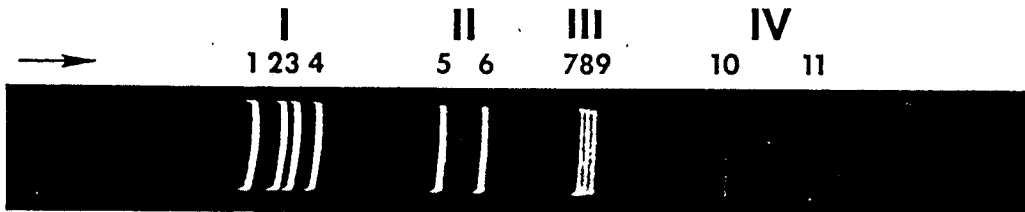


Figure 3: Polyacrylamide gel electrophoresis pattern of RNA from human rotavirus strain D (serotype 1, subgroup II). The 11 segments of double stranded RNA comprising the rotavirus genome (35,63).

When this basic pattern is not seen, the rotavirus being analysed may be (1,13,63);

- a. a non-group A virus
- b. a group A virus that contains rearrangements within individual genome segments.
- c. a new, unique group A virus.

Analysis of genomic electropherotypes is a relatively easy rapid and popular technique for virus detection and for molecular epidemiology studies to monitor virus outbreaks and transmission.

Some rotaviruses do not display the characteristic RNA migration pattern described above. In some human rotavirus strains, the 10th and 11th RNA segments migrate more slowly than usual, yielding a "short" pattern characteristic of almost all subgroup I human rotaviruses (which are, in almost every instance, serotype 2). A human strain with a "long" RNA pattern was recently described which exhibits the unusual combination of subgroup I and serotypes 3 antigenic specificities. Moreover, several "super short" (ultra short) patterns of RNA migration in which the 10th segment migrated even more slowly than the "short" pattern have been observed (29).

The nomenclature of the viral proteins (as originally proposed for SAII proteins) designated structural proteins as viral proteins (VP) followed by a number, with VPI being the highest molecular-weight protein; proteins generated by cleavage of larger precursor are indicated by an asterisk (VP4 is cleaved to produce VP5* and VP8*) (29).

The major structural component of virus particles is VP6, which is located on the surface of single-shelled particles. Removal of VP6 from single-shelled particles results in a loss of polymerase activity. It is highly antigenic and immunogenic, but it remains unclear whether VP6 plays a role in inducing protective immunity (29).

VP4 is the haemagglutinin in many virus strains and proteolytic cleavage into VP5* and VP8* results in enhancement of viral infectivity. Cleavage of VP4 enhances penetration (but not binding) of virus into cells. VP4 is also associated with restriction of growth of certain rotavirus strains in tissue culture cells and with protease-enhanced plaque formation. It

also induces neutralising Ab, and Ab directed to VP4 neutralise the virus in vitro. VP4 has also been shown that it effectively induces protective immunity in animals and immunogenic in children and animals (29).

VP7 is an outer capsid glycoprotein that is the second most abundant (30 %) protein species on the virion, and its normal site of synthesis and processing is exclusively in the endoplasmic reticulum (ER). It is the major neutralization antigen of rotaviruses detected by hyper immune antiserum and serves as the basis of determination of serotypes. Four serotypes of human rotaviruses that can be differentiated by neutralization tests have been described and are designated as serotype 1-4 (35,52).

REPLICATION CYCLE

Rotavirus replication has been studied primarily in continuous cell cultures derived from monkey kidneys and not from their natural host cells, the enterocytes of the small intestine (29,35,63).

Adsorption - The initial stages of replication is attachment of the double shelled virus particle and this occurs via VP7. The identity of the cellular receptor for rotaviruses is not known and this adsorption is sodium dependent, pH insensitive between pH 5.5 and 8, and is dependent on sialic acid residues in the host cell membrane; at 4⁰ C, virus binds but is not internalized (29,35,63).

Penetration - Following adsorption, the virus is internalized. The enhancement of the rotavirus infectivity by proteolysis is reportedly not due to increased efficiency of virus attachment to host cells but is due, instead to a facilitation of the uncoating step. Penetration of the virus is an active step and so will not take place at 0 - 4⁰ C (29,35,63).

Uncoating - Upon penetration, the virus in the cytoplasm enter the lysosomes by a receptor mediated endocytosis. Uncoating might occur with the lysosomal enzymes (29,35,63).

Transcription - The synthesis of viral transcripts is mediated by viral RNA-dependent polymerase (transcriptase) that has a number of enzymatic activities (enzyme complex). The intracellular site of transcription is unknown but is thought to be in the cytoplasm. Rotavirus transcription requires a hydrolyzable form of ATP. The synthesis of positive and negative strand RNA is detectable initially at 3 hours post infection, and reaches maximum after 9-12 hours (29,35,63).

Translation - The synthesized mRNAs elaborate viral proteins and the RNA templates are converted to dsRNA. This conversion to dsRNAs suggest specificity of viral proteins in recognition and replication of rotaviruses mRNA (29,35,63).

Assembly - The distinctive features of rotavirus morphogenesis is that subviral particles, which assemble in cytoplasmic viroplasms, bud through the membrane of the rough endoplasmic reticulum, with maturing particles being transiently enveloped. The envelope acquired in this process appears to be lost as particles move towards the interior of the ER, and is replaced by a thin layer of protein that ultimately comprises the outer capsid of mature virions (29,35,63).

Most of the rotavirus structural proteins and all of the nonstructural proteins are synthesized on the free ribosomes. In contrast, the glycoprotein VP7 and NS28 are synthesized on ribosomes associated with the membrane of the ER (29,35,63).

Rotavirus maturation reportedly is a calcium dependent process, and that virus yields are decreased when produced in calcium-depleted medium. Viruses produced were found to be

exclusively single shelled, and budding of virus particles into the ER was not observed (29,35,63).

Virus Release - EM studies have shown that the infectious cycle ends when progeny virus is released by host-cell lysis (29,35,63).

EPIDEMIOLOGY

Rotaviruses were first discovered in humans about 20 years ago by Bishop et al (3,4) by the examination of duodenal biopsy of a group of Australian children hospitalized with non-bacterial gastro-enteritis. Infection occurs worldwide in both sporadic and epidemic forms (4,7).

Rotaviruses are responsible for approximately 50 % or more of the gastroenteritis in hospitalized paediatric patients during the cooler months of the year in parts of the world that have temperate climate (5,9,14,20,21,24,30,39,43). Cases occur year round in tropical areas (52,53). However no distinct seasonal variation occurred in reports from tropical countries including Ecuador (13,53), Venezuela (15) and South Africa (47), whilst rotavirus infection tended to peak during the dry season in Nigeria (43), Southern India (36), Bangladesh (6), Indonesia (50) and Costa Rica (48).

Dryness may have some effect on the spread of the virus, although in some tropical countries any variation in temperature and humidity may be too slight to be of any significance (13).

Transmission of rotavirus is from person to person by the faecal-oral route with an incubation of one to three days (14,24,50). The virus is detectable in faeces during acute illness and is commonly shed for up to eight days after the onset of the disease and occasionally longer (34).

Infection within families is not uncommon, with both adults and siblings becoming infected (22,58). Nosocomial infection has also been described (18,45). Asymptomatic rotavirus infection and viral carriage occur frequently and have been studied by several investigators (11,12,61). Rotavirus is also prevalent in day-care centres and can be spread to family contacts, thus propagating the infection in the community (31). Many day-care children are asymptomatic, indicating large reservoir of infection (13).

Infection in Neonates

It is likely that human rotavirus (HRV) is introduced into a nursery by staff or new patients during the annual winter outbreak among young children. The factors that allow the endemic establishment of the virus are not known, although the fact that not all nurseries are affected allows speculation that admission policies and nursing practices may be involved. The neonates, though susceptible to infection, are not symptomatic as often as older children (31,41,52,62).

Infection in Adults

It appears that adult HRV disease is often mild, perhaps because these episodes are re-infections. Several studies have shown seroconversion with few or no symptoms in adults, raising the possibility of asymptomatic carriage and transmission of the virus by the adults (52).

Infection in the elderly

Rotavirus gastro-enteritis has been more severe in the elderly, according to reports from nursing homes and other institutions for the elderly (13).

Re-infection

In an analysis of children who were re-infected, sequential

infections usually involved different serotypes, and illness caused by one serotype did not provide resistance to illness caused by other serotypes (13).

PATHOGENESIS

Rotaviruses infect the small intestine and they have tropism for mature enterocytes at the tips of the villi (13). Histological studies have shown that there is blunting of the small intestinal villi, with virus particles in the villous cytoplasm during the acute phase of the illness. Histochemical examination shows that the activities of the disaccharidases enzymes are depressed, and in vitro the response to sodium/potassium pump to glucose is reduced. The net result is transduction of fluids into the intestinal lumen with consequent dehydration and loss of electrolytes (29).

Most, but not all children, with acute rotavirus gastroenteritis have lactose malabsorption and intolerance (25). Loss of fluids and electrolytes in rotavirus gastroenteritis can lead to severe dehydration and even death; it requires fluid and electrolyte replacement therapy. In developing countries, recurrent bouts of gastroenteritis can lead to a vicious cycle of protracted diarrhoea, food intolerance and malnutrition (56).

IMMUNOLOGY

The mechanisms responsible for immunity to human rotavirus infections and illness are poorly understood (29). This area of study is of interest, since rotavirus infections appear to be repetitive. What role various immunoglobulins and other factors play in the protection against rotavirus infection is important to know, since oral vaccines are being developed that may confer immunity to rotavirus infection (10,13).

TABLE 3: SERUM ROTAVIRUS IMMUNOGLOBULIN RESPONSE TO INFECTIONS (13).

