

ROLE OF ROTAVIRUS IN ACUTE GASTRO-ENTERITIS IN  
CHILDREN IN JOS

BY

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## DECLARATION

The author hereby declares that the work presented here for the Master of Science Degree in Microbiology was carried out under the supervision of Drs N.E. Gonwalk and J.U. Umoh. No part of it has been presented for any award or degree and reference to published literature is adequately acknowledged.

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CERTIFICATION

This thesis entitled *Role of rotavirus in acute gastro-enteritis in children in Jos* by Lohya Ielfum Gosham meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

  
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**DEDICATION**

to my beloved, principled Uncle, Chief E.S. Yusufu

and

My beloved parents, Emmanuel Telfum and Tabitha Chakcit  
for spurring me in life towards the fear of God and academic  
pursuits.

*"The glory of young men is their strength; and the beauty of  
old men is the gray head"*

- Proverbs 20:29.

Thanks to God for without the gift of life and strength this  
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#### ABSTRACT

Four hundred and forty two (442) stool samples collected from infants and young children 0-5 years old with acute gastro-enteritis attending clinics and hospitals in Jos, Nigeria between May 1986 and April 1987 were examined for the presence of rotavirus using the Enzyme-linked Immunosorbent Assay (ELISA). Out of the 442 diarrhoeic stool samples examined, 146 were positive giving a prevalence of 33 per cent. Rotavirus was found to occur throughout the year with higher prevalence during the dry (58.7 per cent) than rainy season (21.4 per cent). This suggests an apparent seasonal variation in the occurrence of rotavirus with low relative humidity being the most influential climatic factor. No clear-cut relationship between the prevalence of rotavirus and mean minimum and mean maximum temperatures were observed. Rotavirus was found most prevalent in the 0-6 months age group children and this decreased with age. The most significant risk factor associated with rotavirus infection was the type of feeding. The presence of children less than two years old in the household, type of community (urban and rural), socio-economic status (high and low), source of water for home use and mode of excreta disposal were not associated with rotavirus infection.

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## CHAPTER 1

INTRODUCTION

Rotavirus gastro-enteritis is generally a disease of the infants and young children and appears to have a world wide distribution (WHO, 1980). Rotavirus is a major cause of deaths in infants in many developing countries where its effects are compounded by malnutrition and inadequate medical care (Jolik *et al.*, 1980). Rotavirus was first detected and reported in man in Australia in 1973 by thin section electron microscopic examination of duodenal biopsies obtained from children with acute diarrhoea. Since then, human rotavirus has been incriminated as the most important pathogen associated with non-bacterial acute sporadic gastro-enteritis (Maiya *et al.*, 1977; Samantary *et al.*, 1982) as well as epidemic forms of diarrhoea among infants and young children (Sengupta *et al.*, 1982). A high proportion of Nigerian children suffering from acute gastro-enteritis have been shown to be infected by rotaviruses (Dossator *et al.*, 1979; Gonwalk *et al.*, 1984; Paul and Paul, 1986; Fagbami *et al.*, 1987). Rotavirus is less common in the first six months of life or after two years but affects most commonly the 6-24 months aged children (Mahalanabis, 1980; Soenarto *et al.*, 1981).

Rotavirus infection has been shown to be influenced by climatic conditions (Moe and Shirley, 1982). Relative

humidity has an appreciable effect on the survival of rotaviruses. Rotavirus in faeces survives best at the two extremes of relative humidity rapidly loosing infectivity at intermediate ranges. The lower the temperature, the better is the survival of rotavirus (Moe and Shirley, 1982). In developed countries with a temperate climate, prevalence of rotavirus shows marked seasonal variation with peak during winter season (Davidson *et al.*, 1975; Bryden *et al.*, 1975; Kapikian *et al.*, 1976). Rotavirus infection in the tropics is poorly documented (Suzuki *et al.*, 1981). The question of seasonality of rotavirus in the tropics is not completely resolved (Fagbami *et al.*, 1987, Personal Communication). There have been few reports regarding tropical areas. In studies from Costa Rica and Venezuela little or no seasonal variation in occurrence was observed. However in surveys from India and Bangladesh, rotaviruses have been found mostly in stool samples from diarrhoea cases during the dry season (WHO, 1980).

Rotavirus infection has been associated with dog-ownership and the presence of children less than two years old in the household. Dogs may serve as either reservoirs of human rotavirus or sources of faecal contamination to the household (Engleberg *et al.*, 1982).

#### AIMS AND OBJECTIVES

This study is aimed at determining the prevalence of rotavirus, the possible predisposing factors and seasonal

variations in the occurrence of rotavirus infection in Jos.

The study is important and necessary since there is a dearth of information on the epidemiological marker of rotavirus infection in the country.

## CHAPTER 2

REVIEW OF LITERATURE2.1 Biological characteristic of rotavirus

Rotavirus include some major etiologic agents of infantile diarrhoea in humans and several other animals. In humans, they cause acute gastro-enteritis in infants and young children (McNulty, 1978; Kapikian *et al.*, 1976).

The virus derives its name from a latin word "rota" meaning wheel-like due to the appearance of the intact particle which is suggestive of a wheel with a hub, short spokes and a well defined rim (Flewett *et al.*, 1974; WHO., 1980).

Rotavirus is morphologically similar to other members of the family such as reovirus, orbivirus and cytoplasmic polyhedrosis virus (Mitsumori *et al.*, 1983), however rotaviruses are slightly smaller than reoviruses. The capsomere arrangement of the outer capsid of orbiviruses is often obscure (Mathews *et al.*, 1979). Human rotaviruses are morphologically similar and share certain common antigens with animal rotaviruses. Complement fixation test has shown that the human rotavirus is closely related to at least four animal rotaviruses namely: Nebraska calf Diarrhoea virus (NCDV), Epizootic diarrhoea of infant mouse (EDIM), Simian Agent (SA11), and the Offal agent (O) of calves and sheep. Infact the four antigens can be used as complement

fixation antigens for the detection of rotavirus infection in man (Kapikian *et al.*, 1975).

Rotavirus is 70 nm in size and has a double-stranded RNA in linear segments (WHO, 1980). The genome consists of 11 segments (Mitsumori *et al.*, 1983; Rodger *et al.*, 1981; Gorzilia *et al.*, 1983; Rixon *et al.*, 1984). The genomic double-stranded RNA is enclosed within a double-layered protein capsid formed by five structural polypeptides (Novo and Esparza, 1981; Ericson *et al.*, 1982). Rotavirus possesses naked nucleo-capsids with two clearly defined capsid shells, both having icosahedral symmetry. Outer capsid is probably composed of 32 large ring-shaped capsomeres as revealed by fine structure electron microscopy. The capsid is composed of numerous subunits arranged in the ring-shaped (hexagonal) patterns and many of these subunits are shared by adjacent cells (Jollik *et al.*, 1980). There is an electron dense centre about 38 nm in diameter referred to as the core. The two particle types are the complete, smooth or outer shelled particle measuring 70 nm and the incomplete, rough or inner shelled particle measuring about 10 nm (Bridger and Woode, 1978). Suzuki *et al.*, (1981) reported three types of virus particles; double-shelled particles 75-85 nm in diameter, single-shelled particles 64-68 nm in diameter, and electron dense nucleoids or cores 32-40 nm in diameter. Tubular structures, similar in diameter to the single-shelled particles have been reported in the cytoplasm and nucleus of infected MA104 cells (Suzuki *et al.*, 1981).

The virus particles have been observed in the dilated reticulo endoplasmic reticulum (RER), nuclear envelope (perinuclear space), viroplasm and lysosome-like body. The outer shell has been shown to have been acquired by budding through the membrane of the dilated reticulo endoplasmic reticulum (Suzuki *et al.*, 1981).

