PREVALENCE AND EPIDEMIOLOGY OF ROTAVIRUS INFECTION IN INFANTILE DIARRHOEA AT THE DIARRHOEAL TRAINING UNIT OF THE UNIVERSITY TEACHING HOSPITAL OF LUSAKA, ZAMBIA.

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BY

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

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SUMMARY

In the present study undertaken at the Diarrhoeal Training Unit of the University Teaching Hospital of Lusaka (Zambia), Rotavirus was isolated in 29 out of 152 infants (19.08%) with acute diarrhoea and in 1 out of 78 (1.28%) infants without diarrhoea. Rotavirus detection rate was related to humidity, but not to ambient temperature, and was independent of sex. The highest incidence of rotavirus infection occured in the age group 7-12 months. No clinical features could distinguish infants with rotavirus diarrhoea from those with non-rotavirus diarrhoea. Biochemical and haematological profiles of the two groups of patients were not significantly different. No high-risk factor seemed to predispose infants to human rotavirus infection, but it occured predominantly in well nourished infants.

INTRODUCTION

Diarrhoeal disease remains a worldwide source of deep concern as far as child morbidity and mortality is concerned. It accounts for about 4.0 millions of annual deaths of children under five years, that is 28% of all major causes of death[1]. In Zambia, diarrhoeal disease is among the Top Ten causes of admissions and deaths in hospital[2]. For the year 1988 alone, 9870 children under 1 year and 10,415 children aged 1-14 years were admitted in hospital suffering from diarrhoeal disease. Of these, 863 (8.7%) children under 1 year and 971 (9.3%) children aged 1-14 years respectively died. For infants under 1 year, diarrhoeal disease as a cause of death comes second only to Malaria (13.2%) and for children aged 1 to 14 years, it comes third after Acute Respiratory Infection (ARI) and Malaria (13%). In the Department of Paediatrics at the University Teaching Hospital of Lusaka, diarrhoeal disease accounted for 15% of the total number of admissions and 12% of deaths for the year 1990(3) Figure 1 shows the number of admissions and deaths at the diarrhoeal training unit from November 1989 to March 1991. This reflects the magnitude of diarrhoeal disease as a health problem in Zambia.

In a number of hospital-based studies carried out in infants and young children in developed and developing countries, Rotavirus has been detected in approximately 50% of diarrhoea cases, sometimes with seasonal variation[4]. In the early 1980's, ROTAVIRUS was accepted as the single most important cause of childhood gastro-enteritis (5,6,7).

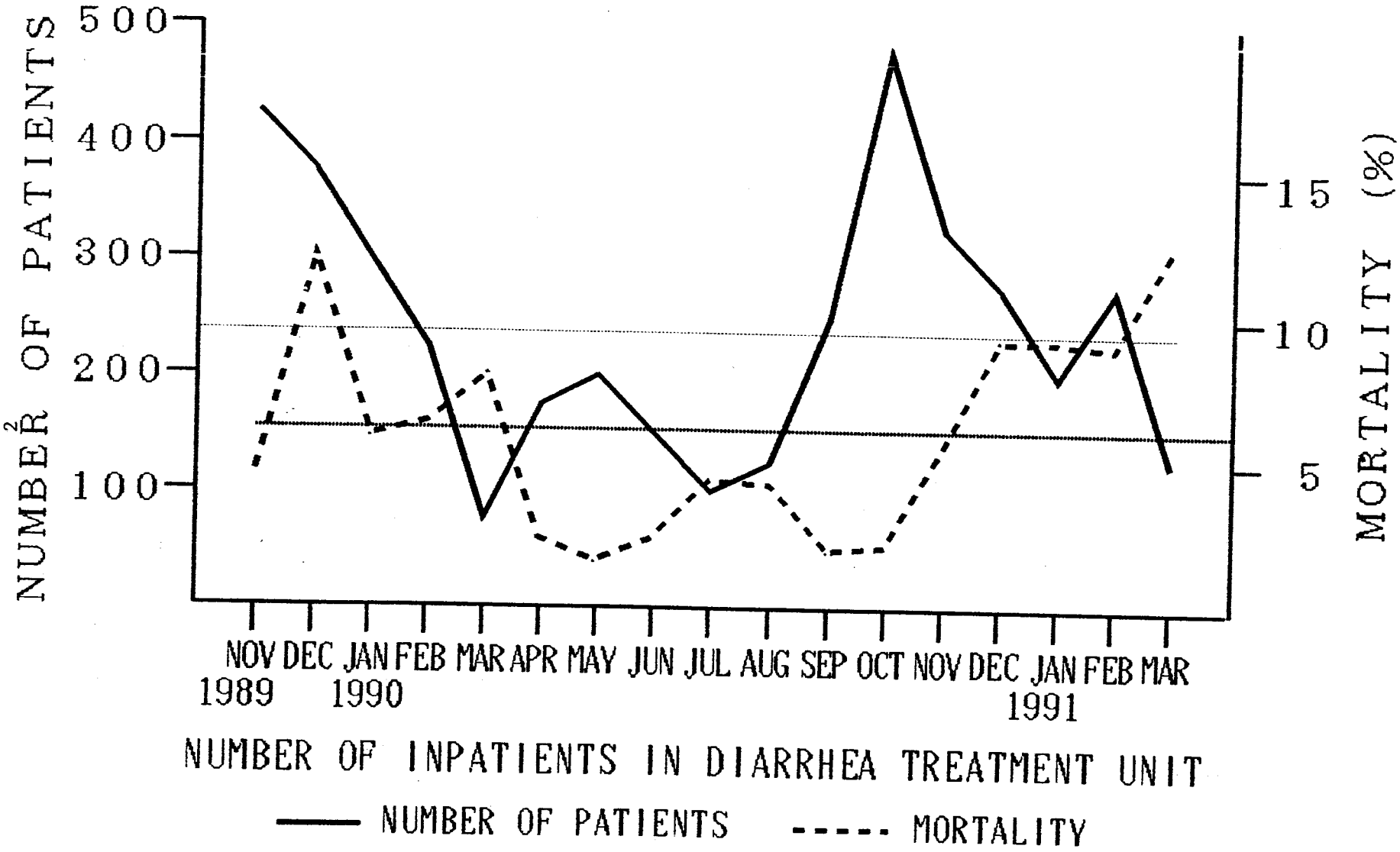

NUMBER OF PATIENTS
500
400
300
200
100
MORTALITY (%)
15
10
5
NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR
1989
1990
1991
NUMBER OF INPATIENTS IN DIARRHEA TREATMENT UNIT
NUMBER OF PATIENTS
MORTALITY

Figure 1

In 1980, the WORLD HEALTH ORGANISATION (WHO) scientific working group on Rotavirus and other viral diarrhoea made some recommendations for research[4]. Among other things, they resolved that:

1. Studies were neeeded to define more completely the morbidity and mortality attributable to rotavirus diarrhoea in different geographic areas. Any factors, either host or environmental, that affected the severity of the disease needed to be identified.
2. The epidemiological characteristics of Rotavirus diarrhoea in different geographical areas also needed elucidation. Studies should seek to define, among other things, the natural history of the disease and the relationship between nutritional status and the incidence of disease.
3. Studies should be carried out to determine the influence of breast-feeding on the natural history of Rotavirus infection. Epidemiological, immunological and social factors should be investigated.

A considerable work has already been done in some countries of East, Central and Southern Africa towards the improvement of our knowledge of Rotavirus infection in terms of its incidence, seasonality, diagnosis and prevention [9-10]. Since the W.H.O. scientific group issued these recomendations, only one study has been done in Zambia that included the Rotavirus[8]. This was a review of the etiologies of diarrhoea in children under five years at the Tropical Diseases Research Centre in Ndola.

The aim of the present study is to survey the prevalence and the epidemiology of rotavirus infection in infantile diarrhoea at the University Teaching Hospital of Lusaka.

I hope that the present study will be the first one in a series of many more that will help us confront the problems of infantile diarrhoea with much success. Knowledge gained from these studies will then be used in our ultimate goal which is the reduction of child morbidity and mortality due to diarrhoeal diseases.

At a time when the World Health Organisation/Control of Diarrhoeal Diseases (WHO/CDD) Programme is encouraging the search for a safe and efficacious vaccine against rotavirus[19], it is our task to assess the magnitude of rotavirus infection in our area and identify the most prevalent serotypes in order to adequately use any potential rotavirus vaccine that is likely to appear on the market in the near future.

CHAPTER ONE

THE VIRUS

1. 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). The Human Rotavirus (HRV) is spherical and 70nm in diameter (Figure 2). It possesses a double-layer of icosahedral protein shells with a core of double-stranded ribonucleic acid (ds RNA)[6,20].

TABLE 1: CLASSIFICATION OF THE FAMILY REOVIRIDAE (5)

GENUS	HOST	REPRESENTATIVE DISEASE
Reovirus	Vertebrates	Colorado Tick Fever
Orbivirus	Vertebrates Insects	Colorado Tick Fever
Rotavirus	Mammals	Gastro-enteritis
Phytoreovirus	Plants	Rice Dwarf
Fijivirus	Plants	Maize Rough Dwarf
Cytoplasmic Polyhedrosis Virus	Insects	Polyhedrosis

In a natural infection, both single-shelled and double-shelled forms of particles are observed in faeces[6]. The surface of the complete virus particle is composed of 32 capsomeres that radiate from a central core[6].

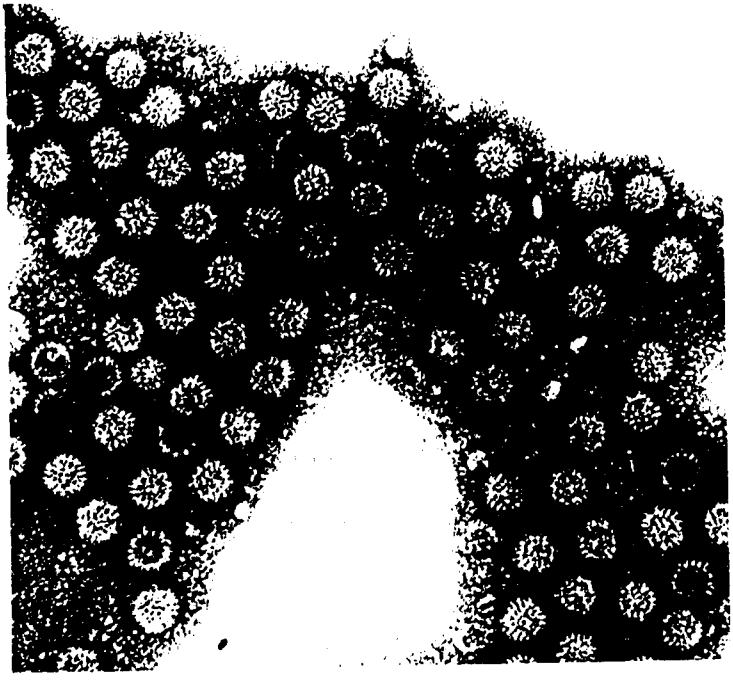


Figure 2:

Rotavirus particles in human stool. Electron micrograph by Dr. J. Mwansa, used with permission. (Original magnification x 150,000)

The name of the virus is derived from the latin "Rota", meaning wheel, because the circular outer capsid resembles the rim of a wheel connected to short spokes that radiate from a wide hub [6, 21].