TYPE OF ANTIBODY	TIME ANTIBODY Ist DETECTED	LENGTH OF TIME ANTI- -BODY PERSISTED
Neutralizing antibody	During convale- -scence	Protection for about one year
Neutralizing antibody	During convale- -scence	Not given
IgM	In acute phase, elevated	Decreased in conva- -lescence
IgG	In convalescence, elevated	Detectable at least 6 to 12 months
IgA	Not given	About 12 months
IgA	Within 1st two weeks	About 6 months
ScIg	Within 1 to 2 wk	About 4 months
sIgA	4 to 10 days	4 to 10 days

TABLE 4: INTESTINAL ROTAVIRUS IMMUNOGLOBULIN RESPONSE TO INFECTION (13)

LOCATION OF ANTIBODY	TYPE OF ANTIBODY	TIME ANTIBODY 1st DETECTED	LENGTH OF TIME Ab PERSIST
Duodenal fluid	ScIg	Within 1 wk level similar to that in serum	Not given
Faeces	IgA	Not given	About 6 months
Faeces	ScIg	Not given	About 6 months
Faeces	IgA	Acute phase	Throughout convalescence
Duodenal secretions	IgA	Acute phase, low	Throughout convalescence, higher than in acute phase
Duodenal secretions	IgM	Acute Phase, high	Throughout convalescence, lower than in acute phase
Faeces	IgA, IgM, IgG	Low levels at onset; increased by 1 to 2 wks	Peaked at 3 to 5 wks; lasted about 2 mo.
Faeces	IgA	Maximum level by 7 days	Not given
Faeces	IgA	Detected by 9 days	Peaked at 2 to 6 wk; then declined
Faeces	Ig A	Primary response: detected by 7 days Anamnestic response detected sooner	Peaked at 3 to 5 wk; then gradual decline Peak levels lasted longer

Several studies measured the levels of rotaviral immunoglobulins in the colostrum and milk of normal mothers, and in the stool of their newborn infants (Table 4).

**TABLE 5: ROTAVIRUS IMMUNOGLOBULIN IN NORMAL MOTHERS
AND NEWBORNS (13).**

Location of antibody	Type of antibody	Time Ab 1st detected	Length of time Ab persisted
Colostrum/ milk	IgG, IgM	Not given	Dropped off by 3 to 5 days postpartum
Colostrum/ milk	IgA, ScIg	Not given	Dropped off by 3 to 4 days postpartum to steady low level
Faeces of breast-fed infants	IgA	At 2 days	Not given
Colostrum/ milk	IgA, ScIg	Not given	Declined from 3 to days postpartum to 2 wk later; then remained unchanged
Infant duodenal fluid	IgA, ScIg	At 3 to 4 days	At least 2 wk
Colostrum/ milk	ScIg	Not given	Fell to low but detectable levels 1 to 2 wk postpartum and remained unchanged for 2 wk
Colostrum/ milk	IgA	Not given	Continued dropping off for 7 to 30 days

While the above tables show the levels of various rotavirus-specific immunoglobulins in the body fluids, these levels were not correlated with their possible role in the protection against, or modification of, rotavirus infection and disease (13).

In neonates and infants, two routes of passively transferred rotavirus antibody have been identified, serum and colostrum-milk. However, serum antibodies have doubtful protective capacity (59). Rotavirus specific IgA and ScIg can survive proteolysis in the gut. Thus, frequent breast feeds have a possible protective effect (13).

A number of potential rotavirus vaccines are currently undergoing efficacy trials. These vaccines include the following; bovine rotavirus vaccine (RIT4237 strain), bovine human reassortant rotavirus vaccine, rhesus-human reassortant vaccine, and nursery strain vaccine (M37). The major aim of all these vaccines is to modify the course of HRV infection by making diarrhoea less severe (10,59), than to prevent diarrhoea (16,49).

CLINICAL FEATURES

The incubation period of rotavirus infection usually extends from 24 to 72 hours (13,14,48). The two most prominent features are vomiting and diarrhoea, usually of sudden onset (13). The vomiting may precede the diarrhoea in many cases (13,52,56). The diarrhoea is watery; mucous is found in the stool in up to 25 % of cases, but blood is rare (35). Fever occurred in about 30-50 % of cases in some studies and in 60-100 % in other studies (13,52).

There have been conflicting reports on the role of rotavirus in causing respiratory infection and symptoms (13). Some studies suggest that rotaviruses may sometimes cause respiratory symptoms (13,46), but several groups have failed to show any significant role of rotavirus in respiratory infection (7,52). The fever and

vomiting resolve in the first day or two, the diarrhoea may last up to eight days (7,52).

Mild to moderate dehydration is frequently seen in HRV patients (52). The dehydration is of isotonic type (54). In the more severe cases seen at treatment centres, severe dehydration and electrolyte imbalance have been observed (62). Most hospitalized children recover within a week of admission (18,48, 54). Death due to rotavirus, albeit rare, has occurred in infants and young children, and is usually due to dehydration and electrolyte imbalance (13).

LABORATORY DIAGNOSIS

1. ANTIGEN DETECTION METHOD

Numerous methods are available for detection of rotavirus in the stool specimens.

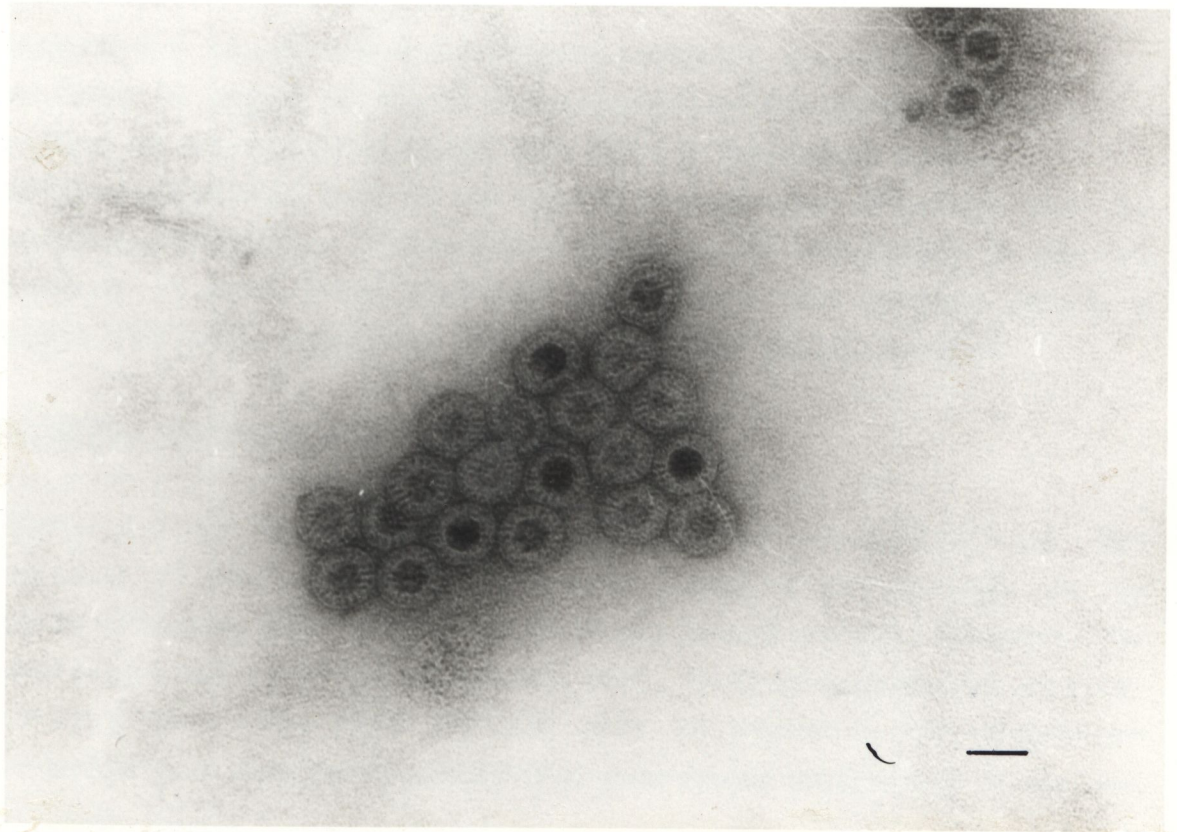
The commonest method used is the Enzyme Linked Immunosorbent Assay (ELISA), since it is highly sensitive, easy to perform and does not require specialised equipment (54).

Most of the ELISAs utilize a three-layer double-antibody sandwich technique (13,54). This method is ideal when large samples are being examined for rotavirus. A microplate is employed and the reaction can be read by a spectrophotometre. However, current kits identify group A rotavirus only. Therefore, the negative specimens can be subjected to Electron Microscopy technique. Other ELISA kits are not commercially available.

Latex agglutination tests (LAT) are also available. In this technique, latex particles coated with specific antibody react with rotavirus antigen present in a stool specimen to give agglutination.

2. ELECTRON MICROSCOPY (E.M.)

Initially, direct visualization of stool material by E.M was employed for rotavirus detection (23). It has the advantage of high specificity because rotavirus have a distinctive morphologic appearance. because faecal specimens can be negatively stained with either Phosphotungstic acid or Uranyl acetate and examined directly within a few minutes of collection (13, 27, 27).



Intravenous therapy is needed for severely sick children, who are:

- (i) in shock and unable to drink fluids
- (ii) persistently vomiting
- (iii) having stool losses of more than 100ml/kg/hour

Figure 4: Electron Micrograph of rotavirus particles (X40000)
(taken by the author; JEM 100-SX)

E.M continues to be the mainstay in the diagnosis of rotaviral diseases and is frequently used as the final arbiter when discrepancies occur with other methods. When only a few specimens are to be examined for rotavirus, E.M is the most rapid diagnostic method because faecal specimens can be negatively stained with either Phosphotungstic acid or Uranyl acetate and examined directly within a few minutes of collection (13,23,27).

Direct E.M examination of stools permits detection of rotavirus in about 90 % of virus-positive specimens. If the specimen is ultracentrifuged and pellet examined after negative staining, E.M is as sensitive as any other method for rotavirus detection. It has the advantage of being able to detect non-group A rotavirus that do not share the common group A antigen (13,23), which is detected by the ELISA method.

TREATMENT, CONTROL AND PREVENTION

There is no specific antiviral therapy available for HRV infection (7,52). Thus the primary purpose of therapy is to provide adequate hydration, to maintain blood volume, electrolyte homeostasis and acid-base balance (25). Oral glucose electrolyte solutions have been used for the past 25 years in the treatment of dehydration due acute childhood diarrhoea including rotavirus diarrhoea (13).

Intravenous therapy is needed for severely sick children, who are:

- (i) in shock and unable to drink fluids
- (ii) persistently vomiting
- (iii) having stool losses of more than 100ml/kg/hour
- (iv) unable to tolerate oral fluids

In underdeveloped or developing countries where there are limited medical resources and where malnutrition is common, oral rehydration therapy and feeding are advocated for (25).

Control of rotavirus diarrhoea is at the moment very difficult. Rotavirus vaccine has potential to control this most important pathogen in childhood diarrhoea. The main role of the vaccine will be to modify the severity of diarrhoea rather than to prevent diarrhoea (16,27).

PART 2: ENTERIC ADENOVIRUSES

CLASSIFICATION AND MOLECULAR BIOLOGY

A variety of classification systems have been proposed for human adenovirus (Table 1). Currently, 41 human adenoviruses are divided into six subgenera; A,B,C,D,E,F. Subgenus F was created to accommodate the newly characterised enteric adenoviruses types 40, 41 and possibly 42, recently recovered from the faeces of a 14 month old boy with bowel atresia (11).