Much work on the polypeptide composition of rotaviruses has been concerned with human and calf rotaviruses. The structure of rotaviruses has revealed a range of 8-10 polypeptides with molecular weights between 15-130  $\times 10^6$  (Dieh, 1984). Two proteins present on the outer capsid of rotavirus Vp3 and Vp7 elicit antibodies capable of neutralizing virus infectivity (Bastardo *et al.*, 1981; Matsuno and Inuoye, 1983; Kalica *et al.*, 1981). The major polypeptide component of the outer capsid layer - Vp7 is glycosylated (Rodger *et al.*, 1981) and coded for by genomic segment 8 or 9 depending upon the virus strain (Both *et al.*, (1984). More than one polypeptide may be involved in the neutralization reaction (Beards *et al.*, 1980). The group antigen is located in the inner capsid layer (McNulty, 1978). Cross-neutralization tests show that apart from group antigen, species specific rotavirus antigens also exist and reside on the outer capsid layer of the virus (WHO, 1980).

Serotypes are probably numerous and cross-react. Serotypes as described by Kapikian *et al.*, (1981); Zissis and Lambert, (1978) were based on differences in the major inner capsid polypeptide which is specified by the sixth genomic

segment. They proposed that rotaviruses distinguished on the basis of differences in the sixth genomic segment by complement fixation tests should belong to different subgroups while those distinguished by serum neutralization should be said to be of different serotypes. Serotypes of rotavirus are defined by neutralization of viral infectivity and to date seven serotypes have been identified, four of which (serotypes 1-4) are found in humans and five (serotype 3-7) in animals. Two different serotypes of rotavirus have been identified in pigs (Bohl *et al.*, 1984).

The virus antigen has been detected in the cytoplasm 12 hours after infection and cytopathic effects occurred 24 hours after infection (Suzuki *et al.*, 1981). Intracytoplasmic inclusion bodies of different sizes and shapes have been demonstrated in MA104 cells (Hoshino *et al.*, 1982). Replication of the virus in cell cultures is dependent on trypsin (McNulty *et al.*, 1979). The virus is released by budding leading to steady-state infection (Jollik *et al.*, 1980).

## 2.2 Clinical manifestations

Clinical signs of naturally occurring rotavirus infections in animals have been described (McNulty, 1978). Limited information on experimental infections in humans is available. Rotavirus infections in human neonates are often asymptomatic (Fagbami *et al.*, 1987 Personal Communication), whereas those occurring in older children are usually

associated with gastro-enteritis. Infection in adult animals and humans is less severe (McNulty, 1978; WHO, 1980). Furthermore, evidence now suggests that rotaviruses may also be involved in causing severe gastro-enteritis in adults, particularly the very old (Echeveria *et al.*, 1978).

The incubation period for rotavirus infection ranges from 1-7 days but it is usually less than 48 hours (WHO, 1980). McNulty (1978) reported a relatively shorter incubation period of about 15 hours. Characteristically, the onset of the disease is heralded by one or two episodes of vomiting with subsequent appearance of diarrhoea (Jolik *et al.*, 1980). Thus the prominent early symptom of rotavirus infection in man is vomiting (Flewett and Woode, 1978). The predominant symptoms in man and animals include diarrhoea which occurs 2-4 days after onset of disease, depression, anorexia, severe dehydration and electrolyte imbalance (WHO, 1980; Engleberg *et al.*, 1982). Otitis media and respiratory symptoms have been reported although there was no evidence to indicate they were caused by the virus. The disease can be fatal in both humans and animals (Snodgrass *et al.*, 1976; WHO, 1980).

The course of the disease is usually 4-8 days with virus shedding for up to 10 days although Flewett *et al.*, (1975) have described diarrhoea in a child which lasted for more than 30 days with virus shedding in faeces for up to 23 days.

Other than acute gastro-enteritis, infection with rotavirus is associated with complications such as intussuscep-

tion of children with gastro-enteritis, self-limiting gastro-enteritis bleeding, development of "fatal Reye's syndrome" and severe central nervous system manifestation, encephalitis and hemolytic syndrome or disseminated intravascular coagulation.

### 2.3 Pathogenesis

Most information about the pathological changes caused by rotavirus infections has come from studies on experimental infections in animals (McNulty, 1978). The pathological studies suggest that virus initially invades the epithelium of the duodenum and upper small intestine. The villous epithelial cells of the small intestine are the principal site of the virus replication (McNulty, 1978). The virus antigen has also been detected by immunofluorescence in the epithelial cells of the colon, and the caecum of infected lambs (Snodgrass *et al.*, 1977). Histopathological changes have been noted in the stomachs and lungs of infected pigs and lambs (Snodgrass *et al.*, 1977). Rotavirus has been isolated in cell culture from extracts of mesenteric lymph nodes from a patient with chron's disease (WHO, 1980). It has been suggested that rotavirus may also cross the placenta and infect the foetus which will be born with infection or actively acquired immunity (WHO, 1980). The histopathological changes in calves and children naturally infected with rotaviruses have been shown to be similar (Bishop *et al.*, 1973). It is documented that rotavirus multiplies with production of

infectious particles in small bowel enterocytes (WHO, 1980). There is no evidence to suggest that this occurs anywhere else. Some patients have had associated respiratory symptoms but in humans, the virus has not been demonstrated in respiratory tissues or gastric contents (WHO, 1980).

From these studies rotavirus infection results in a sequence of events occurring in the small intestine. These include infection of the absorptive villous epithelial cells. Villi are transiently denuded and shortened, replacement of the tall columnar villous epithelial cells with cuboidal or squamous cells, lymphocytic infiltration of the villous lamina propria characterized by increased number of reticulum like cells and repair (McNulty, 1978; WHO, 1980). It has been proposed that upper intestine epithelial cells are initially infected and subsequently the infection progresses posteriorly down the intestine. The changes are said to occur in a cephalocaudal direction and suggest that much of the diarrhoea may be due to loss of absorptive capacity in the small intestine (WHO, 1980; Bohl *et al.*, 1984). Infected cells are lost from the tips of the villi and are replaced by immature cells from the crypts (Snodgrass *et al.*, 1977). These immature epithelial cells have reduced level of disaccharides and possibly also an impaired capacity to absorb glucose and galactose (Bishop *et al.*, 1973). This decreased ability to utilize dietary lactose results in accumulation of lactose in the large intestine, where by virtue of hypertonicity prevents absorption of water from faeces and exert a positive

dehydrating effect (McNulty, 1978). Infected cells are rapidly degenerated which accounts for the appearance of virus-infected cells in faeces a few days after onset of symptoms (Jollik *et al.*, 1980).

#### 2.4 Immunity

Natural resistance to rotavirus is lacking in all ages in humans and animals alike (McNulty, 1978). Antibodies have been detected in persons convalescing from the disease (Engleberg *et al.*, 1982). Sero-epidemiologic studies have shown that most children acquire antibodies to rotavirus before three years of age (Volken *et al.*, 1978).

Most new borns shedding rotavirus in stool have passively acquired antibody in the sera. Serum antibody has little protective effect on rotavirus infection (Fagbami *et al.*, 1987, Personal Communication). This is probably due to the absence of neutralizing antibody in the serum to infecting serotype (Burwith *et al.*, 1981). It is probable that intestinal immunity of a level sufficient to prevent reinfection of the gut lasts for not more than six months following infection especially if the virus is circulating in the adolescent adult population (WHO, 1980; Engleberg *et al.*, 1982). IgA specific antibody has been found in the colostrum and breast of lactating mothers in a number of countries, however the role of breast milk in protection against rotavirus infection is not clear. Mcleans and Holmes, (1981) reported that antirotaviral IgA combined

with inhibitory capacity found in breast milk could effect protection but may be of a short duration.

## 2.5 Epidemiology

Despite the strikingly different sanitary conditions from one part of the world to another, early exposure to rotavirus infection is universal (Engleberg *et al.*, 1982).