Rotaviruses contain a ds RNA genome consisting of 11 segments[6,20]. These 11 segments can be separated by polyacrylamide gel electrophoresis (PAGE)[20].

Different rotavirus isolates frequently exhibit differences in the electrophoretic mobilities of their 11 segments; rotaviruses exhibiting these different electrophoretic mobilities have been termed electrophoretypes [20].

These are excellent markers for identifying and following the spread of viruses from one individual to another in discrete outbreaks; thus, they are good for providing epidemiological information [20].

Rotaviruses can be classified by four main categories: group, subgroup, serotype and strain [6,20].

(i) GROUP SPECIFICITY:

The designation group A, B, C, etc... analogous to influenza virus terminology was used for this purpose.

(a) Group A:

Original, conventional rotaviruses with the common group antigen.

(b) Group B and C:

Atypical rotaviruses possessing other group antigens and genetically different from the 11 gene segments and one-dimensional terminal finger-printing analysis of the RNA segment.

(c) Group D and E:

These are a typical porcine and chicken rotaviruses.

(ii) SEROTYPE SPECIFICITY:

At least 4 human serotypes of rotavirus have been identified: 1, 2, 3 and 4. A possible 5th serotype from Indonesia and a 6th one from the U.S.A have recently been described.

(iii) SUB-GROUP SPECIFICITY:

(a) Sub-Group I:

Includes HRV of the serotype 2.

(b) Sub-Group II:

Includes HRV of the serotypes 1, 3 and 4.

(iv) STRAIN SPECIFICITY:

Several strains have been identified by ELISA and neutralisation techniques, i.e. DS-1, Wa, 69M, W 161 ... The functional relationship between strains remains uncertain.

Rotavirus is structurally stable after prolonged storage at -20^{0}C and after exposure to heat (56^{0}C), ether and mild acids; the virus particle, however, is unstable with acid treatment at a PH of less than 3 (5,6).

2. EPIDEMIOLOGY

Rotaviruses were first discovered in humans 17 years ago by Bishop et al (22,23) by the examination of duodenal biopsy of a group of Australian children hospitalised with non-bacterial gastro-enteritis. Infection occurs worldwide in both sporadic and epidemic forms[6]. 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 climates (24-30). Cases occur year-round in tropical areas (5,31). However no distinct seasonal variation occured in reports from some tropical countries including Ecuador (32), Venezuela (33) and South Africa (34), whilst rotavirus infection tended to peak during the dry season in Nigeria (35), Southern India (36), Bangladesh (37), Indonesia (38) and Costa Rica (26). 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 (20). Transmission of rotavirus is from person to person by the faecal-oral route with an incubation of one to three days (20,25,39). 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 (40).

Infection within families is not uncommon, with both adults and siblings becoming infected (41,42). Nosocomial infection has also been described (43,44). Asymptomatic rotavirus infection and viral carriage occur frequently and have been studied by several investigators (45,46,47). Rotavirus is also prevalent in day-care centres and can be spread to family contacts, thus propagating the infection in the community (48). Many day-care children are asymptomatic, indicating large reservoir of infection (20).

INFECTION IN NEONATES

It is likely that 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 old children (4,5,9)

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 (5).

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 (50).

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 the other serotype (20).

3. PATHOGENESIS

Rotavirus tend to infect the small intestine, as do other Gastro-enteritis viruses (20). In particular, rotavirus replication takes place in epithelial cells on the tips of villi of the small intestine, and infection is confined primarily to these cells (51).
Davidson and Barry found mucosal damage to be quite variable and often patchy (52).

Mucosal changes range from mild to severe; these changes include shortening and blunting of villi, and increased infiltration of the lamina propria with mononuclear cells (22). The destruction of mature enterocytes results in reduced level of disaccharidases and a decrease in the absorptive surface of the small bowel (5).

Most, but not all children, with acute rotavirus gastro-enteritis have lactose malabsorption and intolerance (53). Loss of fluids and electrolytes in rotavirus gastro-enteritis can lead to severe dehydration and even death; it requires fluid and electrolytes replacement therapy. In developing countries, recurrent bouts of gastro-enteritis can lead to a vicious cycle of protracted diarrhoea, food intolerance and malnutrition (54).

a

4. IMMUNOLOGY

Table 2 and 3 show the serum and intestinal responses to rotavirus infection.

TABLE 2: SERUM ROTAVIRUS IMMUNOGLOBULIN RESPONSE TO INFECTIONS(20).

TYPE OF ANTIBODY	TIME ANTIBODY FIRST DETECTED	LENGTH OF TIME ANTI-BODY PERSISTED
Neutralizing antibody	During convalescence	Protection for ≤1 year.
Neutralizing antibody	During convalescence	not given.
IgM	In acute, phase elevated	Decreased in convalescence
IgG	In convalescence, elevated	Detectable at least 6 to 12 mo.
IgA	Not given	≤12 mo.
IgA	Within 1st 2 weeks.	≥6 mo.
ScIg	Within 1-2wk	≤4 mo.
sIgA	4 to 10 days	4 to 10 days

TABLE 3: INTESTINAL ROTAVIRUS IMMUNOGLOBULIN RESPONSE TO INFECTION (20).

Location of antibody	Type of antibody	Time antibody first detected	Length of time antibody persisted
Duodenal fluid	ScIg	Within 1wk; level similar to that in serum.	not given
Feces	IgA	not given	≥6 mo
Feces	ScIg	not given	≤6 mo.
Feces	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.
Feces	IgA, IgM IgG	Low levels at onset; increased by 1 to 2 wk.	Peaked at 3 to 5 wk; lasted ≤2 mo.
Feces	IgA	Maximum level by 7 days	Not given
Feces	IgA	Detected by 9 days declined.	Peaked at 2 to 6 wk; then
Feces	IgA	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 4: ROTAVIRUS IMMUNO-GLOBULIN IN NORMAL MOTHERS AND NEWBORNS (20).

Location of antibody	Type of antibody	Time antibody first detected	Length of time antibody persisted
Colostrum/ milk	IgG, IgM	Not given	Dropped off by 3 to 5 days post-partum.
Colostrum/ milk	IgA, ScIg	Not given	Dropped off by 3 to 4 days post-partum to steady low level.
Feces of breast-fed infants	IgA	At 2 days	Not given
Colostrum/ colostrum/ milk	IgA, ScIg	not given	Declined from 3 to 4 days post-partum to 2wk later then remained unchanged.
Infant duodenal fluid	IgA, ScIg	At 3 to 4 days	At least 2 weeks
Colostrum/ milk	ScIg	Not given	Fell to low but detectable levels 1 to 2 wk post-partum 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 body fluids, these levels were not correlated with their possible role in the protection against, or modification of, rotavirus infection and disease (20).

In neonates and infants, two routes of passively transferred rotavirus antibody have been identified, serum and colstrum-milk. However, serum antibodies have doubtful protective capacity (55). Rotavirus specific IgA and ScIg can survive proteolysis in the gut. Thus, frequent breast meals have a possible protective effect (20).

Resistance to rotavirus disease is most clearly associated with the level of type specific rotavirus intestinal antibody (6).

5. CLINICAL FEATURES

The incubation period of rotavirus infection usually extends from 24 to 72 hours (4, 25, 39). The two most prominent features are vomiting and diarrhoea, usually of sudden onset (20). The vomiting may precede the diarrhoea in many cases (4, 5, 20). The diarrhoea is watery; mucus is found in the stool in up to 25% of cases, but blood is rare (4). Fever occured in about 30-50% of cases in some studies and in 60-100% of patients in other studies (4,5).

There have been conflicting reports on the role of rotavirus in causing respiratory infection and symptoms (20). Some studies suggest that rotaviruses may sometimes cause respiratory symptoms (56,57,58), but several groups have failed to show any significant role of rotavirus in respiratory infection (50,60,61). The fever and vomiting resolve in the first day or two, the diarrhoea may last up to eight days (5,6).

Mild to moderate dehydration is frequently seen in HRV patients (5). The dehydration is of isotonic type (62). In the more severe cases seen at treatment centres, severe dehydration and electrolyte imbalance have been observed (4). Most hospitalized children recover within a week of admission (39,62,63). Death due to rotavirus, albeit rare, has occured in infants and young children, and is usually due to dehydration and electrolyte imbalance (64).

6. DIAGNOSIS

6.1 Cultivation:

Culture of human rotaviruses is usually not carried out in diagnostic laboratories since the virus is found in large quantities in stool specimens and can be rapidly detected by antigen detection tests (20). However, some research laboratories have cultivated rotaviruses by using various manipulations e.g. culture in African

green monkey kidney cells, fetal rhesus monkey kidney cells and human embryonic fibroblasts (65,66).

6.2 Electron Microscopy (E.M):

Initially, rotaviruses were detected directly in stool sample by the E.M. of virus particles negatively stained with photofungistic acid, and this method is still used as the standard (30,67).

6.3 Enzyme Immuno-Assays (EIA's and Latex Agglutination Tests (LAT).

Since EM procedures are time-consuming to perform for a large number of samples, other testing procedures were developed to detect rotavirus or rotaviral antigens. The more universally used tests today are the LAT and the EIAs (20). In the present study, the WELLCOME ROTAVIRUS LAT was used and it will be discussed in detail below.

6.4 Other Dectection Methods:

These tests are primarily used as research tools. They are not commercially available at this time, and there is probably no great demand for them since their primary use is not as diagnostic tests and they do not influence management anyway (20).

a) Antigen Dectection:

- Counterimmunoelectrophoresis (CIE) and Complement Fixation Test (CF). These have been abandoned at present due to low sensitivity (20).
- Radioimmunoassay (RIA) is a sensitive test, but requires expensive equipment (20).
- Polyacrylamide Gel Electrophoresis (PAGE) is very useful for epidemiological studies.