TABLE 6: PROPERTIES OF HUMAN ADENOVIRUS SEROTYPES OF SUBGENERA A-F (2).

Subgenus	Serotype	Tropism/Symptoms
A	12,18,31	Cryptic enteric infection
B:1	3,7,16,21	Respiratory disease
B:2	14,11,34,35	Persistent infections of the kidney
C	1,2,5,6	Respiratory disease persists in lymphoid tissue
D	8-10,13,15,17, 19,20,22-30, 32,33,36,37, 38,39,42-47	Keratoconjunctivitis
E	4	Conjunctivitis,Respiratory disease
F	40,41,(42)	Infantile diarrhoea

Conventional serotypes, particularly 1-7, may be isolated readily in cell culture from faeces and appear to be associated with diarrhoea only occasionally. After electron microscopists began examining faecal extracts for rota viruses, they found a considerable number of adenoviruses which did not grow routinely in cell culture whose existence had not been suspected before. These fastidious strains belong to at least two new serotypes 40 and 41, and appear to be linked much more strongly to episodes of diarrhoea (65).

In contrast to conventional adenoviral serotypes, enteric adenoviruses do not produce nasopharyngitis and keratopharyngitis as primary symptoms; conversely conventional adenoviruses do not frequently cause gastroenteritis as a primary isolated clinical feature. It has been difficult to prove that adenoviruses produce gastroenteritis because of the prolonged period of asymptomatic faecal shedding after respiratory tract infection with traditional adenoviruses.

However, laboratory techniques can now distinguish types 40 and 41 from other adenoviral serotypes in faecal samples, and they have thus been associated with gastroenteritis (2).

Apart from failure to propagate in cell cultures which are normally permissive for adenoviruses, these fastidious strains show no obvious differences from those isolated in cell culture from both the respiratory tract and the gut, being identical in appearance to the classic strains (65).

CHEMISTRY

Adenoviruses retain infectivity for several weeks at 4°C and for months at 25°C. The optimum pH for infectivity occurs at 5.6 after 2.5 to 5 minutes of adsorption. Adenoviruses are resistant to lipid solvents because of the absence of lipids within their structure. The capsid is sensitive to rapid disruption by 0.25 % dodecyl sulphate.

Unexpectedly, a single band has been found close to the origin in PAGE following electrophoresis of stool extracts containing adenoviruses detectable by electron microscopy. This is probably intact DNA although it is surprising that DNA of this size would migrate into the gel (65).

This single band is neither unequivocal evidence of adenovirus nor, does it provide further information about individual strains. Digestion of viral DNA present in the faeces by restriction endonucleases which act on particular nucleotide sequences produce fragments whose sizes are characteristic for that strain. After separation by electrophoresis on agarose they give reproducible and recognisable patterns (65).

STRUCTURE

Adenoviruses are DNA viruses and are nonenveloped, regular icosahedron that are 65-80 nm in diameter. A structure called fibre projects from each of the vertices. The length of the fibre varies with the adenovirus serotype. The capsid is composed of 252 capsomeres (17).

Adenoviruses contains 13 % DNA and 87 % protein, have no membranes or lipids, and are therefore stable in solvents like ether and ethanol (17).

The viral dsDNA is 23 x 10 daltons for Ad 2 and varies slightly in size depending on the serotype. The DNA is associated with a number of internal proteins which assist in maintaining genome integrity (2,17).

Attachment, Penetration, and Uncoating

Because most of the particles of the input virus are not infectious, studies of the early events of adenovirus - cell interaction are as problematic as they are for all other virus eukaryotic - cell infections. A number of interesting studies

have been done in adenovirus - infected cells to suggest that the fibre of the virus attaches to specific receptor on the cell membrane (2,17,65).

The virus bound to its receptor migrates within the plasma membrane to clathrin - coated pits that form endocytic vesicles or receptosomes. There are approximately 10^4 virion receptors and 10^5 fibre receptors per cell, suggesting that the virus undergoes multivalent binding to the cell membrane. Some (but not all) antibodies to fibre neutralise viral infection (17,65).

When the virus reaches the nuclear pores, the DNA enters the nucleus and leaves many of the virion proteins in the cytoplasm. This entire process, which is completed in 2 hours at 37^0 C requires energy in the form of ATP and will not occur at 0^0 C (17,65).

Transcription

Replication of DNA, transcription and maturation of Adenovirus take place in the cell nucleus (17,65).

Early Adenovirus transcription is a complicated series of interrelated biochemical events, but it can be simply defined as the synthesis of viral RNAs from input adenovirus templates before the onset of viral DNA replication (17,65).

As the onset of viral DNA synthesis heralds the beginning of the late phase, the early and intermediate transcription products begin to change dramatically. Newly formed virus structural components are transported into the nucleus, where the assembly of virus particles starts 7 hours after the initiation of DNA synthesis. Up to 100 000 virus particles are assembled in each cell within 30 hours after infection with the most rapidly growing types of human adenoviruses (17,65).

No virus-specific function has been shown to be responsible for the release of infectious virus particles from the cell nucleus. Adenoviruses are therefore considered to be released in connection with the disruption of the cells (17,69). Adenoviruses are therefore cytocidal.

EPIDEMIOLOGY

World wide, it has been documented that adenovirus are the second most common causes of viral gastroenteritis after rotaviruses (8), contributing to 5-20 % of hospitalization for childhood diarrhoea in developed countries (2,38), though most reports indicate 4-10 % (2). Up to 2 % of controls evaluated in some studies are asymptotically affected (2).

In Malawi, adenoviruses diarrhoea accounted for 5 % (43) and 13 % in South Africa (55). On the Zambian Medical scenario, adenoviruses have not been demonstrated yet as aetiological agents of childhood diarrhoea.

Adenovirus diarrhoea does not show any seasonal variations in the temperate climate as rotavirus diarrhoea. Infection occurs throughout the year with no peaks both in the tropics and the temperate regions (17,38).

Peak incidence is among children less than 2 years of age, but older children may be infected, with or without symptoms (60).

Nosocomial outbreaks occur, and in some institutions its a significant problem (2). Infection is transmitted from person to person, with no evidence of other mechanisms of spread (2,38).

PATHOGENESIS

As is common with other viral enteropathogens, adenovirus serotypes 40 and 41 also have tropism for the small intestine.

Adenoviruses infect the epithelial cells on the villi of the small intestine. During the lytic cycle, synthesis of host macromolecules is severely inhibited and this is incompatible with cell survival. The infected cell degenerates in specific ways that help the pathologist diagnose adenovirus infection on biopsy or autopsy tissue (40).

One of the adenovirus structural proteins, the penton, has been shown to be directly toxic to cells other than those intrinsically infected with replicating adenoviruses (40).

The net result is decreased absorption surface with subsequent diarrhoea.

IMMUNOLOGY

Little is known about immunity to fastidious adenoviruses in man, and no experiments have been conducted on resistance to infection. However, a survey for antibody to types 40 and 41 on sera collected from seven countries has shown that antibody to these viruses is widespread in these communities and is acquired early in childhood (38,65). Since there is considerable antigenic overlap between these two serotypes it is difficult to differentiate responses to these two types (43).

CLINICAL PRESENTATION

The incubation period is 3 to 10 days, with illness lasting for more than one week, longer than for other enteric viral pathogens.

Diarrhoea is more prominent than vomiting or fever, and respiratory symptoms are often present (38). Severe dehydration is less common than rotavirus diarrhoea (2).

Person to person transmission is presumably the principal mechanism for the spread of infection. Asymptomatic shedding

has been documented, but generally infectivity parallels symptomatic disease. Food and water have not been reported as vehicles of transmission (38).

LABORATORY DIAGNOSIS

1. ELISA test
2. Electron Microscopy
3. Dot hybridization

TREATMENT, CONTROL AND PREVENTION

The main stay of treatment is the replacement of lost fluids and electrolytes as with rotavirus. The control and prevention of enteric adenovirus diarrhoea is again related to that of rotavirus diarrhoea.

PART 3: SMALL ROUND VIRUSES

Unlike rotaviruses and enteric adenoviruses, the remaining gastroenteritis viruses are small and round. Because they are morphologically similar to one another and refractory to in vitro cultivation and purification, some confusion has existed about their classification. In recent years, however, they have been characterised more fully in detailed morphologic studies. As a result, these small, round viruses can be grouped provisionally into four categories. Norwalk and Norwalk-like viruses, Astroviruses, Caliciviruses, and small, featureless viruses. These groups were initially defined by comparative electron-microscopical studies (13,28).

A) NORWALK VIRUS

The Norwalk virus is the representative agent of a heterogenous group of viruses, also called small structured viruses (SRSV) or the Norwalk-like family of agents. The Norwalk virus is the prototype strain of a group of fastidious 23 to 34 nm nonenveloped viruses associated with outbreaks of gastroenteritis (28).

STRUCTURE

Norwalk viruses are 27 to 32 nm in diameter with a ragged surface outline. They have a single structural protein similar to Caliciviruses. They contain a single-stranded RNA (28).

EPIDEMIOLOGY

Many of the cases of the Norwalk virus gastroenteritis have been associated with groups of patients living in enclosed environments such as schools, recreational camps, or cruise ships (2). Other outbreaks have occurred in individuals ingesting contaminated shellfish or water.

Seroepidemiological studies have been performed in an attempt to determine the age-associated acquisition of Norwalk antibody. Only approximately 20 % living in developed countries such as the United States demonstrate antibody to Norwalk virus by the time they are 5 years old. It is thus unlikely that Norwalk virus is a common cause of infantile gastroenteritis in these geographical populations. However, children living in developing countries such as Bangladesh and Ecuador have a much higher rate of acquisition of Norwalk antibody. A majority of children living in those countries have serological evidence of infection with Norwalk virus by 5 years of age (7).

In addition, a number of children with acute gastroenteritis living in developing countries are documented to exhibit a serological response to Norwalk virus following episodes of acute gastroenteritis (7).

While this agent does not appear to be common among infants hospitalized with severe dehydration, these data indicate that Norwalk virus may be an important cause of mild, infantile gastroenteritis in developing countries.

PATHOGENESIS

The site of infection is the small intestine. There is broadening and blunting of the villi of the proximal intestine, although the mucosa itself is histologically intact. There is also shortening of the microvilli although the epithelial cells are intact.

Marked delay, in gastric emptying was observed in infected volunteers who became ill or who were asymptomatic but developed the typical jejunal lesion. It has been proposed that abnormal gastric motor function is responsible for the nausea and vomiting associated with these viral agents (13,28).