Rotavirus gastro-enteritis is generally a disease of infants and young children and appears to have a world-wide distribution (WHO, 1980). Rotavirus is the single major agent causing gastro-enteritis in children less than two years of age (Mahalanabis, 1980), with 0-6 months age group children being most commonly affected (McCormack, 1982). The virus has been associated with non-bacterial acute sporadic gastro-enteritis (Maiya *et al.*, 1977; Samantary *et al.*, 1982; Saha *et al.*, 1984) as well as epidemic forms (Sengupta *et al.*, 1981; Paniker *et al.*, 1982).

Several species of newborn animals are susceptible to infection with human rotavirus (Yolken *et al.*, 1978). Rotavirus has been demonstrated to cause diarrhoea in mice, calves, piglets, foals, young rabbits, deer, chicken, turkeys, goats, kitten, chimpanzee and gorilla (WHO, 1980), however the epidemiology of rotavirus infection in animals is not well understood. An apparent association of other agents including bacteria, viruses and also environmental stress with increasing severity of the disease in naturally occurring epizootics has been observed (WHO, 1980).

All evidence to date indicates that rotavirus infection spreads by faecal-oral-route through contaminated objects and through cross-infection. Since the incubation period for rotavirus is short (1-2 days), it is probable that most infections in early life occur as a result of post-partum contact with infected persons or environment (WHO, 1980). The intestinal tract is the site of rotavirus multiplication. The virus is excreted in large amounts in faeces. About  $10^7 - 10^{10}$  viral particles can be present per gram of stool (McNulty, 1978). A respiratory mode of spread has also been proposed by some workers and remains a possibility (Lewis *et al.*, 1979; Foster *et al.*, 1980; Kyaw *et al.*, 1983).

Rotavirus will usually affect humans regardless of age and sex. Infection of adult humans and animals has been documented (McNulty *et al.*, 1976). Adult humans infected with rotavirus show mild symptoms of diarrhoea or subclinical infection probably due to active immunity and therefore serve as carriers (Rowland *et al.*, 1978). Neonates infected are often asymptomatic (Fagbami *et al.*, 1987, Personal Communication). Whereas rotavirus infection in young children is usually associated with diarrhoea (Paul and Erinle, 1982).

Climatic conditions have been shown to influence rotavirus survival (Moe and Shirley, 1982). Relative humidity has an appreciable effect on the survival of rotaviruses. Rotavirus in faeces survives best at two extremes of relative humidity, rapidly losing infectivity at intermediate

ranges. As for the effects of temperature, the lower the temperature, the better is the survival of rotavirus. The higher temperature has unfavourable effect on rotavirus survival compared with the lower temperatures (Moe and Shirley, 1982). With no seasonal variation in temperature, relative humidity may influence the epidemiology of rotavirus infections more (Dossetor *et al.*, 1979).

Rotavirus infection in the tropics is poorly documented (Suzuki *et al.*, 1981). There have been few reports regarding tropical and subtropical areas. In Costa Rica and Venezuela, little or no seasonal variation in occurrence was observed (WHO, 1980). While surveys from India and Bangladesh, rotavirus infection was found to occur mainly during the dry season (Maiya *et al.*, 1977; Black *et al.*, 1980). Paul and Erinle, (1982) in Ife, Nigeria demonstrated a higher prevalence of rotavirus during the dry than the rainy season. Dossetor *et al.*, (1979) in Zaria, northern Nigeria showed a prevalence of 61 per cent during the rainy season; while Bonwalk *et al.*, (1984) in Zaria showed an incidence of 18 per cent which was very low compared to Dossetor and co-workers findings. The fairly constant temperature and relative humidity prevailing throughout the year in most tropical and subtropical countries will not cause the survival rate of rotaviruses to vary during most months of the year; explaining the absence of a well-defined seasonal variation (Moe and Shirley, 1982). However in some of the tropical countries where relative humidity is variable

during the year, the incidence of rotavirus tends to increase with relative humidity (Al-Nakib *et al.*, 1980). Differences in epidemiological patterns of childhood rotavirus infections between different tropical countries are minor and probably due to the methods of selection of patients and diagnosis of infection (Soenarto *et al.*, 1981). By contrast, in temperate climates, a clear winter peak of rotavirus infection has been shown, both in North America (Kapikian *et al.*, 1976), Britain (Bryden *et al.*, 1975), and Melbourne, Australia (Davidson *et al.*, 1975). Winter months in these countries have a lower temperature and increased humidity. The low temperature will permit prolonged survival of rotavirus on contaminated surfaces although relative humidity is not optimal (40-60 per cent). This may lead to higher infection rate during winter compared to summer when the temperature is higher and relative humidity not significantly different (Moe and Shirley, 1982).

Dog ownership, presence of children less than two years old, and socio-economic status have been shown to predispose to rotavirus infection (Maiya *et al.*, 1977; Totterdell *et al.*, 1980; Engleberg *et al.*, 1982). A survey carried out during an epidemic of diarrhoea on an India Reservation associated dog ownership and presence of household contact under the age of 2 years with rotavirus infection (Engleberg *et al.*, 1982). Dogs may serve as either reservoirs of human rotavirus or as sources of faecal contamination of the household; while children under the age of two years old

in diapers are likely sources of faecal contamination in a household (Engleberg *et al.*, 1982). A survey in South India revealed that incidence of rotavirus increased in children of low socio-economic level (Maiya *et al.*, 1977).

Several nosocomial rotavirus epidemics involving neonatal nurseries have been described (Totterdell *et al.*, 1980). In one of the outbreaks, it was shown that neither maternally acquired serum antibodies to rotavirus nor antibody to rotavirus in breast milk correlated with protection against rotavirus infection (Totterdell *et al.*, 1980). Breast milk could effect protection against rotavirus infection but may be of a short duration (Mcleans and Holmes, 1981).

#### 2.6 Laboratory Diagnosis

For the determination of viral infections, common methods employed include neutralization test direct, and indirect immunofluorescence, indirect hemagglutination, complement fixation and Enzyme-linked Immunosorbent Assay (Bidwell *et al.*, 1977).

Diagnosis of rotavirus infection is usually based on the detection of the virus or viral antigen in faeces or intestinal contents. In human infections, large number of rotaviral particles are excreted in stool (McNulty, 1978). The optimal period for virus detection is within the first 3-5 days after onset of symptoms (WHO, 1980). Diagnosis becomes easier when virus particles are first of all

concentrated by ultra-centrifugation (Bishop *et al.*, 1974), or when viruses are clumped together by immune electron microscopy using antiserum (Kapikian *et al.*, 1974). The presence of rotaviruses can be detected by electron microscopic examination of faecal extracts obtained during the acute stage of gastro-enteritis. All rotaviruses from whichever species look alike in the electron microscope. Nevertheless, considerable diversity exists among them which are only detectable by immune microscopy (Bridger, 1980), Enzyme Linked Immunosorbent Assay, immune adherence reaction (Kapikian *et al.*, 1981), and serum neutralization (Beards *et al.*, 1980).