Note: Other techniques for detecting rotaviruses in stool samples are being developed.

b) Antibody detection:

Various methods have been used. These include:

- Indirect fluorescent antibody test
- Hemagglutination inhibition
- Immune adherence hemagglutination
- Neutralization: This test has been extensively used for serotyping rotaviral isolates (20).

7. PREVENTION:

7.1 Vaccines

It has been estimated that an effective rotavirus vaccine could:

a) Reduce all diarrhoeal deaths by 30% in the age group 6-24 months.

b) Avert 500,000 - 1,000,000 deaths in children annually (71).

Potential Rotavirus Vaccines:

1. Bovine Rotavirus Vaccines:

a) RIT 4237: it has been withdrawn due to lack of efficacy (71).

b) WC3: it is immunogenic against serotypes 1 (strain Wa) and 3 (strain SA 11).

It is still under evaluation in the Central African Republic and Israel (71,72).

2. Rhesus Rotavirus Vaccine (RRV):

MMU - 18006: It is very immunogenic against HRV serotype 3 only and causes a high reactogenicity.

3. Bovine-Human and Rhesus-Human Reassortant Rotavirus Vaccines:

These combined, multivalent vaccine may result in poorer responses to the individual serotypes.

Better immune responses may be achieved by giving multiple doses or by increasing the amount of virus in each dose. These vaccines are being tested in Peru and the U.S.A. (71,72).

4. Nursery strain vaccines:

These are naturally attenuated HRV. The principle of these vaccines was based on the observation that "Nursery Strains" frequently infect Neonates in maternity wards without causing illness, and the infected infants are later protected, at least in part, against diarrhoea caused by fully virulent rotaviruses (71).

M 37 is currently being tested for safety and immunogenicity in volunteers.

The search continues for a vaccine that could:

(i) induce substantial long-lasting protection against rotavirus diarrhoea in young children following a single oral dose.

(ii) be administered at the age of 2 to 3 months possibly in combination with oral poliovirus vaccine (71).

7.2 Chemical Disinfection:

A group of investigators evaluated 27 disinfectants for their ability to inactivate rotavirus on inanimate objects (68). Only 9 out of the 27

disinfectants used reduced the rotavirus titer by 6 $\log_{10}$ while the others were ineffective. These nine disinfectants are used for specific purposes in a variety of products for home, hospital and food service use. They include: Hydrochloric Acid, Peracetic Acid, Isopropyl Alcohol, Chlorhexidine Gluconate, Glutaraldehyde, Chloramine-T, Povidone-Iodine Complex, Sodium O-Benzyl-P-Chlorophenate and a Quaternary Ammonium Compound.

Since rotavirus was resistant to a wide range of commonly used chemical disinfectants, it is important to use those chemicals that are effective to prevent and control outbreaks of rotavirus diseases (20).

8. TREATMENT

There is no specific antiviral therapy available for HRV infection (5,6). Thus, the primary purpose of therapy is to provide adequate hydration, to maintain blood volume, electrolyte homeostasis and acid-base balance (69). Oral glucose electrolyte solutions have been used for the past 25 years in the treatment of dehydration due to acute infantile diarrhoea including rotavirus diarrhoea (20).

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

a

(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 (54,70).

In underdeveloped or developing countries where there are limited medical resources and where malnutrition is common, oral rehydration therapy and continued feeding have been advocated (54).

CHAPTER TWO

OBJECTIVES OF THE STUDY

1. MAIN OBJECTIVE:

The aim of the present study is to determine the prevalence of Rotavirus infection in infantile gastro-enteritis at the University Teaching Hospital of Lusaka.

2. SPECIFIC OBJECTIVES:

2.1 To assess the impact of the following epidemiological factors on the development and natural course of the disease:

- Age
- Sex
- Education status of the Mother/Guardian
- Breastfeeding
- Nutritional status
- Previous debilitating illness
- Sanitation.

2.2 To review some aspects of the symptomatology associated with rotavirus infection.

2.3 To look at the therapeutic attitudes before presentation at the U.T.H. i.e. home, private surgery and government peripheral clinic therapies.

2.4 To determine the haematological (haemoglobin, total leucocyte count, differential leucocyte count) and biochemical (blood urea, serum sodium and potassium) profiles of patients with HRV infection.

CHAPTER THREE

PATIENTS AND METHODS

1. STUDY SITE AND POPULATION

This study was undertaken at the Diarrhoea Training Unit (DTU) of the University Teaching Hospital (UTH) of Lusaka, the Capital city of the Republic of Zambia. According to the 1990 Census, the population of Lusaka is estimated at about 983.362 inhabitants. The U.T.H is the largest hospital in the country and serves as a referral hospital for patients not only from within the city but also from remote parts of the country.

Sick children who present at the U.T.H. are first seen by a Medical or Clinical Officer in the paediatric out-patient clinic (O.P.D) where a decision is made on whether or not a patient requires admission in hospital. Diarrhoeal patients who need admission are referred for further management to the DTU (WARD A06).

The DTU was opened on 31st October 1989 as part of the Wordl Health Organisation Control of Diarrhoeal Diseases (WHO/CDD) programme and its objective is to serve as a diarrhoeal diseases training centre for health workers in this African sub-region. The number of cases of diarrhoea admitted in the DTU during its first year of existence, from 31st October 1989 to 30th November 1990, was about 2,928 as per the DTU admission book, with an average of 8 patients per day.

The present study covered a period of about 5 months from October 1990 to March 1991. This period was relatively warm and humid (average temperature 22.7^{0}C a average degree of humidity 71.5%).

During the same period, 1,763 children with diarrhoea attended the paediatric out-patient clinic and 1,607 patients were admitted in the DTU.

2. STUDY TYPE:

The present study was cross-sectional.

3. SAMPLING TECHNIQUE:

A convenient, also called warm body or accidental sampling technique was chosen for the purpose of the present study. This means that I used the sample available at the time of selection. It is a well known fact that convenience samples are not representative of a given population and, as a result, there are some limitations as to the applicability of the study discussed.

This handicap was mainly imputable to the time factor. Indeed, the late arrival of the laboratory kits did not give me enough time for the recruitment of study subjects since one of my major concerns was to meet the deadline set for the submission of the present dissertation. In the same vein, I chose to use non-probability sampling because, taking into account the

prevalence rates of rotavirus diarrhoea found in other african studies (9-18), the number of admissions in the D.T.U. per year and assuming a confidence interval of 95% for example, a sample size of at least 500 patients would have been needed if I had used random procedures for selection of a probability sample to ensure the study unit was selected on the basis of chance. Here again, I was forced to use non-probability sampling in order to beat the factor time.

Patients:

Children aged one to thirty-six months referred for admission in the DTU from the OPD, with a diagnosis of acute diarrhoeal disease (ADD) were included in the study. The mother/guardian's definition of diarrhoea was used in the present study. This definition has been used before by other researchers (18).

Neonates were excluded from the study since meconium specimens and stools from neonates have not been sufficiently evaluated, and are not a recommended specimen for the rotavirus latex agglutination tests (LAT).

Stools were collected from children presenting with a history of diarrhoea of one to seven days duration. Collection of samples within this period increases the sensitivity of the LAT (greatest number of rotaviruses

in the stool) and samples collected eight days or more after the onset of symptoms may not contain enough detectable antigen (72).

Controls:

Seventy-eight children including patients admitted in the general paediatric wards with diagnoses other than ADD and some healthy siblings of patients admitted in the same general wards, all aged one to thirty-six months, were used as controls.

The present study not being a case control study, the term "Control" is in fact a misnomer. This group of subjects would rather be referred to as a "comparison group". Healthy sibling controls were included in the study to increase the number of study subjects in the comparison group. Collection of stool from the non-diarrhoeal patients was quite a problem considering the fact that most of the admitted children were constipated and that the manufacturers of the Rotavirus LAT Kit do not recommend samples obtained by rectal swabs or gloved finger. The healthy sibling controls were well babies who could not stay home without their mothers' care and were being nursed in hospital along with their sick siblings.

In the present study, the number of subjects in the comparison group was actually higher than in some of the african studies I came across (9-18).

4. DATA COLLECTION:

A prepared questionnaire was filled in after stool collection. The 8-page questionnaire, comprised about 25 questions related to the specific objectives of the present study (Appendix 1).

Clinical signs of dehydration (7) and nutritional status (Wellcome classification, Appendix 2) were assessed on admission as part of the general physical examination of the patient.

Associated symptoms and past major illnesses were broadly defined to help the interviewer collect the correct information from the mother or guardian.

Associated symptoms:

(i) Fever: A rise in temperature of $\geq 38^{0}C$ on admission or during the patient's stay in hospital.

(ii) ARI: History of cough, sneezing, rhinorrhea or difficulty in breathing of less than two weeks duration.

Major Illnesses in the past 3 months:

Mothers/Guardians were asked whether or not they had taken the child for medical care to either a local government clinic, a private surgery or hospital, and whether or not they were told the nature of the child's illness.

From the mother/guardian's explanation, the interviewer was able to classify the child's illness in a group such as heart disease, ARI, gastroenteritis(GE), anaemia, protein energy malnutrition(PEM), malaria, meningitis, P.T.B., measles and others. Where the mother/guardian did not know the nature of the child's illness, question 13 was marked as "Do not Know".

The following guidelines were used in order to extract information from the mother/guardian:

(i) ARI: See above under "Associated symptoms".

(ii) G.E.: Vomiting and diarrhoea.

(iii) Anemia: lack of blood.

(iv) P.E.M.: swelling of the body, hair and skin changes, loss of appetite, weight loss, high protein diet.

(v) Malaria: fever, chills that responded to anti-malarial drugs.

(vi) Heart disease: "hole" in the heart? "swollen" heart?

(vii) Meningitis: fever, neck stiffness or retraction, bulging fontanelle, excessive crying, refusal to suck, turbid CSF after lumbar puncture.

For diseases such as measles and PTB, no guidelines were required since these are well known in the community.

Outcome of the Disease:

Item 2 of question 28 refers to the transfer of the patient from the D.T.U to any other ward for any problem other than gastroenteritis, i.e. measles, pneumonia, nutrition rehabilitation etc.

5. LABORATORY PROCEDURES

5.1 Stool Investigations:

Stool samples collected in universal sterile containers were examined within 2 hours at the microbiology laboratory which is situated within the U.T.H., at about 500 meters from the DTU.

Stool microscopy for white blood cells, red blood cells, and parasites was done using the FORMOL-ETHER CONCENTRATION method. (Appendix 3). A modified ZIEHL-NIELSON method was used for cryptosporidium (Appendix 4).