Routes of transmission that have been documented include water, food (particularly shellfish and salads), aerosol, fomites, and person to person contact. Infectivity can last for as long as two days after resolution of symptoms. Presymptomatic shedding has been suspected on epidemiologic grounds but not proven in volunteer studies (40).

IMMUNOLOGY

Immunology to Norwalk virus is poorly understood and does not resemble the pattern of most other viruses (13,28).

Studies of volunteers have documented the paradox that persons with the highest preexisting levels of Norwalk antibodies are at highest risk of developing infection. Most persons' antibody levels against Norwalk virus rise after infection; these titres normally peak by the third week and persist until approximately the sixth week, after which they decline. Although preexisting antibody levels correlate with risk of symptomatic illness upon exposure to the virus, acutely elevated antibody levels appear to correlate with resistance to reinfection. The nature of resistance and susceptibility to the Norwalk-like agents is poorly understood (40).

CLINICAL SYNDROME

The incubation period is 24-48 hours and the mean duration of illness is 16-60 hours. Nausea is prominent, with vomiting, no bloody diarrhoea, and abdominal cramps occurring in most cases. These symptoms are experienced by all age groups, but diarrhoea is relatively more prevalent among adults, where as a higher proportion of children experience vomiting.

About 25-50 % of affected persons also report headache, fever, chills and myalgias. Adults have died during illness caused by Norwalk viruses presumably due to electrolyte imbalance (40).

LABORATORY DIAGNOSIS

1) ELECTRON MICROSCOPY

Direct examination of stool material without concentration by ultracentrifugation is of limited value in screening these 27nm particles because they are present in low concentration.

2) IMMUNE ELECTRON MICROSCOPY (IEM)

IEM remains the mainstay for the detection and identification of 27nm gastroenteritis agents in stools. A stool suspension that fails to yield recognisable viral agent e.g. rotavirus or adenovirus by direct EM should be examined further by IEM.

The patient's stool suspension is incubated for 1 hour with his or her convalescent serum (1:5) or with immune human serum, Globulin (1:5) if convalescent serum is not available. The mixture is then ultracentrifuged at 40,000 rpm for 90 minutes, after which the pellet is resuspended and negatively stained with phosphotungstic acid (PTA) or uranyl acetate. Antibody directed against a particle in the stool can be visualised readily on the surface as a 'fuzzy' coating. In addition, under certain conditions, the antibody may cause aggregates, which further facilitates recognition (28).

3) RADIO IMMUNO ASSAY (RIA) AND ELISA.

RIA, which detects both particulate and soluble Norwalk antigens, is more efficient and sensitive than IEM for detection of Norwalk antigen. This technique is based on differential binding of Norwalk virus antigen (present in faeces) to microtitre wells coated with convalescent phase or preinfection serum. Iodine labelled Norwalk antibody is added and if the radiation count is more than a certain limit this is considered positive (28).

An ELISA which is based on the same principles as the RIA but does not employ radioactivity labelled reagents has recently been developed for detection of the Norwalk virus (28).

4) RIA BLOCKING TEST

The development of an RIA-Blocking test (RIA-BL), (and more recently, an ELISA-BL test) for Norwalk virus antibody has made it possible to study a considerable number of gastroenteritis out breaks. This method is based on ability of a test serum to block the binding of the 125 I labelled Ig G fraction of a Norwalk volunteers antigen which is attached to the precoat in the solid phase. These serologic assays detect more Norwalk virus infections than do methods that rely on identification of virus particles or antigen in stool. The RIA and ELISA are more practiced than IEM because they are less time consuming, require less antigen and antibody, and can be carried out routinely.

TREATMENT

Same principle as Rotavirus diarrhoea.

CONTROL AND PREVENTION

Specific methods are not available for the prevention or control of Norwalk virus infection or illness because this infection is highly infectious. Effective hand washing and disposal or disinfection of contaminated material may decrease transmission within a family or institution.

Special care must also be given to the hygienic processing of food in view of the frequency occurrence of food - borne outbreaks of Norwalk virus disease. Measures that increase the purity of drinking water or swimming pool water should also decrease the frequency of Norwalk virus outbreaks (28,40).

B) ASTROVIRUSES

Astroviruses were first isolated from infants with diarrhoea in Great Britain in 1975 by Madeley and Cosgrove, and named for their five- or six- pointed starlike appearance as seen by means of electron microscopy (7).

Astroviruses are spherical particles with a mean diameter of 28 nm. The virion has a single-stranded RNA of positive sense and a size of 7.8 kb, and thus similar to caliciviruses. They have three structural proteins (molecular weights 31 000 to 34000, 29 000 to 31 500 and 20 000 to 24 000). There are five human serotypes of astroviruses (2).

Astroviral disease is most frequent in children from infancy to seven years of age but adults can be infected and suffer a mild disease. Although similar to rotaviral illness, it appears to be less severe in a limited number of clinical studies reported (2). Studies of hospitalized children suggest that astroviruses may account for 3-5 % of admissions of diarrhoea (40). A study in Malawi showed that astroviruses accounted for 1.5 % to the aetiology of diarrhoea under 5 years (32) in outpatient department. In Japan and elsewhere, an association has been demonstrated between astrovirus and an outbreak of gastroenteritis in a kindergarten (13,32). Astroviruses are an important agent of epidemic acute nonbacterial gastroenteritis in school aged children and adults in Japan (32). Out breaks of astroviral disease occur in residential facilities for the elderly also (40).

PATHOGENICITY

Although the pathogenicity of astroviruses is not fully understood, several epidemiological studies have suggested an association between astroviruses and epidemics of mild gastroenteritis involving infants, children or adults. Studies of volunteers have also suggested that astrovirus is a

transmissible infection, even though of low pathogenicity for adults (7,32,40).

IMMUNOLOGY

The characteristics of immunity to astrovirus have not, as yet, been identified. Since reported outbreaks have involved only children and the elderly, young adults may have resistance to infection (40).

CLINICAL PRESENTATION

The incubation period is between 24 to 36 hours, with illness lasting 1-4 days. Gastrointestinal symptoms are nonspecific, consisting of vomiting, diarrhoea, fever, and abdominal pain (40).

DIAGNOSIS

Until recently, electron microscopy has been the only diagnostic tool as the viruses are only found in large numbers (10^{10} per gram of stool). An ELISA has recently been developed that uses group-reactive monoclonal antibodies that detect all astroviral serotypes in stool, which should facilitate epidemiologic studies.

TREATMENT

Same principle as rotavirus diarrhoea.

CONTROL AND PREVENTION

Same principle as with Norwalk viruses.

C) CALICIVIRUSES

Human caliciviruses are poorly understood agents that have not been administered to volunteers, produced disease in animals, or been serially propagated in cell culture (8). They were discovered in 1976 in human faeces, although strains infecting pigs, cats, sea-lions and fur-seals had been identified several years previously (65).

Caliciviruses are approximately 33 nm in diameter, and contain a single positive strand of RNA. They are round although the edge of the particle as seen under the electron microscope is indistinct, and this makes exact measurement difficult. Mature virus is cup-shaped with indentations on the surface, and have a single structural protein (8,65).

There are at least 3 serological distinct strains as determined by immuno-electron microscopy (8,65).

The most frequent form of caliciviral disease occurs in infants and young children and is characteristically indistinguishable from mild rotaviral illness (8). It has an incubation period of one to three days.

This disease pattern occurs in the general paediatric population, in schools and orphanages and among children hospitalized for diarrhoea. Vomiting and diarrhoea are common, with upper respiratory symptoms and fever occurring less frequently. Infections in the elderly have also been documented (8,65).

Seroprevalence studies from many areas indicate that antibody is acquired by most people during early childhood, in a pattern similar to that for group A rotavirus but quite different from that for Norwalk virus. Serum antibody may protect against illness, unlike antibody to Norwalk virus, according to a study of caliciviral infantile diarrhoea in Japan (8,65).

Detection of the virus in stool by electron microscopy has been the mainstay of diagnosis until the recent development of immunoassay techniques (8).

CHAPTER 3

OBJECTIVES OF THE STUDY

1. MAJOR OBJECTIVE

To establish the prevalence of viral gastroenteritis at the University Teaching Hospital, Lusaka.

2. SPECIFIC OBJECTIVES

- 2.1 To compare EM and ELISA based assays used in the detection of viral agents of gastroenteritis.
- 2.2 To determine the seasonal distribution of viral gastroenteritis at the University Teaching Hospital.
- 2.3 To evaluate clinical aspects like dehydration and nutritional status in childhood viral gastroenteritis.
- 2.4 To determine the outcome of viral gastroenteritis in relation to aetiology.
- 2.5 To come up with recommendations on treatment regimes and control of viral agents causing childhood diarrhoea.

CHAPTER 4
PATIENTS AND METHODS

STUDY SITE

The University Teaching Hospital (UTH) is a 2000 bed hospital that acts as a referral facility for the whole Zambia. However, most of the patients come from within Lusaka, which has a population of over a million people. The Department of Paediatrics of the hospital has 500 bed spaces. This study was carried out at the Diarrhoea Training Unit (DTU) of the Department of Paediatrics. All children with diarrhoeal diseases are admitted to this unit. In this unit, mothers participate in the rehydration of their children with oral rehydration salt (ORS) after receiving instructions from the nursing staff.

STUDY TYPE

Prospective study.

PATIENTS

Children under 5 years old with acute diarrhoea admitted to the DTU during the day from Monday to Friday were recruited to the study. Clinical information on the patients was collected by the nursing staff, and physical examination was done by a clinical officer. The information was entered in the questionnaire form and subsequently entered in the computer on EPI programme.

DIARRHOEA STUDY FORM

Study No _____ Hospital No _____ Date of Admission/./..

Name _____ Age ____ Y ____ M Sex M / F

Residential Address _____

Diarrhoea duration in days _____

Fever Y / N If yes duration in days _____

Vomiting Y / N If yes duration in days _____

Nutritional Status Normal nutrition ()
 Mild PEM ()
 Moderate PEM ()
 Severe PEM ()

Weight in Kg _____

Temperature on admission _____ C

Hydration Status No dehydration ()
 Some dehydration ()
 Severe dehydration ()

Feeding Exclusively breast fed ()
 Breast milk and other feeds ()
 Not breast fed ()

When did child stop breast feeding ____ Y ____ M

Other physical findings _____

Type of treatment in Hospital

ORS Y / N Intravenous Fluid Y / N
Antibiotics Y / N Antimalarial Y / N
Others Y / N

Stool Specimen: Date collected ____/____/____

 Appearance - Watery () - Mucoid ()
 - Bloody () - Others ()

Outcome Discharge: Date ____/____/____ Died: Date ____/____/____

Laboratory Diagnosis - Rotaclone () - Adenoclone ()
 E.M: - Rotavirus () - Adenovirus ()
 - Small Round Virus () Type _____

A maximum of ten children were enrolled per day. Acute diarrhoea was defined as an increase in frequency and or a change in consistency (loose or watery) of the stool of less than 14 days duration. Dehydration was graded as no, some, or severe.