For many specimens containing little or no live virus, serotyping methods are not applicable. For the measurement of rotavirus antibody and detection of rotavirus strain and serotypes, a variety of tests which include radial immunodiffusion, indirect immunofluorescence, complement fixation test, immune electron microscopy and Enzyme-linked Immunosorbent Assay are used (WHO, 1980). Radial immunodiffusion is used for group specific rotavirus antibody while indirect immunofluorescence is used for measuring antibody titers against human rotavirus (WHO, 1980). Enzyme-linked reaction with suitably absorbed antisera gives rapid methods for determining both the subgroup and serotype of rotavirus in faeces (Thouless *et al.*, 1978). The Enzyme-linked Immunosorbent Assay (ELISA) is a sensitive, reproducible test for the detection of rotavirus antigen (Ekern *et al.*,

1980). It utilizes an enzyme as the immunoglobulin marker. Reagents used are suitable and non-hazardous. The technique has no concern about the concentration of virus present in the sample (Ekern *et al.*, 1980). The principle of ELISA depends upon two assumptions: that the antibody attaches to the solid phase support yet maintains immunological activity and that the antibody complex can be linked to an enzyme and retain immunological and enzymatic activity (Ekern *et al.*, 1980). The double sandwich ELISA yields objective results and is extremely sensitive for the detection of rotavirus (Volken *et al.*, 1978). ELISA is a more sensitive technique to use for detection of rotavirus infection, particularly in collaborative studies in which transport of specimens may be a problem (Soenarto *et al.*, 1981).

The counter-immunoelectrophoresis (CEIP) technique is convenient, rapid, reliable for selecting rotavirus antigen and more sensitive than electron microscopy (Graubelle *et al.*, 1977). This procedure cannot be used to quantitate antigen but as a screening tool.

Immunoelectrophoresis is a simple and sensitive diagnostic procedure. This problem of non-specific fluorescence may occur though this may be overcome by separating the antigen by immune precipitation prior to staining. This makes the method laborious and time consuming when done on large scale (WHO, 1980).

Radioimmunoassay gives comparable results with immune

electron microscopy (Kalica *et al.*, 1978). This technique however, requires radioactive, unstable, hazardous reagents and a sophisticated, expensive equipment.

Hemagglutination and complement-fixation tests are quite sensitive, but not all rotavirus isolates will hemagglutinate and sometimes faecal material will be anticomplementary (Mohammed *et al.*, 1978). One limitation of the complement fixation test is that it does not distinguish between antibodies of different species origin (WHO, 1980).

Radial immunodiffusion (RID) is another simple and cheap method for detection of group-specific rotavirus antibody (WHO, 1980).

Early attempts to propagate rotavirus of human origin in cell cultures were difficult and unsuccessful. Studies on human rotavirus have been hampered by the difficulties of *in vitro* cultivation and especially of its serial passage in cell cultures. However, these difficulties have now been largely overcome by incubating rotavirus inoculum with trypsin in the maintenance medium. The beneficial effects of trypsin for the isolation and propagation of rotaviruses have been exploited by some workers (Urasawa *et al.*, 1981; McNulty *et al.*, 1979). There are few reports of successful isolation and growth of rotavirus from calves, dogs, chicken and infants without the use of trypsin (Hasegawa *et al.*, 1982; Hoshino *et al.*, 1982). The need for trypsin as an aid in the isolation and replication of rotavirus from field specimens depend to some extent on the strain of rotavirus

(Djeh, 1984). Human rotavirus has been isolated in cultures of MA104 cells a stable cell line derived from embryonic rhesus monkey kidney (Sato *et al.*, 1981). Intracytoplasmic inclusion bodies of different sizes produced by rotavirus on MA104 cells have been reported (Hoshino *et al.*, 1982). Human rotavirus (Wastrain) has also been cultivated in African green monkey kidney (AGMK) cells (Alteburg *et al.*, 1980).

#### 2.7 Prevention and control

The control of gastroenteritis in developing countries is focused on the reduction of incidence, morbidity, and mortality of disease by means of socioeconomic improvement, education and child health care. Immunoprophylaxis against a target enteropathogen such as rotavirus becomes scientifically rational (Schoub and Berkowitz, 1983). Reduction in death rate could be realized by adequate oral rehydration of infected children especially the severely dehydrated patients with intractable vomiting (Sack *et al.*, 1978). Oral-glucose electrolyte solution is normally being administered. Breast-milk has been shown to contain antirotaviral IgA and trypsin inhibitory content which could effect protection against rotavirus infection for a short duration (McLearns and Holmes, 1981). Rotavirus infection in infants receiving low levels of antirotaviral IgA and low trypsin inhibitory capacity is similar to that of bottle-fed infants. While infants receiving high levels of both antirotaviral IgA and

lacteal trypsin inhibitory contents have substantially reduced infection (McCleans and Holmes, 1981).

Studies in animals have clearly demonstrated that local antibody to rotavirus in the lumen of the small intestine rather than serum antibody is of primary importance to resistance to rotavirus-induced diarrhoeal illness (WHO, 1980). Passive protection requires making efforts to maintain rotavirus antibody in the intestinal lumen, in addition to the blood stream of the neonate (Bridger, 1980).

A volunteer study also revealed that intestinal fluid response to rotavirus can be transient as observed in animals (Kapikian *et al.*, 1980). Kapikian *et al.*, 1983 orally challenged volunteers with human rotavirus strain D (Subgroup 2, serotype Wa) and diarrhoeal illness occurred two to four days after inoculation.

Viral shedding was detected in some volunteers whereas some developed serologic evidence of infection. The presence of preinoculation serum immunofluorescent antibody to rotavirus strain D or high levels of neutralizing antibody to Wa human rotavirus correlated with resistance to diarrhoeal illness.

A heterotypic human or animal rotavirus might be useful in immunization of humans against rotavirus (Kapikian *et al.*, 1983). Knowledge of the factors influencing susceptibility to illness due to rotavirus in humans remains scanty and such information is essential for successful development of immunoprophylaxis (Kapikian *et al.*, 1983).

## CHAPTER 3

MATERIALS AND METHODS3.1 Materials

- 1 Microtitre pipettes of 50  $\mu$ l, 100  $\mu$ l, 1 ml and a 10 ml (Flow laboratories, U.K).
- 2 Sterile bijoux bottles for stool samples collection.
- 3 Bottles for storage of reagents.
- 4 Gallenkamp centrifuge.
- 5 An incubator set at 37°C.
- 6 An Enzyme-linked Immunosorbent Assay reader (litretek Multiskan, Flow laboratories, Irvine Scotland, U.K).
- 7 A suction device for faecal extracts aspiration.
- 8 Test faecal samples and rotavirus negative faecal sample
- 9 Reagents:

Reagents used for the test were obtained from Hoechst U.K Ltd., Pharmaceutical Division produced by Behring Institute.

Reagents for antigen detection by Enzyme-linked Immunosorbent Assay (ELISA), include the following:

- i) Enzygnost Rotavirus (Ag'), test plate consisting of 96 reaction wells (coated with simian rotavirus (SA11) antiserum for rabbit.
- ii) Rotavirus antigen (Ag') for Enzygnost (positive Ag'

control

iii) Anti-Rotavirus/Alkaline phosphatase conjugate for  
Enzgnost Rotavirus (Ag').

iv) Supplementary Reagents for Enzygnost:

(a) Substrate Tablets

P-nitrophenyl phosphate

(b) Substrate Buffer

Diethanolamine 100 ml

MgCl<sub>2</sub>.6H<sub>2</sub>O 102 g (0.5 mmol)

pH was 9.8 and the total volume made up to one litre  
with distilled water.

(c) Substrate Solution

For each test plate, the substrate solution was obtained  
by dissolving two substrate tablets in 10 ml of substrate  
buffer.

(d) Dilution Buffer

Sterile phosphate buffer pH 7.0

Tween 20 40.0 ml

Sodium azide 0.2 g

Bovine protein 5.0 g

The volume was made up to one litre with distilled water

(e) Washing Solution

Phosphate buffer pH 7

Iween (R) 20      200.0 ml

Sterile 20 - fold concentrate

The volume was made up to one litre with distilled water

(f) Stopping solution

2N NaOH.

## 3.2 METHODS

### 3.2.1 Study area

Jos is a town in Northern Nigeria. It is in the guinea savanna region located about 1,159 above sea level and one of the coldest areas in the country. The town experiences significant variations in temperature and relative humidity compared with the southern and western parts of Nigeria. It is characterized by two major seasons; a period of dry season from November - March and a period of rainy season from April - October.