Stool bacteriological cultures were done using the conventional methods for the isolation and identification of common enteropathogenic bacteria, i.e. Escherichia Coli, Shigella and Salmonella Species, Vibrio Cholerae (73).

Isolation of enterobacteria was done on Mac Conkey Agar Desoxycholate Citrate agar (DCA), Selenite Broth, Thiosulphate Citrate Bile Sucrose (TCBS) and in Alkalin Peptone Water from OXOID LTD U.K.

For the identification of organisms, Triple Sugar Iron Agar (TSI), Urea Agar and Indole were used as per flow charts in appendix 5.

Sensitivity patterns to antibiotics were tested on positive bacterial isolates using antibiotic sensitivit discs and diagnostic sensitivity test Agar from Mast Ltd. U.K.

The following antibiotics were included:
Co-Trimoxazole (25ug), Ampicillin (20ug), Cefotaxim (30ug), Gentamycin (10ug), Carbenicillin (100ug), Chloramphenicol (10ug), Tetracycline (10ug).

Stool samples were tested for occult blood using Hematest tablets from AMES LTD, U.K. (Appendix 6).

The rotavirus latex agglutination test (LAT) from Wellcome (England) was used for the isolation of Rotavirus antigens in stools (appendix 7).

13 stool specimens from diarrhoeal cases that tested negative for Rotavirus using the LAT and 60 stool samples from the control group were re-examined for rotavirus antigens using an ELISA test from CAMBRIDGE BIOSCIENCE, U.S.A (Appendix 8).

5.2 Blood Investigations

a) Biochemistry

Blood Urea was determined by Di-acetyl-monoxime (DAM) method on venous blood collected either in a lithium heparin treated container when available or in a plain container (Appendix 9). Serum sodium and potassium were estimated by flame-emission spectrometry (Appendix 10). Blood glucose was measured by the O-Toluidine method on venous blood collected in a potassium oxalate/sodium fluoride treated container (Appendix 11).

b) Haematology:

Venous blood was collected in a potassium EDTA container for haematological investigations. The haemoglobin was estimated using a modified Cyanmethaemoglobin method (Appendix 12).

The Sickling Test was done using the Metabisulphite Reduction method (Appendix 13). Malaria Parasites were looked for in thick smears stained by Field's method (Appendix 14).

The total white cell count was done by visual method (Appendix 15).

Thin blood smears were stained by the May-Grunwald-Giemsa method for differential white cell count (appendix 16).

6. STATISTICAL ANALYSIS

Data were analysed using the paired sample student's T-test (SPSS programme) and ANOVA (EPI info version 5) fo parametric and normally distributed data.

Kruskal-Wallis H test (EPI info version 5) and Chi-square test (Statistix) were used for non-parametric results.

CHAPTER FOUR

RESULTS

1. PREVALENCE OF ROTAVIRUS INFECTION AND OTHER ENTERIC PATHOGENS.

Rotavirus was isolated in 29 (19.08%) patients with diarrhoea and in 1 (1.28%) control (Table 5). The LAT produced a non-specific reaction in 4 diarrhoea cases. Centrifugation was repeated in the 4 cases in order to have a more clear supernatant, but non-specific reactions were again recorded. Due to the late arrival of the ELISA kit and limited storage facilities (63 stool specimens only were kept at - 20^0C), 13 of the diarrhoeal specimens and 50 control samples were re-examined for rotavirus using the ELISA test. All the 63 samples tested negative for rotavirus.

Due to financial constraints, antisera were not available for escherichia coli and salmonella species serotyping. For the same reasons, major enteric pathogens such as campylobacter jejuni and yersinia enterocolitica were not isolated.

Rotavirus was isolated in association with escherichia coli in 10 (34.48%) of the rotavirus positive cases. Escherichia coli was isolated in association with vibrio cholerae in 2 diarrhoea cases and in mixed infection with salmonella in 2 controls (Table 6). Vibrio cholerae were found to belong to the serotype ogawa.

TABLE 5: ENTERIC PATHOGENS ISOLATED IN 152 PATIENTS WITH DIARRHOEA AND 78 CONTROLS

Pathogens	Cases N	(%)	Controls N	(%)
Escherichia coli	77	(50.65)	72	(92.30)
Rotavirus	29	(19.08)	1	(1.28)
Cryptosporidium	3	(1.97)	1	(1.28)
Salmonella	2	(1.31)	2	(2.56)
Vibrio Cholerae	2	(1.31)	0	(0.00)
Ascaris Lumbricoides	0	(0.00)	1	(1.28)

TABLE 6: MIXED INFECTIONS IN 152 DIARRHOEA CASES AND 78 CONTROL

Pathogens	Case N	Controls N
E. Coli/Rotavirus	10	1
E. Coli/V. Cholerae	2	0
E. Coli/Salmonella	1	2
E. Coli/Cryptosporidium	1	1
E. Coli/A. Lumbricoides	0	1

No helminth was identified amongst the diarrhoea cases but ascaris lumbricoides ova were identified in one control.

No patient was found to have a positive slide for malaria parasites.

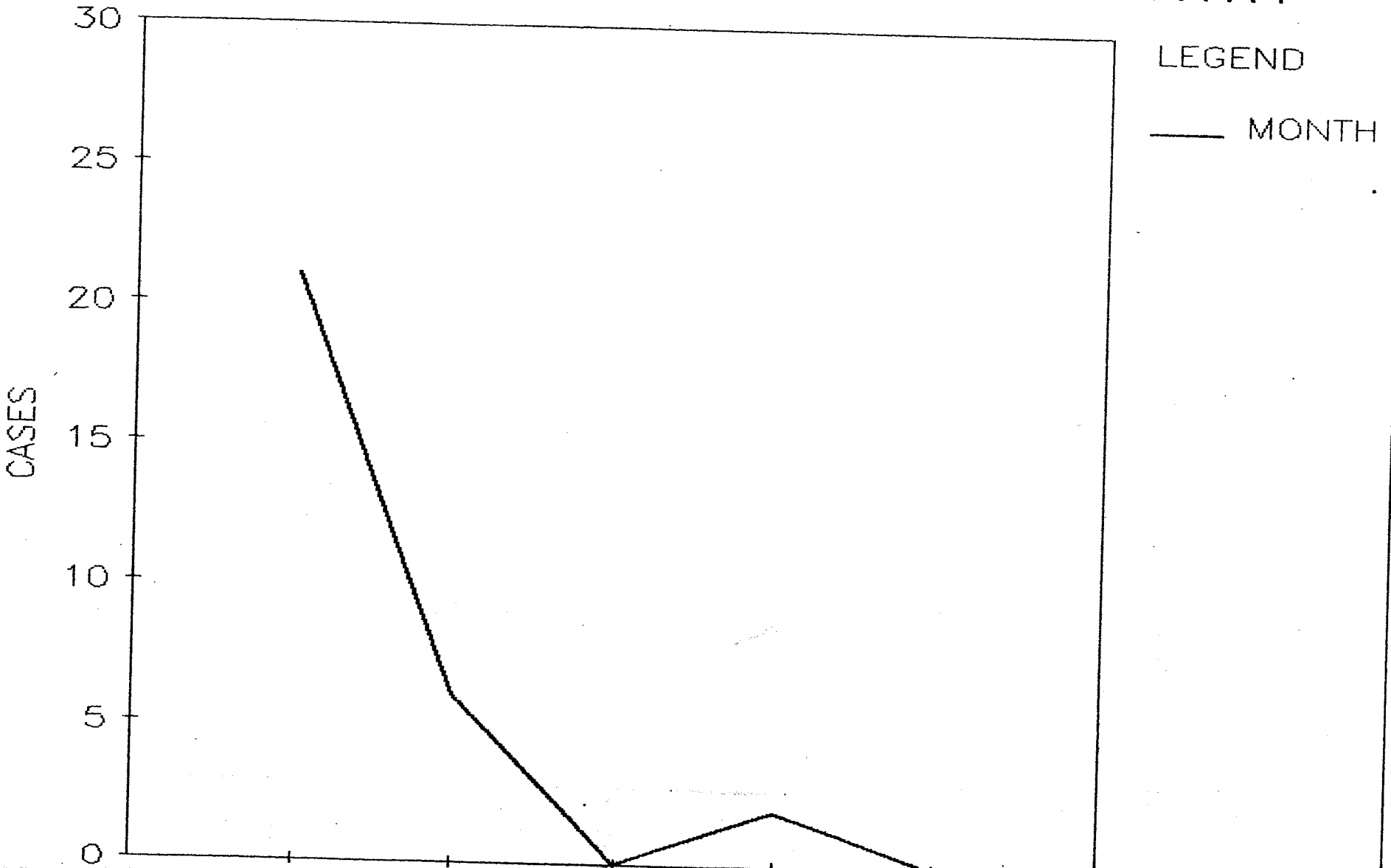
The peak rotavirus detection rate ocurred in October (Figure 3) the month which also recorded the lowest percentage of humidity (49%). The monthly average humidity increased from 49% in October 1990 to 84% in March 1991. The mean ambient temperature did not vary significantly during the study period (22.7^{0}C on average and did not have any considerable relationship with the rotavirus detection rate (Figure 4).