CONTROLS

The control subjects were recruited from children admitted to the general paediatric wards with disease conditions other than diarrhoea by the investigator. In addition they had not had diarrhoeal disease or vomiting in the preceding one month.

The stool specimens were collected in sterile plastic containers by the nursing staff on the ward, and transported to the virology laboratory within 24 hours of collection and stored at -20° C until tested.

LABORATORY PROCEDURES

Stool samples stored at -20° C were prepared to 10 % suspension by the following procedure.

PREPARATION OF STOOL SUSPENSION

1. Approximately half a gram of stool was thawed and suspended in 5 mls of minus Phosphate Buffered Saline (-PBS) to make a 10 % stool suspension.
2. This was thoroughly mixed either by shaking or vortexed, on a vortex machine.
3. This was then centrifuged at 3 000 rpm (Kubota 5100, Bench centrifuge) for 10 minutes.
4. The stool suspension supernatant was now ready for ELISA assay and E.M. studies.
5. The remainder of the stool suspension was restored at -20° C.

1. ELISA-Rotaclone antigen detection kit.

Rotaclone [Cambridge Biosciences (USA)], is a rotavirus

antigen detection kit which is specific for group A rotavirus. The group specificity is located on the outer surface and antibody to it has formed the basis of a number of diagnostic tests. This is coded for by VP 6 (19). The kit uses a monoclonal antibody which is specific against VP6. All samples were be tested by this method.

The details of the procedure is indicated below;

ELISA ANTIGEN DETECTION KITS - ROTACLONE AND ADENOCLONE (according to the manufacturers manual, Cambridge Biosciences, Inc., USA)

1. 100 *uI* of 10 % stool suspension were dispensed in microwells previously coated with either rotavirus antigen or adenovirus antibody.
2. Positive control, code number 2 (rotavirus antigen or adenovirus antigen), two drops, was dispensed to one well. For the negative control, the sample diluent 100 *uI* was used.
4. Code number 3 (enzyme conjugate) was then added to all the wells.
5. This was then incubated at room temperature for one hour.
6. The microplate was then washed with distilled water two to three times.
7. Codes number 4, and 5 were then added to all the wells.
8. This was allowed to stand for 10 minutes, and then code number 6 (stop solution) was added to all the wells.
9. The Optical Density (OD) was read at 450 nm. Absorbances greater than 0.150 were taken as positive.

2. Electron Microscopy (E.M)

The method employed in Electron Microscopy (E.M.) [JOEL SX200] was negative staining of 10 % stool suspension.

Below are the details of negative staining.

NEGATIVE STAINING METHOD (46)

1. A drop of 10 % stool suspension was carefully delivered on

- to a carbon coated copper mesh with collodion membrane and ironically charged held on an E.M. forceps.
2. This was allowed to stand for two minutes.
 3. After two minutes, excess stool suspension was then absorbed on filter paper.
 4. The copper mesh was then washed with 2-3 drops of distilled water.
 5. A drop of uranyl acetate was applied on to the copper mesh by picking a drop of uranyl acetate previously dispensed on the parafilm with E.M. forceps.
 6. After 1-2 minutes excess uranyl acetate was removed by filter paper, and was allowed to air dry.
 7. The dried copper mesh was finally put in a grid box and was ready for E.M. studies.

Based on their morphology the three groups of viruses; Rotavirus, Enteric Adenovirus, and Small Round Viruses were identified by this technique. E.M. is more sensitive and specific than ELISA kits (29). At least two thirds of the samples were subjected to this method.

Stool samples negative for rota- and adeno- viruses by either ELISA or E.M. were further subjected to ultracentrifugation, and then examined by E.M. for demonstration of Small Round Viruses (SRV).

Details of the procedure of ultracentrifugation are indicated below.

ULTRACENTRIFUGATION (BECKMAN L65)

Stool samples which were negative on rotaclone and negative or rotavirus on E.M. were subjected to ultracentrifugation.

1. Stored stool suspension supernatant at -20°C was thawed and put in 5 mls centrifuge tubes.
2. The initial centrifugation was done at 10,000 rpm for 30

minutes.

3. The supernatant was transferred to another 5 mls centrifuge tube.
4. Ultracentrifugation was finally done at 40, 000 rpm for 3 hours at 10⁰C.
5. The supernatant was discarded and the residual was mixed with the minimum supernatant that remained.
6. This was then subjected to negative staining and E.M. studies as described above.

Grading of Rotavirus Density

Grading of rotavirus density was also done by E.M. with rotavirus particles less than 10 per field as grade 1, more than 10 but less than 100 as grade 2, and more than 100 as grade 3.

3. ELISA-Adenoclone antigen detection kit

Antigenic determinants that are important in the serologic classifications of adenoviruses are inherent to the hexon, penton, and the fibre. In this test it is the hexon structural protein that forms the basis of this test (17). Adenoclone is an ELISA based assay and due to limited resources and cost of this kit only 200 samples picked at random will be tested.

Details of the procedure is indicated above along with the ELISA-Rotaclone.

STATISTICAL METHODS

Data was analysed by EPI-INFO, a WHO software.

CHAPTER 5

RESULTS

1. ROTAVIRUS DIARRHOEA

Of 1064 children recruited for the study, 256 were positive for rotavirus antigen, representing 24.1 % of rotavirus positivity. Among those who were positive 26.2 % (155) were male and 21.2 % (100) were female, a ratio of 1.6:1.0 respectively, ($p=0.055$).

Most of the children who presented with acute gastro enteritis were less than two years (85.7 %, 880 / 1064). Rotavirus positive rate was higher in children less than one year (37.6 %, 173 / 460). It was 16.6 % (68 / 420) in children aged one year, and decreased to 6.5 % (3 / 46) in those aged 4 to 5 years, (Table 7 and Figure 4).

Of the children who were tested 43.2 % (460 / 1064) were below the age of one year. Rotavirus positive rate for those aged 0-2 months, 3-5 months, 6-8 months, 9-11 months was 36.4 %, 46.7 % and 39.3 %, and 30.0 % respectively, (Table 8).

Most of the children (85.1 %, 905 / 1064) who were admitted to the diarrhoea unit had diarrhoea for 2-7 days prior to admission and rotavirus positive rate ranged from 17.2 - 31.4 %. Most patients presented after 3 to 4 days of the onset of diarrhoea and the detection rate was higher in these children, (Table 9).

Rotavirus diarrhoea was seen throughout the year. The highest detection rate was noted in the month of May 36.6 % (15 / 41), and the lowest was in the months of November and December 2.6 % (1 / 38) for either month. However, between the months of February and August, the detection rate was well over 25.0 % (Figure 5). In Lusaka, the study site, the cool dry season lasts from April to August, the hot dry season from September to October, and the hot rainy season from November and lasts until

March. Rotavirus positive rate in the dry season (April to October) was 27.6 % (146 / 576) which was significantly higher than in the rainy season (November to March) (19.8 %, 97 / 491) ($p < 0.005$). The peak diarrhoea season in Zambia which occurs between the months of August to December did not seem to coincide with the highest detection rate of rotavirus (Figure 5).

Of the children below one year, 96.0 % (456 / 472) were breast feeding and rotavirus positive rate was not statistically different from those who were not breast feeding, (Table 10).

Clinical findings of rotavirus positive and negative children are summarised in Table 11.

Vomiting was equally present in both groups.

Fever was more common in the rotavirus negative children 54.4 % (441 / 811) than the rotavirus positive group 41.8 % (107 / 256), ($p < 0.005$).

Stool was bloody in 15.6 % (40 / 256) of the rotavirus positive children and in 52.4 % (425 / 811) of rotavirus negative children.

In rotavirus positive children 82.7 % (211 / 253) of the children had dehydration, and this was higher than in the rotavirus negative children (56.2 %, 456 / 794) ($p < 0.001$). However, some dehydration was the most common form of dehydration (79.4 %, 201 / 253) than severe dehydration (3.9 %, 10 / 253) in the rotavirus positive children. This was paralleled by 52.9 % (420 / 794) for some dehydration and 4.5 % (36 / 794) for severe dehydration in the rotavirus negative group, (Table 11).

Rotavirus positivity occurred more frequently in children with normal nutritional status (28.9 %, 162 / 558), than in those who were malnourished (18.9 %, 162 / 558), ($p < 0.003$), (Table 11).

The relationship of rotavirus density and nutritional status is further shown in Table 12. Rotavirus density was defined as graded 1 (rotavirus particles less than 10 per field), grade 2 (rotavirus particles more than 10 but less than 100 per field), and grade 3 (rotavirus particles more than 100 per field). Normal nutritional status was associated with a high grade (grade 3) of rotavirus shedding 17.6 % (13 / 74), and zero for those with frank protein energy malnutrition, (Table 12).

Associated case fatality rate in rotavirus positive children was 6.4 % (16 / 256) and this was one-half, 12.9 % (101 / 811) in the rotavirus negative children. Fatality in the rotavirus positive group was only seen in children less than 2 years (Table 13).

Table 14 shows the distribution of fatality in children less than 12 months of age, with the highest case fatality being in the children below 2 months of age.

Table 15 illustrates the comparison of Electron Microscopy and ELISA rotaclone methodologies in the detection of rotavirus in stool specimens. The ELISA used in this study was evaluated using E.M. as the gold standard. The sensitivity was found to be 81.4 % where as the specificity was 97.1 %. It appears therefore that the ELISA was as good as E.M. in detecting rotavirus in children with acute diarrhoea.

2. ENTERIC ADENOVIRUS DIARRHOEA

Of the 1064 stool samples, 700 samples were examined by electron microscopy. Of these 1.29 % (11 / 700) samples were positive for adenovirus. Random ELISA-Adenoclone assay on 164 samples showed that 3.65 % (6 / 164) were positive. There was no definite distribution of enteric adenovirus diarrhoea but that isolated cases were seen throughout the year.

Dual infection, rotavirus and adenovirus infection was noted in two samples. The clinical presentation in these two cases was not different from those who were only infected by either rotavirus or adenovirus.