### 3.2.2 Study population

The subjects for this study consisted of four hundred and forty two infants and young children with acute diarrhoea 0 - 5 years old attending clinics and hospitals in Jos from May 1986 - April 1987.

### 3.2.3 Faecal sample collection

Fresh stool samples were collected from these children in sterile bi jou bottles. Collection was done by keeping the bottle directly in position of the anus of a defaecating child. A questionnaire was used for obtaining information concerning each patient. The information contained in the questionnaire basically include age, weight, previous diarrhoeal therapy, presence of other children less than two years old in the household, source of water for home use,

type of food, major symptoms presented by diarrhoeic children (diarrhoea was defined as the sudden onset and passage of 3-4 loose and watery stools per day), type of community and the socio-economic status of the patients was grouped broadly into high and low. Children of the low socio-economic status were from poor rural, and urban families whose income per month was less than two hundred and fifty naira.

The stool samples collected were stored at  $-20^{\circ}\text{C}$  initially in the various clinics and hospitals where they were collected. However, at the end of each month, samples were brought out and transported to the Department of Microbiology, Ahmadu Bello University, Zaria in a Coleman cooler lined inside with ice or freezer packs. Time taken for specimens transportation each month was approximately three hours.

On arriving Zaria, specimens were again brought out and stored at  $-20^{\circ}\text{C}$  until when required for analysis.

#### 3.2.4 Preparation of faecal samples

Samples stored at  $-20^{\circ}\text{C}$  were brought out and allowed to thaw after which 0.1 ml faeces was mixed with 0.4 ml of the dilution buffer in a MacCarthy bottle. This was then shaken to form a good suspension. The suspension was centrifuged at approximately 2000  $\times g$  for about five minutes. The supernatant was kept in the refrigerator overnight and the Enzyme-linked Immunosorbent Assay used to test the samples.

### 3.2.5 Preparation of the Reagents

Reagents were prepared in accordance with Behring Institute instructions for Enzygnost Rotavirus Antigen detection by Enzyme-linked Immunosorbent Assay.

#### (i) Enzygnost Rotavirus (Ag') Test plate

The test plate was opened from the narrow side of the bag, removed from the pack, labelled and allowed to stand for about five minutes at room temperature before use.

#### (ii) Rotavirus Antigen

Using dilution buffer, 1:640 dilution of the antigen was made. The remaining undiluted rotavirus antigen was dispensed into sterile biou bottles using sterile pipettes. These were stored at -20°C in aliquots until when required.

#### (iii) Anti-Rotavirus/Alkaline phosphatase conjugate for Enzygnost Rotavirus

A 1:35 dilution of the conjugate was carried out by mixing 0.1 ml of alkaline phosphatase conjugate with 3.4 ml dilution buffer. The remaining undiluted conjugate was dispensed into sterile biou bottles and stored in aliquots at -20°C until when needed.

(iv) Supplementary Reagents

These include substrate tablets, substrate buffer, dilution buffer, washing solution and stopping solution.

(a) The substrate tablets and substrate buffer were used for the preparation of the substrate solution. For each test plate employed 10 ml of substrate buffer was pipetted into a sterile bottle, allowed to come to room temperature and then two substrate tablets were dissolved in it.

(b) Dilution buffer

This was removed from the refrigerator and brought to room temperature. Using sterile pipettes, the required amount for use was withdrawn.

(c) Washing solution (concentrate)

This was warmed before use by swirling in tepid water in order to re-dissolve crystals present. Then 1:20 dilution was made by taking one part by volume of concentrate to 19 parts by volume of sterile distilled water.

(d) Stopping solution

The stopping solution contained 2N NaOH and 0.05 ml of this was added to each test well.

### 3.2.6 Procedure for Enzyme-linked Immunosorbent Assay

Firstly, 0.15 ml of the centrifuged supernatant from the negative control sample was each placed into the A1 and A12 wells of the test plate using a pipette. Later, 0.15 ml each of the centrifuged supernatants from the test samples was pipetted into each reaction well. Then 0.15 ml of the diluted rotavirus antigen (positive control) each was dispensed into the last wells of the first and twelfth columns respectively. The test plate was incubated for two hours at 37°C in a humidified incubator on top of a plastic container which was a poor conductor of heat.

The faeces dilutions were suctioned-off at the end of incubation period and 0.2 ml of the diluted washing solution was pipetted into each well. This was suctioned off and the process repeated twice. The washing solution was allowed to stand for 1-2 minutes to prevent non-specificity of tests. The next step was the addition of 0.05 ml of the diluted enzyme conjugate solution to each well after which the plate was incubated for one hour in the humid incubator set at 37°C. The enzyme conjugate was suctioned-off and washing was carried out thrice using the washing solution. Substrate solution was added in 0.1 ml amount to each reaction well and incubation done for 30 minutes in humid incubator set at 37°C.

At the end of the period, enzyme reaction was stopped by the addition of the stopping solution (2N NaOH) and the yellowish-green colour reaction was evaluated within one

hour. Test samples were evaluated both visually by reading in indirect day light against a white background. A sample was considered positive when a yellowish-green colour developed in the test well and the negative well remained colourless. However, the degree of positivity of the samples were further confirmed by taking photometric readings using an ELISA reader (Titretek Multiskan, Flow laboratories, Irvine Scotland, U.K).

The absorbance of the liquid content of each well was read at wavelength of 405 nm against 0.1 ml of substrate solution plus 0.05 ml of stopping solution as reference solution. The reference solution was in a normal, uncoated microtitre plate with round-bottomed wells for adjustment of the photometer.

A reaction was considered positive when the absorbance was at least 0.15 A above the value measured for the negative control.

### 3.3 Data analysis

Data was analysed statistically by means of odds ratio and chi-square ( $X^2$ ) tests. A probability of 0.05 or less was regarded as significant for the  $X^2$  - test while a value greater than one was regarded as significant for odds ratio.

## CHAPTER 4

RESULTS

The monthly prevalence of rotavirus in Jos during the period of study May 1986 - April 1987 is as shown in Table 1. Out of the four hundred and forty two diarrhoeic stool samples analysed, rotavirus was detected in one hundred and forty six (33.0 %). The prevalence of rotavirus correlated with climatic changes. The prevalence of rotavirus was found to be higher during the dry season 58.7 % (November - March) than during the rainy season 21.4 % (April - October); although two peaks (November - March) and (July - August) corresponding to dry and rainy seasons were observed. The risk of rotavirus infection was high in the months of March (5.1), January (4.1), November (3.6), February (3.4), August (1.9), July (1.3) and December (1.3); while infection was low in the other months.

The prevalence of rotavirus varied with mean relative humidity (Figure 1). A significant relationship was noted between the mean relative humidity and prevalence of rotavirus ( $r = 0.65$ ;  $P < 0.05$ ). It seemed that the two extremes of mean relative humidity had influence on the prevalence of rotavirus. In this study, it was noted that as mean relative humidity decreases, rotavirus detection rate increases. There was no clear-cut relationship between the prevalence of rotavirus and mean minimum and mean maximum temperatures (Fig.2).