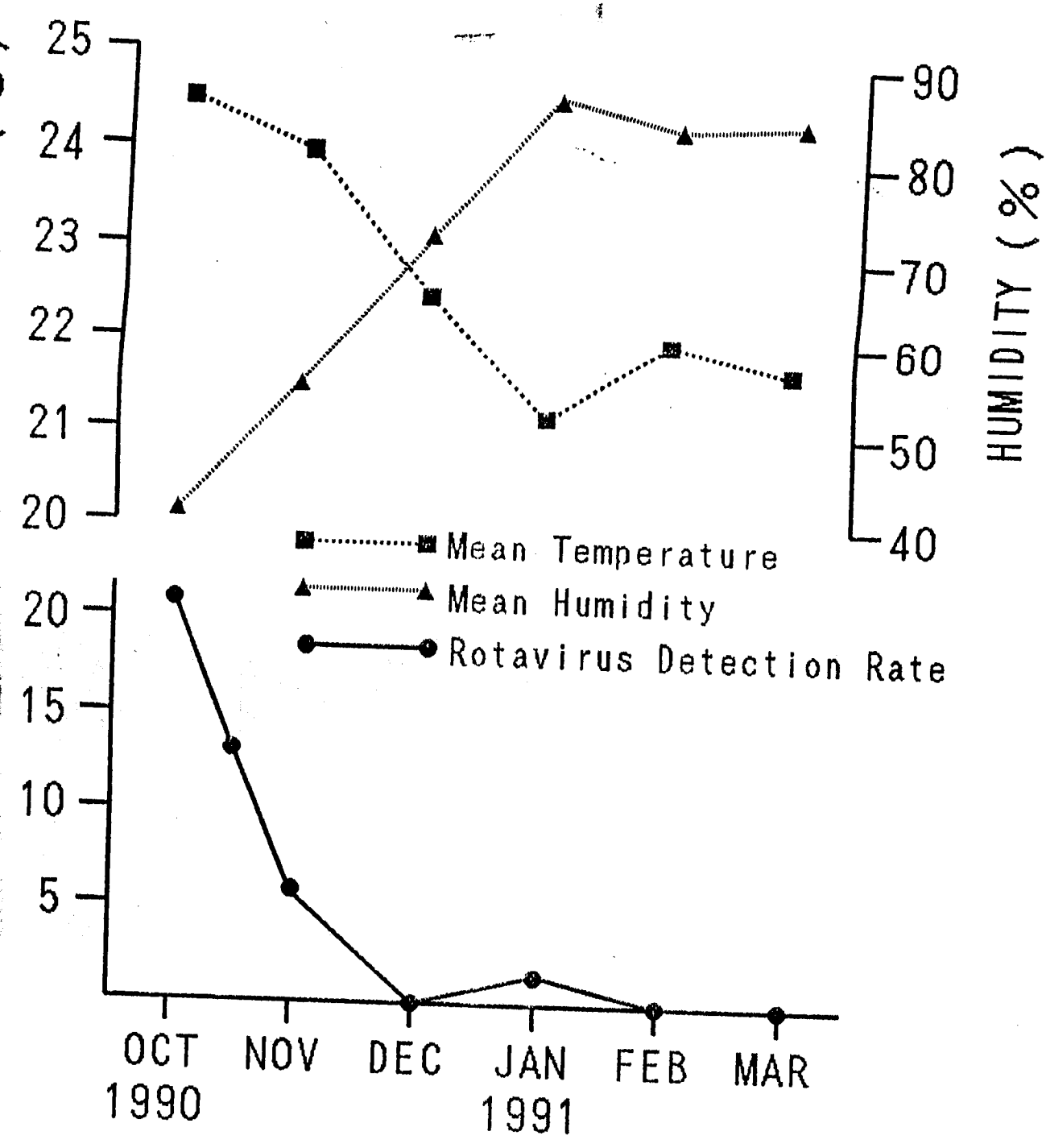
2. AGE DISTRIBUTION

The mean (SD) age of the rotavirus positive cases, 11.68 months (±5.80) and the mean (SD) age of the negative cases, 11.52 months (±5,29), showed no variance for the paired sample T-test.

The highest prevalence of rotavirus infection was found in the age group 7-12 months (34.47%) Infants aged 19-27 months were the least affected by rotavirus infection. (Figure 5).

ROTAVIRUS POSITIVE CASES BY MONTH

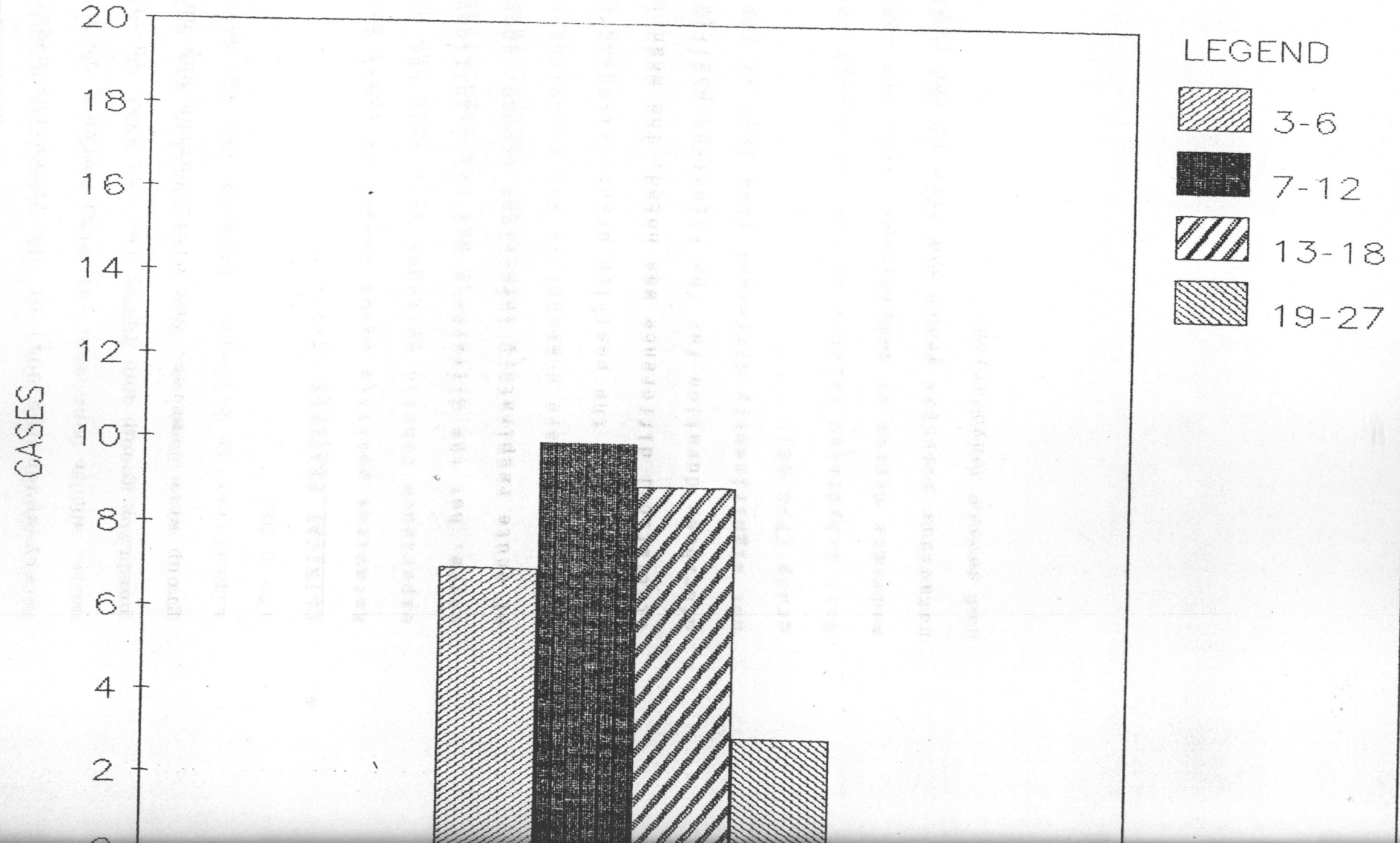




Mean monthly Temperature and Humidity in Lusaka from October 1990 to March 1991 and Rotavirus Detection Rate.

Figure 4.

DISTRIBUTION OF ROTAVIRUS POSITIVE CASES BY AGE IN MONTHS



3. SEX DISTRIBUTION

Fifteen (51.72%) of the rotavirus positive infants and sixty-seven (56.30%) of the rotavirus negative were male, while fourteen (48.98%) infants in the rotavirus positive group and fifty-two (43.69%) in the negative group were females. Sex distribution was not significantly different between the two groups ($P > 0.05$).

4. CLINICAL FEATURES (Table 7)

Rotavirus positive cases tended to vomit and to experience febrile episodes more than the negative cases, but the difference was not significant. Symptoms of acute respiratory infections (cough, sneezing, rhinorrhea) were commoner in the rotavirus negative group than in the positive group, although no statistical difference was noted. The mean (SD) diarrhoea duration for the rotavirus positive group, was not significantly different from that of the negative group ($P>0.05$).

Most dehydrated infants in the two groups had mild to moderate degree of dehydration. Only one case in the rotavirus positive group and five in the negative group had severe dehydration.

TABLE 7: CLINICAL FEATURES OF 29 ROTAVIRUS POSITIV AND 119 ROTAVIRUS NEGATIVE CASES.

Clinical Features	Rotavirus* Positive n (%)	Rotavirus Negative n (%)	
Symptoms			
Vomiting	27 (93.10)	103(86.55)	NS**
fever	17 (58.62)	64(53.78)	"
respiratory	13 (44.82)	68(57.14)	"
Dehydration			
some (mild to moderate)	24 (82.75)	105(88.23)	"
severe	1 (3.44)	5 (4.2)	"
Duration of Diarrhoea			
mean (SD) days	3.93 (0.88)	4.07(1.27)	"
Stool Frequency in the Past 24 hours			
mean (SD)	6.62 (4.33)	6.84(4.21)	"
Stool			
watery	16 (55.17)	61(51.26)	"
yellow	23 (79.31)	83(69.74)	"
mucus	8 (27.58)	34(28.57)	"
macroscopic blood	0 (0.00)	2(1.68)	--
occult blood	16 (55.17)	71(59.66)	NS
white blood cells	9 (31.03)	39(32.77)	--
red blood cells	8 (27.58)	27(22.68)	NS

* include 10 cases with mixed infection (E.coli/ Rotavirus)

** NS - Not significant, P>0.2

There was no significant difference in the mean (SD) stool frequency in the 24 hours preceding admission in hospital between the rotavirus positive cases, 6.62 (±4.33), and the negative cases, 6.84 (±4.21) ($P>0.05$). Stool consistency did not vary considerably between the two groups of patients with about half of the cases in each group producing watery/liquid stool.

The stools tended to be more yellow in colour in the rotavirus positive group than in the negative group. The two groups could not be differentiated in terms of the presence of macroscopic/occult blood or mucus in their stools.

White blood cells and red blood cells were similarly found in the stool of the two groups of infants. This similarity may have been due to the fact that one-third of the rotavirus positive cases had mixed infection (Rotavirus + E coli).

5. BIOCHEMICAL AND HAEMATOLOGICAL PROFILES

5.1 Biochemistry

Serum sodium and potassium were measured in 27 rotavirus positive cases, and blood urea in 28 positive cases. Serum sodium and potassium were estimated in 108 and 112 rotavirus negative cases respectively, and blood urea in 104 negative cases.

Blood glucose was determined in 27 rotavirus positive cases and 116 negative cases.

The mean blood glucose for the rotavirus positive cases and that for the negative cases were not significantly different (Table 8). No rotavirus positive case had a blood sugar below 3.0 mmol/L. Three negative cases (2.58%) had blood sugar levels below 3 mmol/L.

The mean blood urea levels of the 2 groups were not significantly different (Table 8).

The mean serum sodium and potassium levels of the 2 groups showed no variance for the paired sample T-test (Table 8).