3. SMALL ROUND VIRUSES

The investigator failed to demonstrate any Small Round Viruses (SRV) in the stools by E.M. method after ultracentrifugation of the first 100 samples. Suspicious electron micrographs were however taken and sent to the Virus Research Centre, Sendai National Hospital, Sendai, Japan for further scrutiny. They recommended Immuno Electron Microscopy (IEM) upon examination of these electron micrographs. Samples sent to the Virus Research Centre, Sendai, Japan also failed to yield any SRV by direct Electron Microscopy. IEM was however not done on these samples.

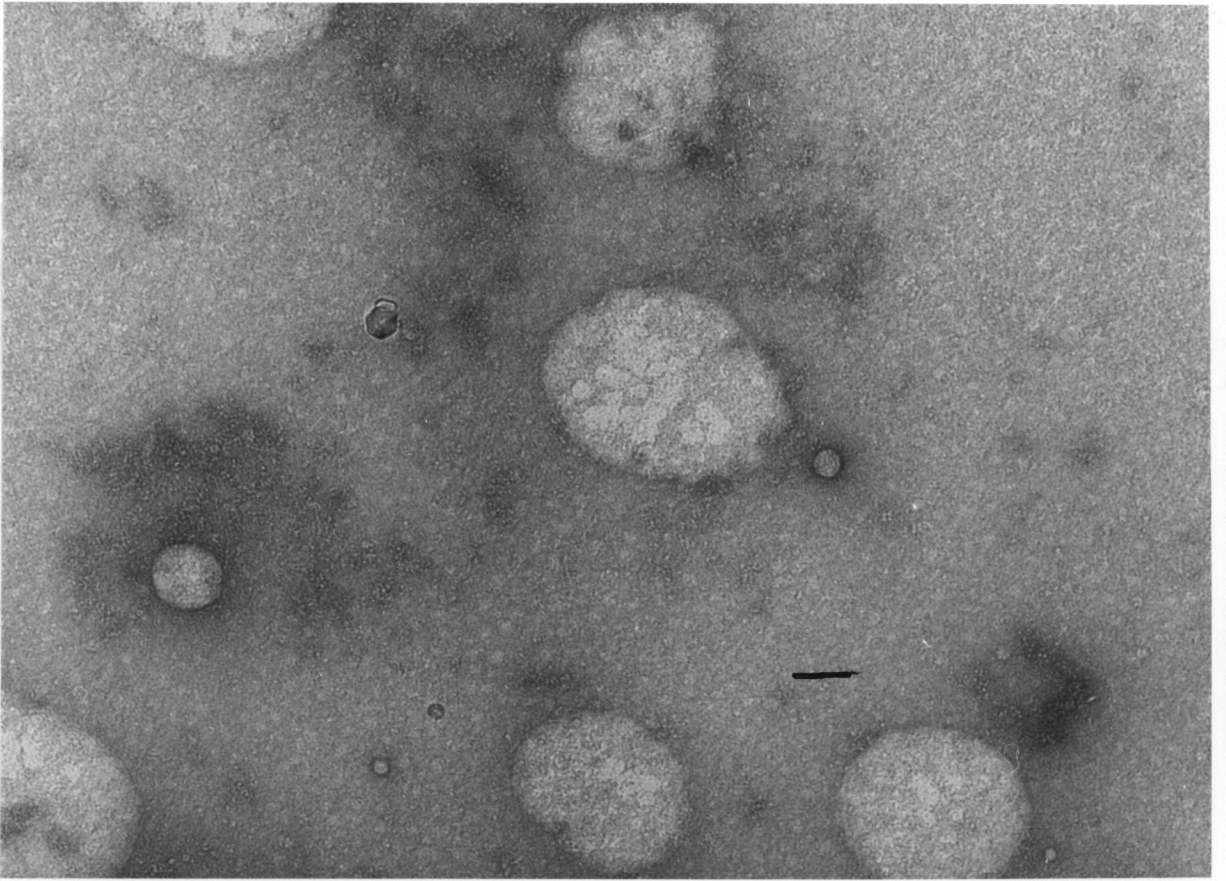
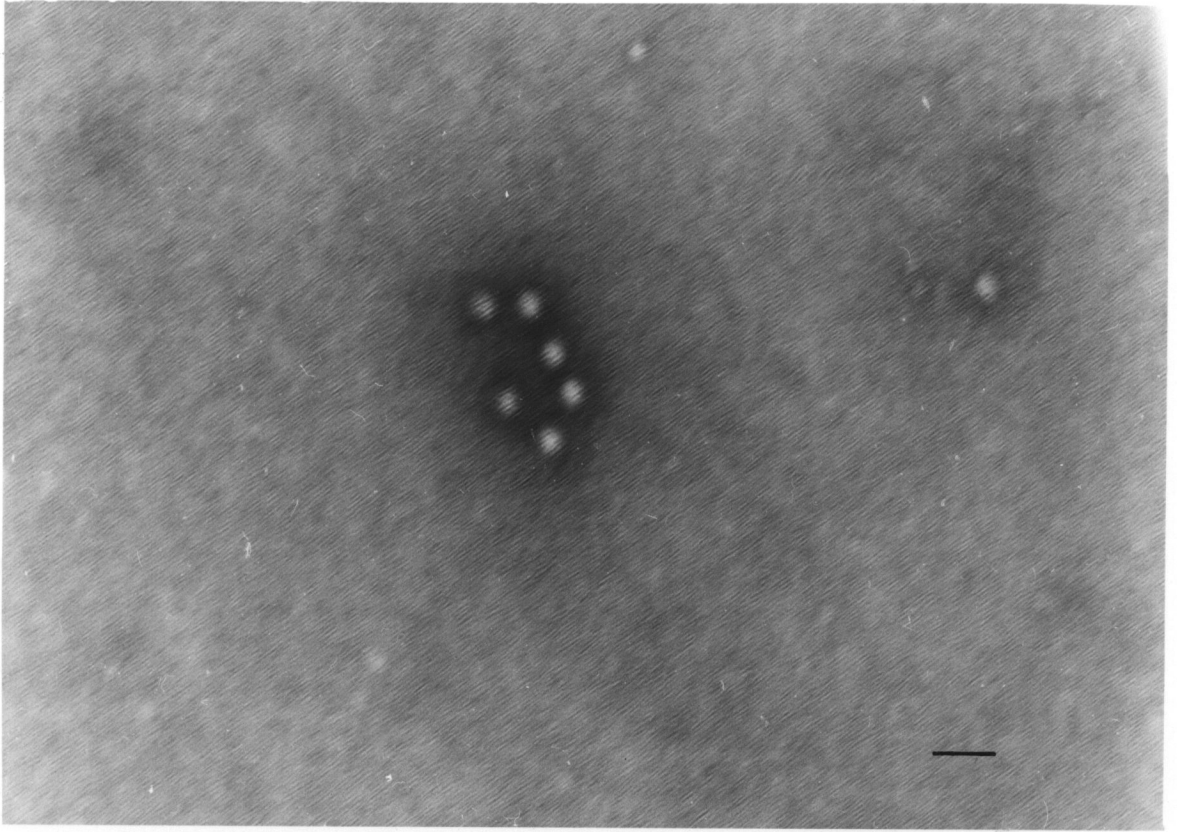


Figure 5: Electron Micrograph of suspected SRV (X40000) (taken by the author) (b - enlarged electron micrograph)

CONTROL SUBJECTS

Out of 341 children with medical conditions other than diarrhoeal disease 2.3 % (8 / 341) were positive for rotavirus on Rotaclo. No viruses were noted on E.M.

CHAPTER 6

DISCUSSION

Rotavirus is the single most common entero-pathogen found in surveys of hospitalized children with acute gastro-enteritis in both developed and developing countries (3,4). Worldwide, the prevalence rate of rotavirus diarrhoea ranges from 20 to 60 % (4,7). In this one year study of hospitalised children, 24.1 % were positive for rotavirus. However, during this period there was an epidemic of *Shigella dysenteriae* and this could have decreased the rotavirus positivity rate. Rotavirus shedding in bloody diarrhoea was 15.6 % but was as high as 84.4 % in non-bloody diarrhoea. Limited studies in Africa have been done on the prevalence of rotavirus the whole year round. In Kenya and Malawi where the study samples were limited prevalence rates of 41 % and 42 % were shown respectively (42,44). In Nigeria, (20), this was found to be 33 % with a higher prevalence in the dry season (59 %) than in the rainy season (21 %).

The peak age of infection was between 3-5 months 46.7 % and decreased to 30.5 % by 9-11 months (Table 8). Most of the rotavirus infections occurred between 6 months and 2 years (20). This suggests that there is an earlier age of exposure to rotavirus before the age of 6 months in this study population. Probably this reflects a high load of circulating rotavirus in the community. It also suggests that the infants may be losing their maternal antibodies much earlier. Immunity to HRV builds up with age (20), but does not prevent reinfection.

Rotavirus positive rate was high below the age of one year 37.6 % (173 / 460). This observation was most likely due to primary infection in children and after the age of one year rotavirus diarrhoea was probably due to reinfection (16.6 %, 68 / 420), (Table 7). Reinfection after the primary infection probably resulted in mild diarrhoea and was most likely to be treated in the out patient department, at local health facilities or indeed at home. Therefore, primary infection of rotavirus

diarrhoea in children less than one year tended to be severe warranting admission to hospital.

It has been suggested that breast feeding protects against rotavirus diarrhoea by providing specific secretory IgA and non-specific inhibitors (26). It has also been shown that human breast milk can modify the course of rotavirus diarrhoea by making it milder and of shorter duration (24,37). However, in this study 96.0 % (456 / 472) were breast feeding and rotavirus positivity was 36.4 % (Table 10). Therefore, breast feeding did not seem to prevent or modify rotavirus diarrhoea nor did it seem to reduce the mortality. The non-protective role of breast feeding to rotavirus diarrhoea may indicate the low levels of sIgA in breast milk and probably the nutritional status of the mothers.

Rotavirus detection was high 17.2-31.4 % (924 / 1051) in children who presented within 2-7 days of onset of diarrhoea (Table 9). The duration of diarrhoea and the time of presentation to hospital is crucial for the detection of rotavirus. However, longer rotavirus shedding was also observed in a few patients 9.4 % (3 / 32), (Table 9). These three patients had continued to shed after 14 to 20 days after the onset of diarrhoea.

Rotavirus diarrhoea was seen throughout the year with higher positive rates in the dry season (28.9 %) than in the rainy season (19.6 %), (Figure 5). In temperate climates, there is a strong seasonal variability in the incidence of rotavirus the peak incidence tends to be in the winter months (3,4,). In contrast, rotavirus is detected all year round in the tropics (7). In Nigeria, seasonal distribution was similar to this study where rotavirus positive rate was 53 % in the dry season and 21 % in the rainy season (20). Strong seasonal variations with very low or no prevalence of rotavirus in the rainy season were reported in Gabon (49) and the Gambia (21).