Table 1 Monthly prevalence of rotavirus in children with acute diarrhoea in Jos between May 1986 and April 1987

Month	Number of stool samples tested	No. positive for rotavirus	Percentage positive	Odds ratio
May	35	5	14.3	0.34
June	29	1	3.4	0.07
July	34	13	38.2	1.30
August	30	14	46.7	1.90
September	89	22	24.7	0.67
October	72	8	11.1	0.25
November	36	23	63.9	3.60
December	36	14	38.9	1.30
January	21	14	66.7	4.10
February	24	15	62.5	3.40
March	21	15	71.4	5.10
April	15	2	13.3	0.31
	442	146	33.0 %	

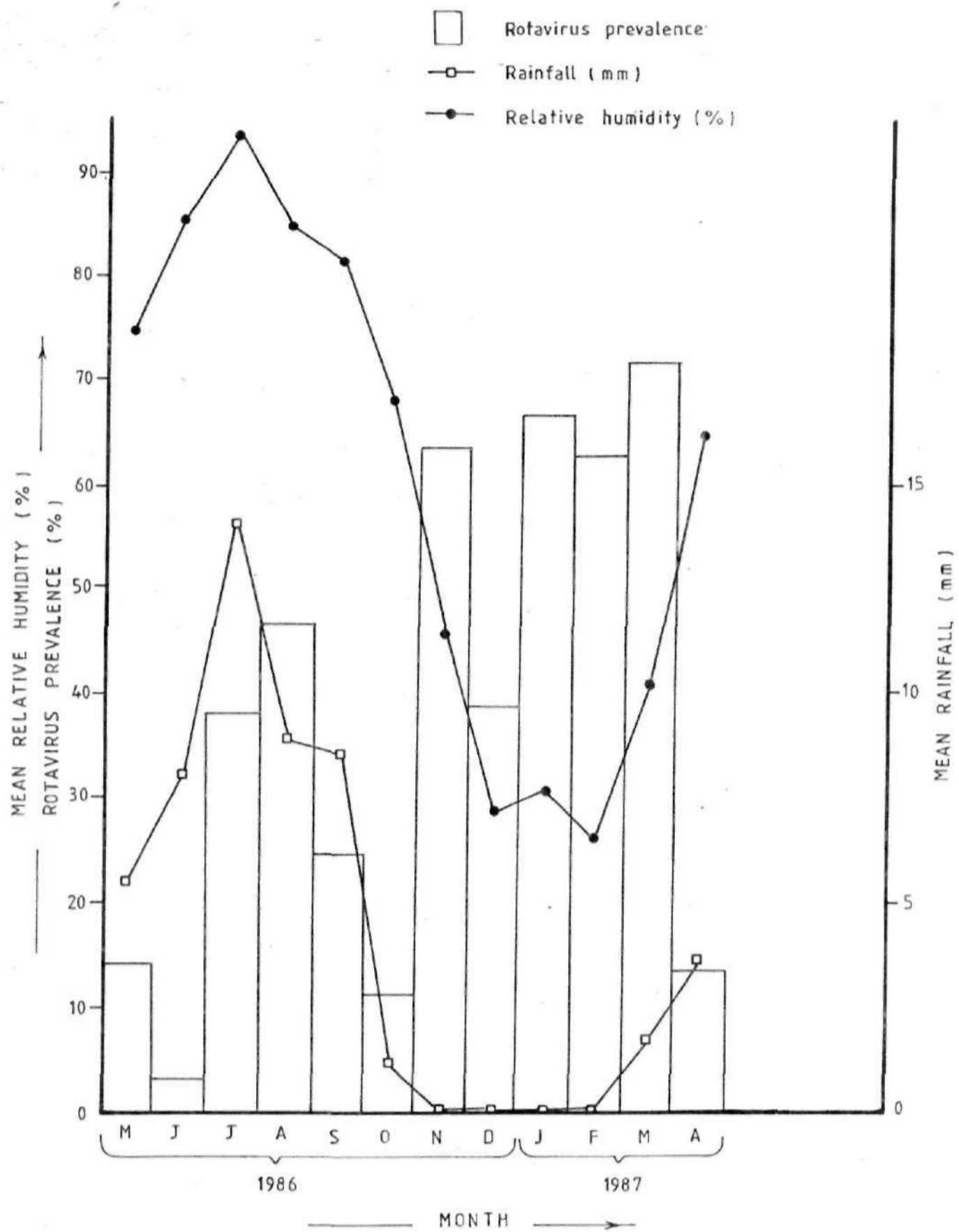


Fig. 1 Prevalence of rotavirus in children with acute gastro-enteritis in Jos from May 1986 to April 1987 in relation to relative humidity and rainfall.

Rotavirus was detected in stools of infants and children of all age groups (Figure 3). Age had a significant statistical influence on the prevalence of rotavirus ( $P < 0.05$ ). The highest percentage of rotavirus positives was found in infants 0-6 months (44.2%). With increase in age, rotavirus detection rate was found to decrease except in the age group 25-36 months. Children 0-6 months old had the highest risk of infection (odds ratio = 1.61).

Sex of the child had no significant influence on the frequency of rotavirus ( $P > 0.05$ ). Both sexes were equally affected with frequency being 35.1 % and 30.1 % in males and females respectively (Table 2).

The most significant risk factor for rotavirus infection was the type of feeding. The frequency of rotavirus infection in the breast-fed, formula-fed, pap-fed and adult food-fed infants and children is as shown in Table 3. Out of the 189 breast-fed diarrhoeic children tested, 80 (42.3 %) were positive for rotavirus. The frequency of rotavirus was 15.9% formula-fed, 25 % pap-fed, and 22 % in adult food-fed infants and children. Breast-fed infants and children had the highest risk of infection (odds ratio = 1.17).

Animals ownership (Table 4), presence of children less than two years of age (Table 5), source of water for home use (Table 6), excreta disposal (Table 8), socio-economic status (Table 9), type of community (urban and rural), Table 10 were not significantly associated with rotavirus infection ( $P > 0.05$ ).