No significant difference in terms of electrolyte disturbances were found between the patients and the comparison group (Table 9).

5.2 Haematology

The mean haemoglobin levels in the positive group and in the negative group were not significantly different (Table 10).

TABLE 8: BIOCHEMICAL PROFILE OF ROTAVIRUS POSITIVE AND NEGATIVE CASES.

	Rotavirus + Cases Mean (range)	Rotavirus - Cases Mean (range)
Serum sodium mmol/L	134.3 (124-155)	134.4 (120-155)
Serum potassium mmol/L	3.1 (1.3-5.5)	3.2 (1.0-5.5)
Blood urea mmol/L	6.3 (2.4-16.2)	5.0 (1.0-28.1)
Blood sugar mmol/L	5.4 (3.1-9.0)	5.6 (1.75-16.0)

TABLE 9: ELECTROLYTE DISTURBANCES IN ROTAVIRUS POSITIVE AND NEGATIVE CASES.

	Rotavirus + Cases n (%)	Rotavirus - Cases n (%)
Serum Na > 150 mmol/L	1 (3.70)	4 (3.70)
Serum Na < 130 mmol/L	9 (33.33)	24 (22.22)
Serum K > 5.5 mmol/L	0 (0.00)	0 (0.00)
Serum K < 3.0 mmol/L	14 (51.85)	54 (48.21)
Blood urea >13.0 mmol/L	3 (10.71)	5 (4.80)

TABLE 10: HAEMATOLOGICAL PROFILE OF ROTAVIRUS POSITIVE AND NEGATIVE CASES.

	Rotavirus + Cases n (%)	Rotavirus - Cases n (%)
Haemoglobin in g/dl Mean (range)	10.0 (6.2-18)	10.1 (6.8-17.6)
Hb < 9 g/dL	7 (41.17)	22 (30.13)
Hb > 15 g/dL	2 (11.76)	7 (9.58)
Total white cell count.		
< 2.5 x 10^9/L	1 (4.00)	1 (1.26)
> 19.5 x 10^9/L	0 (0.00)	1 (1.26)
Differential white cell count		
Polymorphs < 35%	18(69.23)	62 (62.62)
Lymphocytes ≥ 70%	17(65.38)	52 (52.52)

Patients with rotavirus diarrhoea tended to be more anaemic than those with non-HRV diarrhoea (Table 10). Both groups did not have significant leucocytosis or leucopenia, but had relative neutropenia (Table 10). Lymphocytosis was more a feature of rotavirus diarrhoea than of non-rotavirus diarrhoea (Table 10).

6. PROGNOSIS

The mean (SD) hospital stay for the rotavirus positive group, 1.37 days (± 0.62), did not vary significantly from that of the rotavirus negative group, 2.07 days (±1.65) ($P>0.3$).

Two rotavirus positive patients and Twenty-five rotavirus negative patients, well hydrated, were transferred to the general paediatric wards for problems other than dehydration, e.g. protein energy malnutrition, persistent fever, anaemia, acute respiratory infection... Of all the 152 diarrhoea cases included in the study, 5 died.

One rotavirus positive patient, well hydrated, was transferred to the general paediatric ward where he died of pneumonia.

One rotavirus negative case died of complications of protein-energy malnutrition.

Another rotavirus negative case with failure to thrive died in the DTU. This was the only case that had a positive sickling test. Electrophoresis of the haemoglobin (Hb) showed Hb AS.

Two rotavirus negative cases were discharged from the DTU, but were re-admitted five days later. One died of complications of protein-energy malnutrition and the other died of aspiration pneumonia.

7. OTHER EPIDEMIOLOGICAL FACTORS

7.1 Educational Status of mother/guardian

Most infants were being looked after by their mothers with very few being nursed by their grand-mothers or aunts during the period of 3 months preceding the onset of the acute diarrhoeal disease.

In the rotavirus positive group, 62.7% of the mothers/ guardians had some form of primary school education as compared to 52.94% in the negative group.

Among the positve cases, 27.59% of mothers/guardians attended a secondary school as opposed to 30.25% of the negative cases.

The education status of the mother/guardian did not have an impact on the prevalence of rotavirus infection ($P>0.05$).

7.2 Previous debilitating illness

Sixteen (55.17%) rotavirus positive cases and seventy-four (62.18%) negative cases had at least one episode of acute diarrhoeal disease in the three months preceding their inclusion in the study.

Four (13.79%) rotavirus positive cases and fourteen (11.76%) negative cases experienced a rise in body temperature in the same three-month period. Most mothers attributed the rise in temperature to malaria since the majority of infants responded well to antimalarial treatment administered at the local clinic.

No major debilitating illness seemed particularly to predispose to rotavirus infection.

7.3 Breast-feeding

Twenty-two (75.86%) rotavirus positive infants and eighty-seven rotavirus negative infants were being breastfed at the time of their inclusion in the study. They were all partially breast-fed. Other types of food given to these infants included milk formula, porridge, nshima, fruits, eggs and a variety of relish. Partial breast-feeding did not protect against rotavirus infection ($P>0.05$).

7.4 Nutritional status

Two (11.11%) rotavirus positive and thirty-three (45.83%) rotavirus negative infants were underweight as per their under five card.

Six (20.69%) rotavirus positive infants and fifty-eight (48.74%) rotavirus negative infants were clinicall underweight (Wellcome Classification).

No infant was found to have kwashiorkor or marasmic-kwashiorkor.

Rotavirus infection occured predominantly in well nourished infants ($P<0.01$).

7.5 Sanitation

Twenty-seven (93.10%) families in the rotavirus positive group and one hundred and eleven (93.28%) in the negative group had access to tap water for domestic consumption.

Fourteen (48.27%) families in the rotavirus positive group and thirty-seven (31.09%) in the negative group used flushing toilets, while the rest used pit-latrines. Amongst those families that used pit-latrines, these were covered in the case of 10 (66.66%) families from the rotavirus positive group and in the case of 53 (64.63%) families from the negative group.

Access to tap water for domestic consumption, the use of a flushing toilet or a covered pit-latrine did not prevent against rotavirus infection ($P>0.05$).

A positive history of contact with a person/s having diarrhoea up to one week prior to the infants inclusion in the study was noted in 8 (27.58%) rotavirus positive cases ans 24 (20.16%) negative cases. The difference between the two groups was not significant ($P>0.05$).

8. TREATMENT ADMINISTERED TO INFANTS BEFORE ADMISSION IN THE U.T.H

Oral rehydration solutions (glucose/electrolytes solutions) were the most common form of treatment used (54%).

Oral rehydration solutions were given to infants either alone (20%) or in combination with anti-diarrhoeal mixture (14%), with traditional medicine (14.67%) or with antibiotics (5.33%).

Sugar-salt-solution (home-made) were also administered to infants, alone (5.33%) or in combination with traditional medicine (7.33%).

Traditional medicine in the form of roots (46.15%), leaves (30.77%) or bark (21.15%) was soaked in water (33.96%), boiled (28.30%) or pounded (24.53%) and mostly administered orally to infants (81.13%).

Sitz baths were used in a few cases (9.43%) in combination with the oral route.

CHAPTER FIVE

DISCUSSION

The incidence of rotavirus infection as a cause of infantile diarrhoea varies widely from country to country (5, 20, 100). It has already been demonstrated that climatic factors affect the infection rate (20, 100). The incidence in a given country may vary depending on the type of study, whether hospital or community-based (9,91). In a particular area, different age groups selected for epidemiological research purposes may give different prevalence rates (100).

The method used for detection of human Rotavirus in stool is as important as the factors already mentioned.

The sensitivity and specificity of different diagnostic procedures have an influence on the detection rate.

Immune electron microscopy (IEM), electron microscopy (EM) and enzyme immuno-assays (EIA's) are more sensitive than latex agglutination tests (LAT).

Nevertheless, the LAT's are promising, inexpensive, simple and reliable assays (17, 98). The inclusion of a preimmune-serum latex control reduces the number of equivocal test results (96). In comparison with EIA's, LAT's are the simplest and most rapid way of diagnosing rotavirus infection. They require the minimum of equipment and technical skills, and are therefore particularly suited to third world countries where recurrent rotavirus infections commonly cause a high degree of morbidity and mortality in young children.

In Kenya, Mutanda (9), using the Bryden et al tissue culture system and immunofluorescent unit microscopy, isolated rotavirus in 14% of the diarrhoeal cases.

In the Central African Republic, Georges et al (14) isolated rotavirus in 17.6% of children below 15 years presenting with diarrhoea.

Fagbami et al (79) found rotavirus in 21% of children presenting with acute gastroenteritis in Ibadan, Nigeria.

Rotavirus was isolated in 15.4% of diarrhoeal cases in a community-based study by Loening W.E.K. et al (82) in Durban, South Africa.

In Alexandria (Egypt), rotavirus was found to be present in 13.1-16.9% of infantile diarrhoea in a study by Massoud et al (83).

In Ife-Ife, Nigeria (84), 15.2% of children with acute diarrhoea had rotavirus isolated from their stool.

In the present study,collection of stool samples was done within 7 days of diarrhoea onset in order to enhance the sensitivity of the test (72). The prevalence of rotavirus infection in infantile diarrhoea at the U.T.H of Lusaka correlates well with some of the studies mentioned above. Other African studies give higher prevalence rates ranging from 25% in South Africa (12) to 29% in Kenya (9), 27.8% in Ethiopia (11), 33% in Nigeria (86), 39.4% in Zambia (8), 39.5% in Egypt (85) and 42% in Malawi (18).

Some researchers found much lower prevalence rates. Dowling and Hilary Wynne did not isolate any rotavirus in a group of 94 children under 2 years presenting with diarrhoea in Lebowa (12).

Nathoo et al reported a 1% incidence of rotavirus infection in Harare (80). De Mel et al (101) isolated rotavirus in only 4% of children presenting with diarrhoea in Johannesburg.

In the present study, the age group 7-12 months was the most affected with rotavirus infection (34.47%) followed by the 13-18 months age group (13.05%). This is in agreement with a number of studies (11,14,18,82,84).

Other researchers found that rotavirus infection reached a peak in the age group 0-6 months (79,86,87).

Some studies agree that rotavirus infection is predominant during the first year of life (9,18), although it was found to be more prevalent in children above one year according to a few studies (82,83).

In the present study, rotavirus infection occured independently of sex. This finding confirms reports by Mutanda (9), Massoud et al (83), EL-Mouzi et al (85) and Mourad et al (87).

On the other hand, Olusanya and Taiwo (84), and Gomwalk (86) reported that male infants were more predisposed to rotavirus infection.