In Lusaka, the study site, the cool dry season lasts from April to August and this period coincided with the peak of rotavirus diarrhoea (Figure 4). However, this contrasted sharply with the peak diarrhoea period due to bacteria pathogens which occurs between September and December, the hot dry season to the early hot rainy season. The aetiology of this bacterial diarrhoea during the diarrhoea season has not been fully studied in Zambia. However, in recent years this has been associated with out breaks of bacillary dysentery and cholera towards the beginning of the hot rainy season. In neighbouring Zimbabwe, the peak of rotavirus diarrhoea was seen in the cool dry season (May and June, 31.1 % and 20.5 % respectively) (57).

In children with bloody diarrhoea, rotavirus positive rate was 8.6 % whereas in those with non-bloody diarrhoea this was 36.4 %. During the months of November and December in the year under study there was an epidemic of cholera along side an epidemic of *Shigella dysentery*. Maybe this double outbreak of bacterial diarrhoeas decreased the incidence of rotavirus in the months of November and December, (Figure 5).

In this study, more children had dehydration in the rotavirus positive group 82.4 % (211 / 256) than the rotavirus negative group 56.2 % (456 / 811). It has been reported that rotavirus diarrhoea is significantly associated with dehydration (29). In temperate regions, rotavirus diarrhoea is associated with more severe dehydration than other entero-pathogens (24,52). However, this study shows that the majority of the children had some dehydration 79.4 % (201 / 253), (Table 11), than severe dehydration 3.9 % (10 / 253). These results suggest that rotavirus though a major cause of gastroenteritis does not seem to cause frequent severe dehydration in regions where the infection is detected throughout the year. In Malawi, severe dehydration due to rotavirus diarrhoea accounted for less than 5 % (44). Therefore, severe dehydration due to rotavirus is a less frequent finding in Africa.

Vomiting was observed in equal proportions in those children who were positive and negative for rotavirus (Table 11). Fever was more common in rotavirus negative children than in rotavirus positive ($p < 0.005$). However, these clinical features are consistent with other findings (44) that there are no clinical signs associated specifically with rotavirus gastroenteritis.

Children with normal nutritional status 63.3 % (162 / 256) were significantly excreting rotavirus more frequently than those with malnutrition (36.3 %, 93 / 256), (Table 11). Rotaviruses have tropism for the healthy young enterocytes (14) than the shortened villi which is a feature in malnutrition. Therefore, children with normal nutritional status are more likely to be infected with rotavirus than those with malnutrition. The high rotavirus density 17.6 % [13 / 74, (grade 3)] in well nourished children further suggests that the virus replicates more efficiently in the healthy enterocytes than the shortened villi in children with malnutrition 0.0 % [0 / 3, (grade 3)], (Table 12).

The associated case fatality rate of 6.4 % associated with rotavirus diarrhoea in this study was high compared to less than 1 % in the developed countries (6). The case fatality rate in rotavirus negative children of 12.1 % was double that in rotavirus positive group. High mortality rate in the rotavirus negative children may have been due to antibiotic resistant *Shigella dysenteriae*. Most of the deaths associated with rotavirus diarrhoea were in children less than one year (Table 13). HIV infection should also have been associated with the case fatality rate. HIV is highly endemic in Zambia, and HIV sero-positive rate in hospitalised children with acute diarrhoea at UTH is 24 % (42). The case fatality rate in HIV sero-positive children was higher than the sero-negative. Furthermore, the UTH is a referral hospital, and most children are referred from peripheral clinics and hospitals in and around Lusaka. Patients are often coming to UTH in the late stage of their diseases. This also contributed to the high case fatality. The high mortality in the less than one year old was probably due to HIV infection

and associated early age of exposure to rotavirus in primary infection. Most deaths 72.7 % (8 / 11) occurred in children less than eight months (Table 14). Mortality tended to decrease with age and this was probably due to reinfection with rotavirus and a build up in the partial immunity against rotavirus (Table 14). However, in the rotavirus negative group a case fatality of 2.3 % was noted.

In this study, enteric adenovirus contributed 3.65 % to the aetiology of acute gastroenteritis in hospitalized children and this is comparable to 5.0 % in Malawi in non-hospitalised children (44). No mortality was associated with this group in this study. This suggests that enteric adenovirus does not seem to cause severe morbidity to need admission to hospital. Other studies have also shown that significant dehydration is an uncommon feature of adenovirus diarrhoea, and that most of the patients had mild symptoms (63). May be most of the children with enteric adenovirus diarrhoea were seen in the local health centres and were never referred to hospital or were seen in OPD and never admitted. However, in developed countries, most studies show that 4-10 % of hospitalization for childhood diarrhoea are due to enteric adenovirus (51).

Children with enteric adenovirus diarrhoea were sporadically seen during the study period, and no defined seasonal distribution was noted. This observation is consistent with findings from Sweden (63) that enteric adenovirus diarrhoea was seen throughout the year and displayed no marked seasonal pattern, in contrast to the winter prevalence of rotaviruses. No mortality was noted in these children with enteric adenovirus diarrhoea.

Small Round Viruses (SRV) were less likely to be detected by direct E.M. after ultracentrifugation as was attempted in this study. The suspicious electron micrographs were thought to have been caliciviruses by the experts and they recommended immuno-electron microscopy (IEM). IEM was however not done due to the

unavailability of convalescent sera of these patients.

Finally it is concluded that rotavirus is the commonest cause of acute childhood diarrhoea and that rotavirus is seen throughout the year. The commonest circulating HRV is group A. Furthermore this study highlights the early age of exposure of HRV and the high mortality in children less than 6 months. This study report also shows that malnourished children are rather resistant to rotavirus diarrhoea than those with normal nutritional status. Enteric adenovirus is documented for the first time in Zambia as an aetiological agent for acute diarrhoea. The ELISA antigen detection kits for rotavirus and adenovirus were just as good as the E.M. The sensitivity of the ELISA was 81.4 %, with a specificity of 97.1 %.

CHAPTER 7
RECOMMENDATIONS

1. More education is needed to the parents and health workers on the use of Oral Rehydration Salts and that they should be made readily available to children with acute watery diarrhoea.
2. A community based survey be conducted on the aetiology of viral gastroenteritis, both in urban and rural settings.
3. Molecular analysis of rotaviruses be done on the positive stored stool samples.
4. With this base line data on rotavirus diarrhoea, rotavirus vaccine trial be initiated in Lusaka for possible incorporation in the Expanded Programme on Immunization.
5. Secretory Immunoglobulin A against rotavirus be assayed in breast feeding mothers.
6. Immuno-Electron Microscopy technique be experimented with in an attempted to demonstrate the SRVs in Zambian children with acute watery diarrhoea.
7. Antibiotic and sulphonamide use should be discouraged in acute watery diarrhoea because of the development of resistance factors.

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TABLE 7: AGE DISTRIBUTION AND ROTAVIRUS POSITIVITY

AGE (YRS)	n	+ve	%
0	460	173	(37.6)
1	420	68	(16.6)
2	96	10	(10.0)
3	32	2	(6.3)
4	24	2	(5.9)
5	22	1	(4.1)
	1064	256	24.1