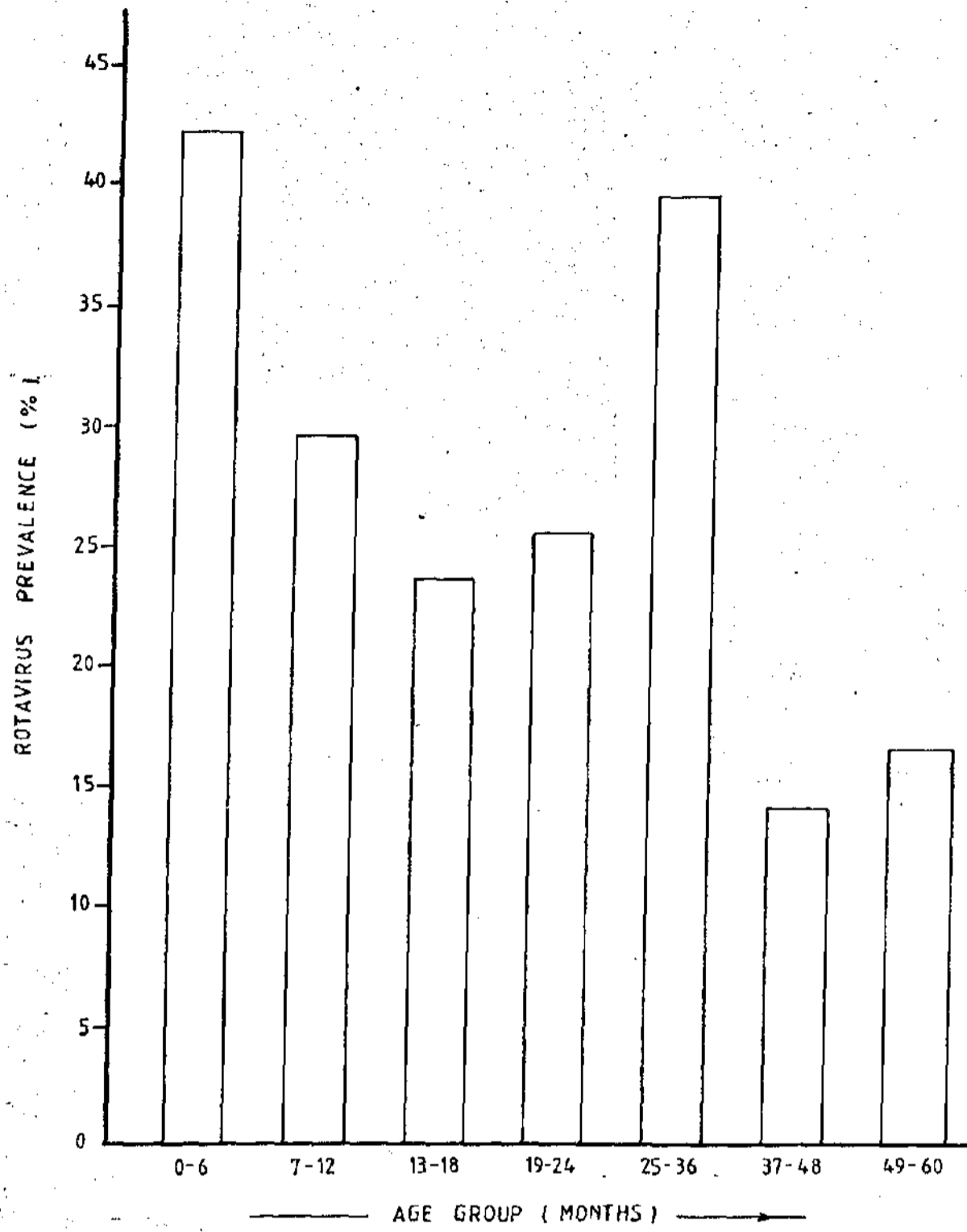


Fig. 3 Age distribution of the prevalence rates of rotavirus infection in children with acute gastroenteritis in Jos from May 1986 to April 1987.

Table 2 Age and sex distribution of rotavirus cases

Age (months)	Sex					
	Male			Female		
	No tested	No positive	% positive	No tested	No positive	% positive
0 - 6	76	38	50.0	62	23	37.1
7 - 12	90	27	30.0	68	20	29.4
13 - 18	38	9	23.7	21	5	23.8
19 - 24	23	8	34.8	16	2	22.5
25 - 26	19	8	42.1	9	3	33.9
37 - 60	13	1	7.7	7	2	28.6
	259	91	35.1	183	55	30.1

Table 3 Rotavirus infection in relation to type of feeding

Feeding	No. of stool samples tested	No. positive for rotavirus	% positive	Odds ratio
Breast	189	80	42.3	1.17
Breast, formula, Pap and Adult food	27	9	33.0	1.01
Breast & formula	73	22	30.1	0.87
Breast and Pap	62	18	29.0	0.83
Pap	4	1	25.0	0.68
Adult food	41	9	21.9	0.57
Formula	44	7	15.9	0.87

Table 4 Rotavirus infection in relation to animals in the household

Animals in the household	No. tested	No positive for rotavirus	% positive
Donkey	2	2	100.0
Cat and Dog	9	6	66.7
Chicken	12	5	41.7
Sheep	13	5	38.5
Any other (s)	33	11	33.3
No animals	258	84	32.6
Dog	64	20	31.3
Cat	45	12	26.7
Goat	5	1	20.0
Cattle	1	0	00.0

Table 5 Rotavirus infection in relation to children less than two years old in the household

Children less than 2 yrs old	No. tested	No. positive for rotavirus	Percentage positive
Not stated	7	1	14.3
Children present	138	45	32.6
No. of children absent	297	100	33.7

Table 6 The prevalence of rotavirus in infants and young children with and without previous therapy

Therapy	No. tested	No. positive for rotavirus	Percentage Positive
Therapy given	184	60	32.6
No therapy	258	86	33.3

Table 7 Rotavirus infection in relation to source of water for home use

Source of water	No. tested	No. positive for rotavirus	Percentage Positive
Stream	6	3	50.0
Tap	335	112	33.4
Well	98	30	30.6
Not stated	3	1	33.3

Table 8 Rotavirus infection in relation to excreta disposal

Excreta disposal	No tested	No positive for rotavirus	Percentage Positive
Fit latrine	357	121	33.9
Water system	42	13	31.7
Flush	89	11	28.2
Not stated	5	1	25.0

Table 9 Socio-economic status and rotavirus infection

Socio-economic status	No tested	No positive for rotavirus	Percentage Positive
High	33	7	21.2
Low	409	139	34.0

Table 10 Type of community and rotavirus infection

Type of community	No. tested	No. positive for rotavirus	Percentage Positive
Not stated	1	0.00	0.00
Urban	354	116	32.7
Rural	87	30	34.5

The frequency of detection of rotavirus was highest in the Child Welfare Clinic (Table 11). However in general, there was no statistical significant differences between the various locations of sample collection ( $P > 0.05$ ).

Table 12 shows the major symptoms exhibited by the diarrhoeic children. The clinical syndrome was found to consist basically of vomiting, fever and respiratory problems.

Table 11 Rotavirus distribution pattern in different locations (Clinics/Hospitals) in Jos

Location	No. tested	No. positive for rotavirus	Percentage Positive
Vom Christain Hospital	11	2	18.2
Jankwano Hospital	31	6	19.4
Jos Univ. Teach. Hospital	9	2	22.2
Nassarawa Clinic	102	30	29.4
Maternity Hospital	191	65	34.0
Iudunwada Clinic	57	22	38.6
Child-welfare clinic	41	19	46.3

Table 12 Major symptoms exhibited by the diarrhoeic children

Major symptom	No tested	No positive for rotavirus	Percentage Positive
Diarrhoea and respiratory	25	5	20.0
Diarrhoea only	67	20	29.9
Diarrhoea and vomiting	86	27	31.4
Diarrhoea and fever	89	29	32.6
Diarrhoea, fever, vomiting and respiratory	43	15	34.9
Diarrhoea, vomiting, fever	92	34	37.0
Diarrhoea, vomiting, resp.	15	6	40.0
Diarrhoea, fever, respiratory	25	10	40.0

## CHAPTER 5

DISCUSSION

The prevalence of 33 per cent in this study shows that rotavirus is a pathogen involved in acute gastro-enteritis in children 0-5 years old in Jos. Already rotavirus has been established as a cause of acute diarrhoea in man and animals in many parts of the world (McNulty, 1978; Rowland *et al.*, 1980; Dossetor *et al.*, 1979; Davidson *et al.*, 1975; Stintzing *et al.*, 1981; Samantary *et al.*, 1982; Fagbami *et al.*, 1987, Personal communication).

This finding is comparable to those obtained in other areas; 27.8 per cent in Addis Ababa (Stintzing *et al.*, 1981), 38 per cent in Michigan (Gurwith *et al.*, 1981), 38 per cent in Yogyakarta, Indonesia (Soenarto *et al.*, 1981), 40 per cent in Rhodesia (Cruikshank and Zilberg, 1976), and 41.8 per cent in Porto Alegre, Brazil during a 12 month period of study (Jose *et al.*, 1983). In contrast, much higher prevalence of rotavirus infection has been reported in some parts; 51 per cent in an Indian Reservation (Engleberg *et al.*, 1982), 61 per cent in Zaria, northern Nigeria (Dossetor *et al.*, 1979) and 70 per cent in southern India town (Paniker *et al.*, 1983). Lower prevalence of 13.8 per cent in Ife, Nigeria (Paul and Erinle, 1982), 17.6 per cent in Bangui, Central African Republic (Georges *et al.*, 1984) and 22.4 per cent in Calcutta, India (Saha *et al.*, 1984)

have also been reported. In general, a variable rotavirus detection rate of 7-70 per cent has been reported (WHO, 1980).