The monthly detection rate of rotavirus in the present study was clearly related to the average monthly humidity increasing from 49% in October 1990 to 84% in March 1991.

The ambient temperature remained relatively warm throughout the study (22.7^{0}C on average) and did not have any influence on the prevalence of rotavirus infection.

Stintzing et al (11) found rotavirus infection to have no relation to any climatological factor in Ethiopia (11). In South Africa, Dowling and Hilary Wynne reported (12) a rotavirus incidence of 0% in Lebowa and and 15% in Transkei under temperate and dry climatic conditions which were similar in both areas.

In a study in the Central African Republic, rotavirus infection reached a peak during the dry season (14) and similar findings were reported in Gambia (81), in Egypt (83,85,87) and in Nigeria (86).

Gomwalk et al in their study in Nigeria (86) found that relative humidity was the most influential climatic factor. Rotavirus infection was not found to be related to relative humidity in Japan (95).

The present study showed that vomiting and fever were not prominent features of HRV diarrhoea.

Pavone et al (18) found that rotavirus positive infants vomited more than negative infants (71% and 48% respectively), but fever was more common among the negative cases (50% and 83% respectively).

HRV positive patients were more likely to vomit and to experience fever in a study by Hieber et al (26).

El-Mougi et al (85) reported significant vomiting in HRV positive patients than in negative patients, but fever occure similarly in both groups.

Stoll et al in Bangladesh (88), Linhares in Brazil (89), Koopman et al in the U.S.A (91) and Saha et al in India (92), found that vomiting and fever were prominent features of acute diarrhoeal disease due to HRV.

Panicker et al (94) reported in their study that fever was more likely to occur in non-HRV diarrhoea than in HRV diarrhoea.

In the present study, rotavirus negative cases tended to present with respiratory symptoms more than positive cases did, but the difference was not significant.

In the study by Pavone et al (18), HRV negative cases had more respiratory symptoms than positive cases.

Respiratory symptoms were more common amongst HRV positive patients in studies done in the U.S.A. (26) and in Egypt (85).

No significant difference were found for any respiratory symptoms between rotavirus positive and negative cases as reported by Koopman J.S. et al (91).

The issue of rotavirus associated respiratory symptoms remains a controversial one.

The mean duration of diarrhoea at the time infants were included in the study did not differ significantly between the HRV positive and negative cases. A similar finding was reported in the U.S.A (26) and India (94).

In the present study, the majority of HRV positive patients presented with mild to moderate degree of dehydration as it was found in Kenya (10), in Malawi (18), in the USA (26), in Bangladesh (88) and in India (92).

HRV negative patients also presented mostly with mild to moderate degree of dehydration in the present study.

Several authors found both rotavirus positive and negative cases to have watery stools (26, 92, 94), whereas HRV diarrhoea was found to produce more watery stool in Kenya (10), in Malawi (18), in the USA (26), in Egypt (85) and in Bangladesh (88).

Some of the above mentioned reports (10,18,26) claimed that HRV diarrhoea was more likely to produce yellow stool.

In the present study, the 2 groups did not differ significantly in terms of stool colour or consistency.

Gross blood was rarely found in both groups in the present study, but occult blood was found in more than half of the patients in each group. Mucus, pus cells and red blood cells were present in the stools of both groups of patients in similar proportions.

In his study in Kenya, Mutanda (10) found no red blood cells, but reported the presence of pus cells in the stools of 73% of HRV positive cases.

Pavone et al (18) reported that HRV negative patients had mo faecal leucocytes than positive patients.

Hieber et al (26) in Dallas and San Jose (U.S.A) found no gross blood, less mucus but more faecal leucocytes in HRV positive patients than in negative patients.

Stoll et al (88) reported that mucus was a significant featur of HRV diarrhoea.

Saha et al (92) found that HRV positive cases had no macroscopic blood, but had more mucus in their stools than HR negative cases.

Clemens et al (90) stated that gross and/or occult blood and leucocytes were significantly present in the stools of HRV positive patients.

It is noteworthy to say that in the present study, 10 out of 29 (34.48%) HRV positive patients had mixed infection i.e. rotavirus + E.coli. This may have contributed to the presence of blood, pus cells and mucus in the stools of those patients.

El. Mougi et al (85) found no difference in the mean serum sodium levels between rotavirus positive and negative cases. High serum or vitreous humor sodium levels (>150 mmol/L) were found in a group of 21 fatal rotavirus cases analysed by Carlson et al (93).

In a study on the clinical features of infantile gastro-enteritis due to rotavirus at the Rush Green Hospital in England, severe electrolyte disturbance did not occur,

although there was a suggestion of a correlation between the higher blood urea and the number of rotavirus particles in the stool (102).

In the present study, there was no significant difference in the mean serum sodium, serum potassium, blood urea and blood glucose levels between the two groups of patients.

Acute rotaviral gastroenteritis is said to be characterised by isotonic dehydration. In the present study, rotavirus negative cases tended to have isotonic dehydration (74%) more than the positive cases did (63%). Amongst the positive cases 33.33% had hypotonic dehydration as compared to 22.22% in the negative group.

Three HRV positive cases (10.71%) and 5 negative cases (4.80%) had high blood urea levels (>13.0 mmol/L). Rotavirus positive cases tended to have high blood ureas but this was not significant. Leucocytosis (white cell count > 19.5×10^9/L)* or leucopenia (white cell count < 2.5×10^9/L)* was a feature of neither group.

Clemens (90) et al found lymphocytosis and monocytosis to be more a feature of non-watery rotaviral diarrhoea than of watery - liquid rotaviral diarrhoea.

* Defined by age-adjusted values (Nelson Textbook of Paediatrics, 13th edition, Behram and Vaughan, 1987, W.B. Saunders Company

In the present study, rotavirus positive cases tended to have lymphocytosis (>70% lymphocytes*) in their peripheral blood more than did negative cases.

Anaemia (Haemoglobin <9 g/dl*) occured more frequently in the rotavirus positive group (41.17%) than in the negative group (30.13%).

Most authorities agree that the course of HRV gastroenteritis is usually short in hospitalised children and the majority of children recover within a week of admission (39, 62, 63). The average duration of hospitalisation is four days, although it has lasted up to 2 weeks (6). Death due to rotavirus, albeit rare, has occured in infants and young children (93). In the study by Oyejide and Fagbami (106), there was no mortality associated with the cases of rotavirus diarrhoea. In the present study, the mean hospital stay for the rotavirus positive group was not different from that of the negative group.

This lack of significant difference must be considered with caution, taking into account the discharge policies of the DTU. Patients are discharged once they are fully rehydrated and their mothers have proved to be competent in the use of oral glucose/electrolytes solutions. Complete cessation of the diarrhoea is not a rule, so that well managed children do not spend more than 3 days in the DTU independently of the cause of their diarrhoea.

The only fatal rotavirus positive case had clinical evidence of pneumonia and had neither signs of dehydration nor electrolyte imbalance at the time of transfer to the general ward.

Mourad et al (87) found a significantly high rotavirus detection rate among infants with illiterate fathers. In the same study incidence of rotavirus infection was also high among infants whose mothers were manual workers or full time housewives.

In the present study, the educational status of the mothers or guardians did not have any influence on the rotavirus detection rate, although mothers or guardians tended to be better educated in the rotavirus positive group. The illiteracy rate was 3.45% amongst mothers of the rotavirus positive group as compared to 13.45% in the negative group. Human milk has been recognised to provide some degree of protection against HRV, through both specific antibodies and nonspecific factors (5). Breast-feeding did not provide absolute protection in the study by Pavone et al (18) since 64% of the children with gastroenteritis who were infected with HRV and 57% of those who were not were being breastfed at presentation. Soenarto et al (38) reported that there was no significant difference in incidence of rotavirus infection in children aged 6 months to 2 years who were receiving breast milk compared with those who were receiving artificial milk

formulas. Male children and breastfed children were more predisposed to infection with rotavirus than their counterparts in Jos, Nigeria (86). Mourad et al (87) found rotavirus detection rate to be much higher among infants receiving an external source of feeding other than breastfeeding. Rotavirus isolation rate was 20% among breastfeeders and 32.8% among non breastfeeders.

In Guatemala (104), Rotavirus-related diarrhoea and diarrhoea due to other specific pathogens were proportionately fewer and milder during the period of exclusive breastfeeding, but breastfeeding did not provide absolute protection against HRV infection.

In an epidemiological study of rotavirus diarrhoea in a cohort of Nigerian infants there was no difference in the breasfeeding practice for children who had rotavirus diarrhoea and those who did not (106).

In the present study about 75% of HRV patients were being partially breastfed at the time they presented with diarrhoea at the U.T.H. This finding suggests that only exclusive breastfeeding confers a significant protection against HRV infection.

The present study showed that rotavirus infection occured significantly more in well nourished infants than in undernourished ones ($P<0.01$).

In Indonesia, Soenarto et al (38) reported that there was no significant difference in incidence of rotavirus infection between a group of children with good nutritional status and a group with poor nutritional status. Oyejide et al (105) found no significant association between birthweight and rotavirus diarrhoea in a cohort of 115 infants followed up from birth to the age of 12 months in Ibadan, an urban Nigerian community. A few earlier reports claimed that malnutrition and chronic immune deficiency states predispose to a chronic rotavirus infection (108).

The present study showed that the use of a flushing toilet or tap water for domestic consumption did not protect against rotavirus infection.

This is in contradiction with findings by Olusanya and Taiwo (89). In their study in Ife-Ife (Nigeria), the majority of the children that had the rotavirus lived in areas without pipe-borne water and similarly belonged to the low socio-economic level.

Mourad A.J. et al in Egypt (87) found that among their rotavirus positive group, safe and private indoor water supply was available in 31.6% while 26.3% were associated with shared and outdoor water supply. This was insignificant statistically. Evidence suggested a contaminated water supply in an outbreak of gastroenteritis caused by both rotavirus and shigella sonnei in a private school in Rio de Janeiro (103).

The present study showed that the HRV positive group had a relatively better sanitation than the HRV negative group. This could be explained by the fact that populations having better sanitation probably have a lower incidence of bacterial diarrhoea, with an accompanying increase in the percentage of viral diarrhoea cases (107).

CHAPTER SIX

CONCLUSIONS

a. Prevalence Rate of Rotavirus

Rotavirus was isolated in 29 out of 152 (19%) acute infantile diarrhoea cases admitted in the diarrhoea training unit at the U.T.H of Lusaka and in only one of 78 (1.28%) infants without diarrhoeal disease.