**FIGURE 6: AGE DISTRIBUTION AND ROTAVIRUS POSITIVITY
(HISTOGRAM)**

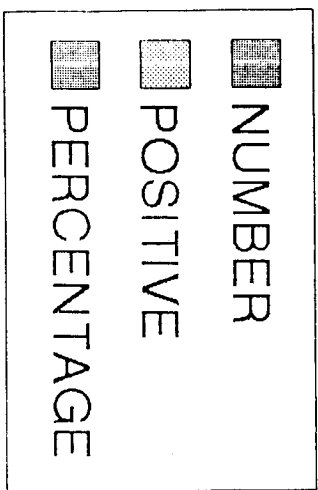
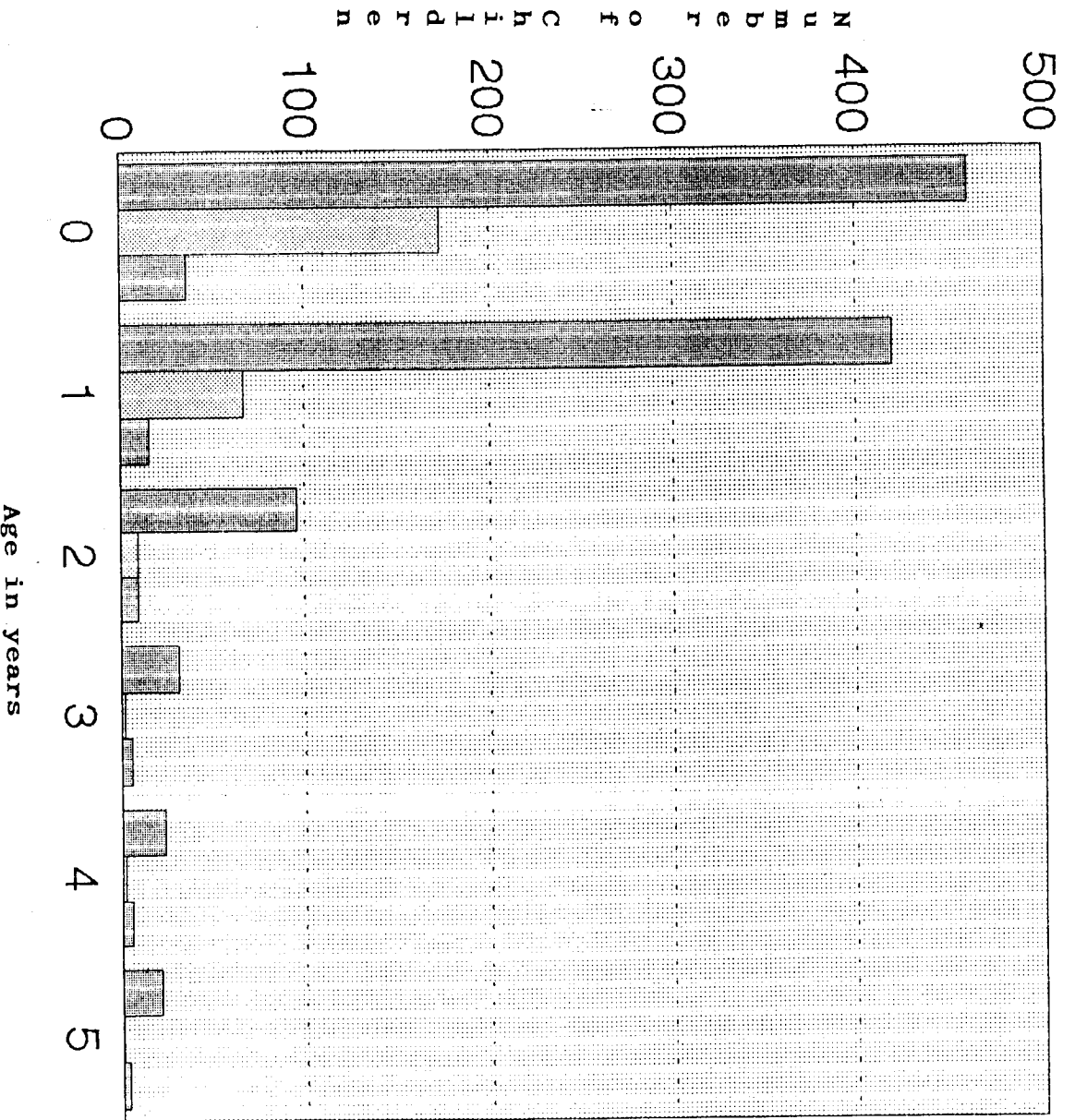
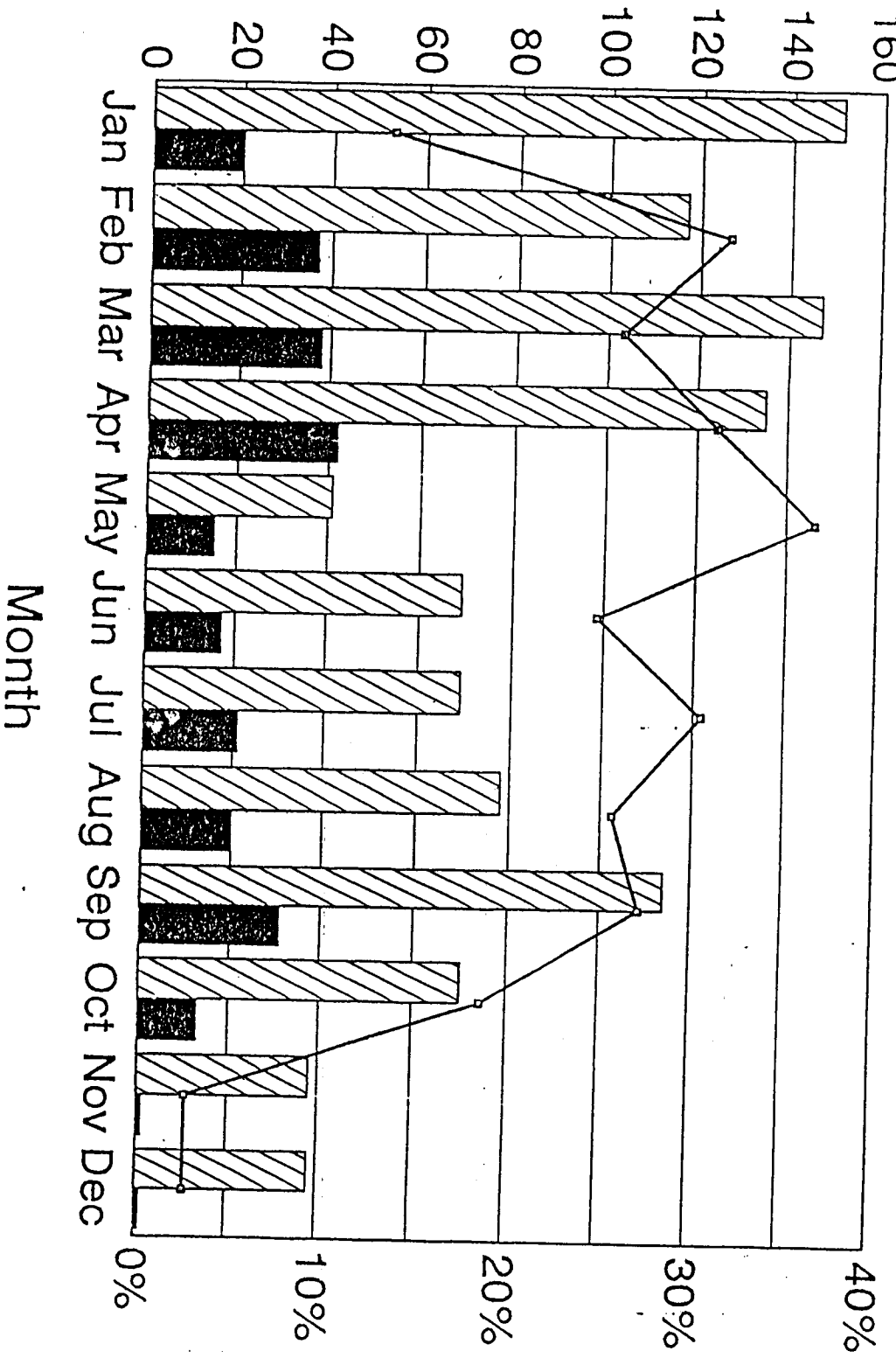


FIGURE 7: MONTHLY DISTRIBUTION OF ROTAVIRUS POSITIVITY AT THE UNIVERSITY TEACHING HOSPITAL






 No. Tested
  No. Positive
  % Positive

TABLE 8: ROTAVIRUS POSITIVITY IN CHILDREN LESS THAN ONE YEAR

AGE (mths)	n	+ve	%
0-2	22	8	(36.4)
3-5	92	43	(46.7)
6-8	173	68	(39.3)
9-11	177	54	(30.5)
	460	173	37.6 (mean %)

TABLE 9: DIARRHOEA DURATION AND ROTAVIRUS POSITIVITY

DAYS	n	+VE	%
1	12	2	16.7
2	87	15	17.2
3	242	76	31.4
4	217	56	25.8
5	151	42	27.8
6	95	20	21.1
7	113	21	18.6
8 -13	95	13	13.7
14-20	32	3	9.4
	1051	250	23.8 (mean %)

TABLE 10: BREAST FEEDING AND ROTAVIRUS POSITIVITY

A. LESS THAN 1 YEAR

BREAST FEEDING	POSITIVE	NEGATIVE	TOTAL
YES	172 (36.4%)	284 (60.2%)	456 (96.6%)
NO	5 (1.1%)	11 (2.3%)	16 (3.4%)
	177 (37.5%)	295 (37.5%)	472 (100.0%)

p-value = 0.60

B. MORE THAN 1 YEAR

BREAST FEEDING	POSITIVE	NEGATIVE	TOTAL
YES	56 (12.5%)	244 (55.7%)	300 (68.5%)
NO	5 (1.1%)	133 (30.4%)	138 (31.5%)
	61 (13.9%)	377 (86.1%)	438 (100.0%)

p-value = 0.0000082

TABLE 11: CLINICAL FINDINGS OF ROTAVIRUS POSITIVE AND NEGATIVE CHILDREN IN HOSPITALISED CHILDREN WITH DIARRHOEA

CLINICAL FINDINGS	ROTAVIRUS POSITIVE (n=256)	ROTAVIRUS NEGATIVE (n=811)	p-value
Vomiting			0.757
Yes	100 (39.1 %)	322 (39.7 %)	
No	156 (60.0 %)	480 (59.2 %)	
Unknown	0 (0.0 %)	9 (1.1 %)	
Fever			0.0004
Yes	107 (41.8 %)	441 (54.4 %)	
No	149 (58.2 %)	362 (44.6 %)	
Unknown	0 (0.0 %)	8 (1.0 %)	
Bloody stool			0.0000
Yes	40 (15.6 %)	425 (56.2 %)	
No	216 (84.4 %)	378 (46.6 %)	
Unknown	0 (0.0 %)	8 (2.1 %)	
Dehydration			0.0000
Yes	211 (82.4 %)	456 (56.2 %)	
No	42 (16.4 %)	338 (41.7 %)	
Unknown	3 (1.2 %)	17 (2.1 %)	
Nutrition			0.0003
Under nourished	93 (36.3 %)	389 (48.0 %)	
Well nourished	162 (63.3 %)	398 (49.1 %)	
Unknown	1 (0.4 %)	24 (3.7 %)	
Outcome			Relative Risk 0.53 (0.33 <RR< 0.85)
Death	16 (6.3 %)	101 (12.5 %)	
Discharge	235 (91.8 %)	680 (83.8 %)	
Unknown	5 (2.0 %)	30 (3.7 %)	

TABLE 12: ELECTRON MICROSCOPY ROTAVIRUS POSITIVE GRADE AND NUTRITIONAL STATUS

NUTRITIONAL STATUS	ELECTRON MICROSCOPY GRADE			TOTAL
	1	2	3	
Normal	28 (37.8 %)	33 (44.6 %)	13 (17.6 %)	74
Under weight	13 (65.0 %)	6 (30.0 %)	1 (5.0 %)	20
Malnutrition	2 (66.6 %)	1 (33.3 %)	0 (0.0 %)	3
TOTAL	43	40	14	97

TABLE 13: ASSOCIATED CASE FATALITY RATE AND AGE IN ROTAVIRUS POSITIVE AND NEGATIVE IN HOSPITALISED CHILDREN WITH ACUTE DIARRHOEA

AGE (years)	ROTAVIRUS POSITIVE			ROTAVIRUS NEGATIVE		
	No. tested	No. of death	CFR	No. tested	No. of death	CFR
< 1	173	11	6.4 %	295	39	13.2 %
1	68	5	7.4 %	357	54	15.1 %
2	10	0	0.0 %	86	4	4.7 %
3	2	0	0.0 %	30	0	0.0 %
4	3	0	0.0 %	43	1	2.3 %
TOTAL	256	16	6.4 % (mean %)	811	98	12.1 % (mean %)

TABLE 14: ASSOCIATED CASE FATALITY RATE IN CHILDREN LESS THAN ONE YEAR

AGE (MONTHS)	ROTAVIRUS POSITIVE	No. DIED	CFR (%)
0 - 2	8	1	12.5 %
3 - 5	42	3	7.1 %
6 - 8	68	4	5.9 %
9 - 11	51	3	5.9 %
TOTAL	169	11	6.5 % (mean %)

**TABLE 15: COMPARISON OF ELECTRON MICROSCOPY AND ELISA ROTACLONE
IN DETECTION OF ROTAVIRUS IN STOOL**

ELISA	ELECTRON MICROSCOPY		TOTAL
	+VE	-VE	
+VE	83	9	92
-VE	19	301	320
TOTAL	102	310	412

ELISA +ve p-value = 0.902
 -ve p-value = 0.997
 sensitivity = 81.4 %
 specificity = 97.1 %