The monthly prevalence of rotavirus varied significantly during the twelve month period of study. Rotavirus was found to occur throughout the year with higher prevalence during the dry than the rainy season. Two peaks of rotavirus activity (July to August) and (November to March) corresponding to rainy and dry seasons were observed. Generally, the average mean temperature varied very little while a significant variation in the mean relative humidity occurred throughout the year in Jos. During the dry season in Jos, the mean relative humidity and temperature are usually low. The results obtained suggest that a seasonal variation in rotavirus activity occurred and also indicate that alterations in relative humidity may influence the survival of rotavirus more than temperature in Jos, Nigeria. The two peaks observed in this study, occurred at the two extremes (high and low) values of relative humidity. The rainy season peak (July to August) may be due to the high relative humidity observed during these months (93.7 per cent and 84.9 per cent). This is similar to the finding of Dossetor *et al.*, (1979) who reported an incidence of 61 per cent in Zaria, northern Nigeria during the last months of the rainy season (September to October). During this period, the mean relative humidity was as high as 70 per cent for each of the two months. The dry season peak (November to March) which was higher than the

rainy season peak, corresponded to low relative humidity (25.6 - 45.5 per cent). This finding is in agreement with similar surveys conducted in Nigeria. Paul and Erinle (1982) in Ife, Nigeria showed higher prevalence of rotavirus during the dry season when relative humidity was low. Fagbami *et al.*, (1987, Personal Communication), showed a significant higher incidence of neonatal rotavirus infection during the dry season (9 per cent) than the rainy season (2 per cent) in Ibadan, south-west of Nigeria. However, this results seem not to agree with that of a pilot study carried out in Zaria during the dry season, in the months of February and March which showed a low prevalence of 18 per cent (Gomwalk *et al.*, 1984). Low relative humidity seems a more important climatic factor than the high relative humidity in influencing the occurrence of rotavirus in Jos. Relative humidity has been shown in a study carried out in Birmingham, Britain to have an appreciable effect on the survival of rotaviruses. Rotavirus in faeces survived best at two extremes of relative humidity rapidly loosing infectivity at intermediate ranges where relative humidity is variable during the year. The incidence of rotavirus infection tends to increase with environmental relative humidity (Al-Nakib *et al.*, 1980).

Recent observations in different settings have revealed the importance of climatic factors in the epidemiology of rotavirus infection (Kapikian *et al.*, 1976; Paul and Erinle, 1982; Konno *et al.*, 1983; Makino *et al.*, 1983).



This could have resulted in the variation of the number of positives obtained.

Rotavirus was found affecting mostly the 0-6 months age group infants, with increase in age, prevalence was found to decrease except in the age group 25-36 months. This finding parallel similar results obtained by some workers. Paul and Paul (1986) in Nigeria showed that babies less than one year, were more affected by diarrhoea caused by rotavirus and that infection was most prevalent in the 2-6 months age group. Georges *et al.*, (1984) found that rotavirus was most frequent among children less than one year old. He also showed that this incidence decreased in children more than one year of age, and rotavirus was not identified in children older than 5 years old. The increase in the prevalence observed in the 25-36 months age group children may be due to the small number of samples that were available in this age group.

Reports from other communities are different. Infants less than six months of age have not been as commonly affected by rotaviruses as young children. Rotavirus has been detected among infants and children of all age groups with higher incidence (54 per cent) in the 6 months to one year age group (Georges *et al.*, 1984). Rotavirus is less common in the first six months of life or after 2 years but affects most commonly the 6-24 months age children (Mahalanabis, 1980; Soenarto *et al.*, 1981). The 0-6 months age group children are most commonly affected probably this indicates

early exposure to the virus as a result of cross-infection in hospital nurseries. Several nosocomial epidemics involving neonatal nurseries have been reported (Potterdell *et al.*, 1980). An increase in the resistance to rotavirus associated disease has been shown to occur with age in animals but variable (Bridger *et al.*, 1980).

Rotavirus infection was more common in infants who were breast-fed than those who were not. It has been shown that breast-milk contains some antibodies that may effect protection of the neonates against various infections during the first few months of life; however this study does not support a protective role for breast-feeding. This finding agrees with the finding of Gurwith *et al.*, (1981) who showed that rotavirus infection was as common in infants who were breast-fed as in those who were not. It contrasts the finding of Mcleans and Holmes, (1981) who showed a significantly lower incidence of rotavirus in breast-fed than bottle-fed neonates and trypsin inhibitors in human colostrum and milk might effect protection of neonates against rotavirus infection in the first five days of life. However the likelihood of rotavirus infection in infants receiving milk containing low levels of anti-rotaviral IgA combined with low trypsin inhibitory content is similar to that of bottle-fed infants. Infants receiving high levels of IgA and trypsin inhibitory content have substantially reduced infection rate (Mcleans and Holmes, 1981).

Presence of children less than 2 years of age was not



proportion of children infected with rotavirus bore no relationship to socio-economic status of the family. Maiya *et al.*, (1977) reported a different finding which showed an increased incidence of rotavirus infection of low socio-economic status.

The major symptoms exhibited by the diarrhoeic children include vomiting, fever and respiratory. Similar observations have been made by Engleberg *et al.*, (1982) and Gurwith *et al.*, (1981). The involvement of respiratory signs suggests a synergistic effect between rotavirus and respiratory viruses. Multiple infections with a variety of potentially pathogenic micro-organisms are more common than infections with a single micro-organism (Bridger, 1980). McNulty (1978) has reported clinical signs which may include depression, anorexia, vomiting, diarrhoea, dehydration and death.

The location of sample collection showed no significant influence on rotavirus infection. Even though some of the clinics/hospitals were located in rural areas, rotavirus infection has been shown to be universal despite the sanitary conditions from one part of the world to another (Engleberg *et al.*, 1982). Fagbami *et al.*, (1987, Personal Communication) reported a significantly higher overall incidence of neonatal rotavirus infection in the rural community (21 per cent) than in the urban (4 per cent) which contrasts with this observation. It appears that the survival of rotavirus is favoured more by climatic factors such as low relative

humidity rather than non-sanitary conditions.

The findings of this study suggests an apparent seasonal variation in the occurrence of rotavirus in Jos, Nigeria with low relative humidity being the most important influential climatic factor. More long term surveys of this kind are important to clarify and resolve the seemly apparent discrepancy in the tropics.





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ROLE OF ROTAVIRUS DIARRHOEA IN CHILDREN, JOS, NIGERIAQuestionnaire

- 1 Sample Number \_\_\_\_\_ Date \_\_\_\_\_
- 2 Hospital Number \_\_\_\_\_
- 3 Name \_\_\_\_\_
- 4 Age \_\_\_\_\_ Sex \_\_\_\_\_ Weight \_\_\_\_\_
- 5 Source of water for home use:  
Well \_\_\_\_\_ Tap \_\_\_\_\_ Others (Specify) \_\_\_\_\_
- 6 Feeding:  
Breast \_\_\_\_\_ Formula \_\_\_\_\_ Others (Specify) \_\_\_\_\_
- 7 Disposal of Excreta:  
Flush toilet \_\_\_\_\_ Pit-latrine \_\_\_\_\_ Bush \_\_\_\_\_
- 8 Major symptom \_\_\_\_\_  
Vomitting \_\_\_\_\_  
Diarrhoea \_\_\_\_\_  
Fever \_\_\_\_\_  
Respiratory \_\_\_\_\_
- 9 Previous diarrhoea therapy:  
Yes \_\_\_\_\_ No \_\_\_\_\_
- 10 Presence of \_\_\_\_\_ children less than two years of age:  
Yes \_\_\_\_\_ No \_\_\_\_\_
- 11 Animals in Household:  
Dog \_\_\_\_\_ Horse \_\_\_\_\_  
Cat \_\_\_\_\_ Donkey \_\_\_\_\_  
Goat \_\_\_\_\_ Cattle \_\_\_\_\_  
Sheep \_\_\_\_\_ Others (Specify) \_\_\_\_\_
- 12 Type of Community:  
Rural \_\_\_\_\_ Urban \_\_\_\_\_
- 13 Socio-economic Status:  
High \_\_\_\_\_ Low \_\_\_\_\_