The prevalence of rotavirus infection was not related to the ambient temperature but to relative humidity.

b. Demographic

The highest rotavirus detection rate was found in the age group 7-12 months but infection ocurred independently of sex.

c. Clinical

Symptoms such as vomiting, fever, rhinorrhoea, cough and sneezing were not features of HRV infection only.

Dehydration caused by HRV diarrhoea was mostly of isotonic type and of mild to moderate degree.

HRV infection did not produce stools with particular characteristics, whether gross or microscopic.

The above findings suggest that infants with rotavirus diarrhoea cannot be distinguished clinically from those with non-rotavirus diarrhoea.

d. Laboratory

Infants with HRV diarrhoea did not have biochemical or haematological pictures different from those with non-HRV diarrhoea, although the former tended to have low haemoglobin levels and relative lymphocytosis.

e. Risk Factors

Diarrhoea due to HRV infection had a good prognosis, with most patients (93%) being discharged from the hospital within 2 days post-admission. No infant died of dehydration and/or electrolyte imbalance due to HRV diarrhoea.

No debilitating illness seemed to predispose infants to HRV infection and the education status of mothers/guardians did not prevent infants from acquiring the infection.

Partial breast-feeding did not offer any protection against HRV and infection significantly occured in well nourished infants.

A better water supply and good sanitation did not offer any additional protection against rotavirus infection.

The lack of a positive history of diarrhoea contact in 72.42% of HRV positive cases suggest that most infants acquire the infection in the community from asymptomatic older children and adult carriers.

f. Therapeutic attitudes

The present study also showed that one out of two mother used oral glucose/electrolyte solutions before making a decision to take her baby to the hospital.

Antidiarrhoeal mixtures, antibiotics and traditional medicine are still widely used despite extensive efforts being made to educate health workers and parents.

CHAPTER SEVEN

RECOMMENDATIONS

1. More community-based and hospital-based studies are needed in Zambia in order to assess the real magnitude of diarrhoeal disease due to HRV.
2. For epidemiological studies, more sensitive diagnostic methods such as electron microscopy and enzyme immuno-assays are required if one is to have a more accurate prevalence rate of rotavirus infection.
3. The rotavirus latex agglutination test is a very useful diagnostic tool in intermediate hospitals and peripheral clinics. Being a rapid and easy procedure, it is helpful in the acute management of patients.
4. Electrophoretic studies should be done in order to identify the different rotaviral strains occuring in one geographical area; this would help in choosing the type of vaccine required in a particular area. Studies to assess the efficiency of the vaccine should then be undertaken.
5. Long term prospective studies must be undertaken to determine whether or not the incidence of rotavirus infection follows a seasonal pattern.
6. Studies with larger sample sizes must be undertaken to assess the impact of specific high risk factors on the development and natural course of the disease.

7. Apart from improving sanitary conditions, emphasis should be put on educating the public about personal hygiene.

8. Health education for health workers and parents regarding the dangers of using antidiarrhoeal mixtures, antibiotics and traditional herbs in acute diarrhoeal disease must continue.

APPENDIX 1

PREVALENCE AND EPIDEMIOLOGY OF ROTAVIRUS INFECTION IN INFANTILE DIARRHOEA AT THE UNIVERSITY TEACHING HOSPITAL OF LUSAKA.

STUDY QUESTIONNAIRE NO: .....
NAME: .....

1. AGE: months (1-36)

2. SEX: 1- Male 2-female

3. EDUCATION STATUS OF MOTHER/GUARDIAN:
   1 - Primary
   2 - Secondary
   3 - College
   4 - University
   5 - None

4. IF PRIMARY SCHOOL STATE GRADE:..... (1-7)

5. ASSOCIATED SYMPTOMS:
   1 - fever
   2 - vomiting
   3 - ARI*
   4 - fever/vomiting
   5 - fever/ARI
   6 - fever/vomiting/ARI
   7 - vomiting/ARI
   8 - ARI/G.E**
   9 - G.E./Malaria
   10 - Other specify
   11 - None

* ARI = Acute Respiratory Infection
** GE = Gastroenteritis

6. DEGREE OF DEHYDRATION:
   1 - some
   2 - severe
   3 - none

7. ARE YOU BREASTFEEDING?
   1 - yes
   2 - no

8. IF YES - EXCLUSIVE/PARTIAL?
   1 - exclusive
   2 - partial
   9 - N/A

9. IF NO, WHEN DID YOU STOP?
   1 - (1-24 months)
   2 - N/A

10. IF PARTIALLY, WHEN DID YOU?
   1 - (1-8 months)
   2 - N/A

11. GROWTH CURVE/UFC*:
   1 - normal
   2 - underweight
   3 - erratic
   4 - card Not available

12. CLINICAL NUTRITIONAL STATUS:
   1 - normal
   2 - underweight
   3 - kwashiorkor
   4 - marasmic kwashiorkor
   5 - marasmus

13. MAJOR ILLNESS IN THE PAST 3 MONTHS:
   1 - heart disease
   2 - pneumonia (ARI)
   3 - bronchitis (ARI)
   4 - gastro-enteritis
   5 - anaemia
   6 - tuberculosis
   7 - malaria
   8 - measles
   9 - P.E.M.**
   10 - meningitis
   11 - ARI/ G.E.
   12 - G.E./malaria
   13 - G.E./P.E.M.
   14 - Malaria/ G.E./ ARI
   15 - Pneumonia/G.E.
   16 - pneumonia/G.E./ Malaria
   17 - ARI/G.E./Malaria
   18 - G.E/Meningitis

*UFC = Under Five Card

**PEM = Protein Energy Malnutrition

19 - pneumonia/measles
20 - G.E./Tuberculosis
21 - G.E./Malaria/PEM
22 - Other specify .....
23 - None

14. SOURCE OF DRINKING WATER:
1 - tap
2 - well
3 - spring
4 - dam
5 - bore-hole
6 - stream/river

15. TYPE OF TOILET:
1 - flush
2 - pit latrine
3 - none

16. IF PIT LATRINE STATE...
1 - covered
2 - uncovered
9 - N/A

17. HAS ANY OTHER PERSON/S WITHIN THE HOUSEHOLD SUFFERED FROM DIARRHOEA WITHIN 1 WK?
1 - YES
2 - NO

18. DIARRHHOEA DAYS DURATION: ------ Days

19. FREQUENCY OF DIARRHOEA IN THE PAST 24 HRS:
------ Times

20. STOOL DESCRIP./CONSISTENCY:
1 - formed
2 - semi-formed
3 - watery

21. STOOL DESCRIP./COLOUR:
1 - white
2 - yellow
3 - green
4 - brown
5 - yellow/green

22. STOOL DESCRIP./CONTENT:
1 - mucus
2 - undigested food
3 - blood
4 - mucus/blood
5 - mucus/undigested food
6 - None

23. TREATMENT PRIOR TO ADMISSION IN A06:

1 - Oral rehydration salts (ORS)
2 - Sugar-salt-solution (SSS)
3 - Anti-dirarrhoeal mixture (ADM)
4 - Antibiotic
5 - Traditional medicine (TM)
6 - ORS/ADM
7 - ORS/TM
8 - ORS/ADM/Antibiotic
9 - ORS/SSS
10 - ORS/SSS/TM
11 - ORS/SSS/TM/Tablets/Injections
12 - SSS/ADM
13 - SSS/Antibiotic
14 - SSS/TM
15 - SSS/ADM/Antibiotic
16 - SSS/ADM/Antibiotic/TM
17 - ADM/TM
18 - ORS/Antibiotic
19 - ORS/ADM/TM
20 - ADM/ Antibiotic
21 - ORS/Antibiotic/TM
22 - All (1-6)
23 - others .....
24 - None

24. IF T.M STATE DETAILS/NATURE:

1 - roots
2 - leaves
3 - bark
4 - roots/leaves
5 - others
9 - N/A

25. IF T.M. / FORM:

1 - boiled
2 - pounded
3 - dried
4 - soaked
5 - pounded/boiled
6 - pounded/soaked
7 - boiled/pounded/dried

26. IF T.M. / ROUTE:

1 - rectal
2 - oral
3 - sitz-bath
4 - scarifications
5 - combination of above
6 - oral/sitz-bath
9 - N/A

27. DURATION OF HOSPITAL STAY:
1 - 14 days

28. OUTCOME OF DISEASE:
1 - discharged
2 - transferred
3 - died

29. IF PT. DIED, CAUSE: (comment file)
1 - yes
2 - no

30. LAB/RESULTS/STOOL/OCCULT BLOOD:
1 - Negative
2 - positive

31. LAB/RESULTS/MICROSCOPY/WBC:
1 - occasional
2 - 1 - 10
3 - many
4 - nil

32. LAB/RESULTS/RBC:
1 - occasional
2 - 1 - 10
3 - many
4 - nil

33. LAB/RESULTS/PARASITES:
1 - cryptosporidium
2 - any other
3 - nil

34. LAB/RESULTS/YEAST CELLS:
1 - 1 +
2 - 2 +
3 - 3 +
4 - nil

35. LAB/RESULTS/CULTURE:
1 - E. Coli
2 - shigella
3 - salmonella
4 - cholera
5 - Other or combination of the above.
7 - nil

36. LAB RESULTS/SENSITIVITY/IF CULTURE/CEFOTAXIM:
1 - S
2 - R
9 - N/A

37. LAB RESULTS/GENTAMYCIN:
1 - S
2 - R
9 - N/A

38. LAB RESULTS/CHLORAMPHENICOL:
1 - S
2 - R
9 - N/A

39. LAB RESULTS/CARBENICILLIN:
1 - S
2 - R
9 - N/A

40. LAB RESULTS/AMPICILLIN:
1 - S
2 - R
9 - N/A

41. LAB RESULTS/TETRACYCLINE:
1 - S
2 - R
9 - N/A

42. LAB RESULTS/COTRIMOXAZOLE:
1 - S
2 - R
9 - N/A

43. LATEX AGGLUTINATION TEST:
1 - Positive
2 - negative
3 - non specific

44. HAEMATOLOGY/HAEMOGLOBIN: .....g/dl

45. BLOOD SLIDE / MP:
1 - negative
2 - positive
9 - N/A

46. SICKLING TEST:
1 - negative
2 - positive
9 - N/A

APPENDIX II

WELLCOME SYSTEM OF CLASSIFICATION OF MALNUTRITION (77, 78).

WEIGHT FOR AGE	WITH OEDEMA	WITHOUT OEDEMA
60 - 80%	KWASHIORKOR	UNDERNUTRITION
LESS THAN 60%	MARASMIC-KWASHIORKOR	MARASMUS

APPENDIX III

FORMOL-ETHER CONCENTRATION TECHNIQUE

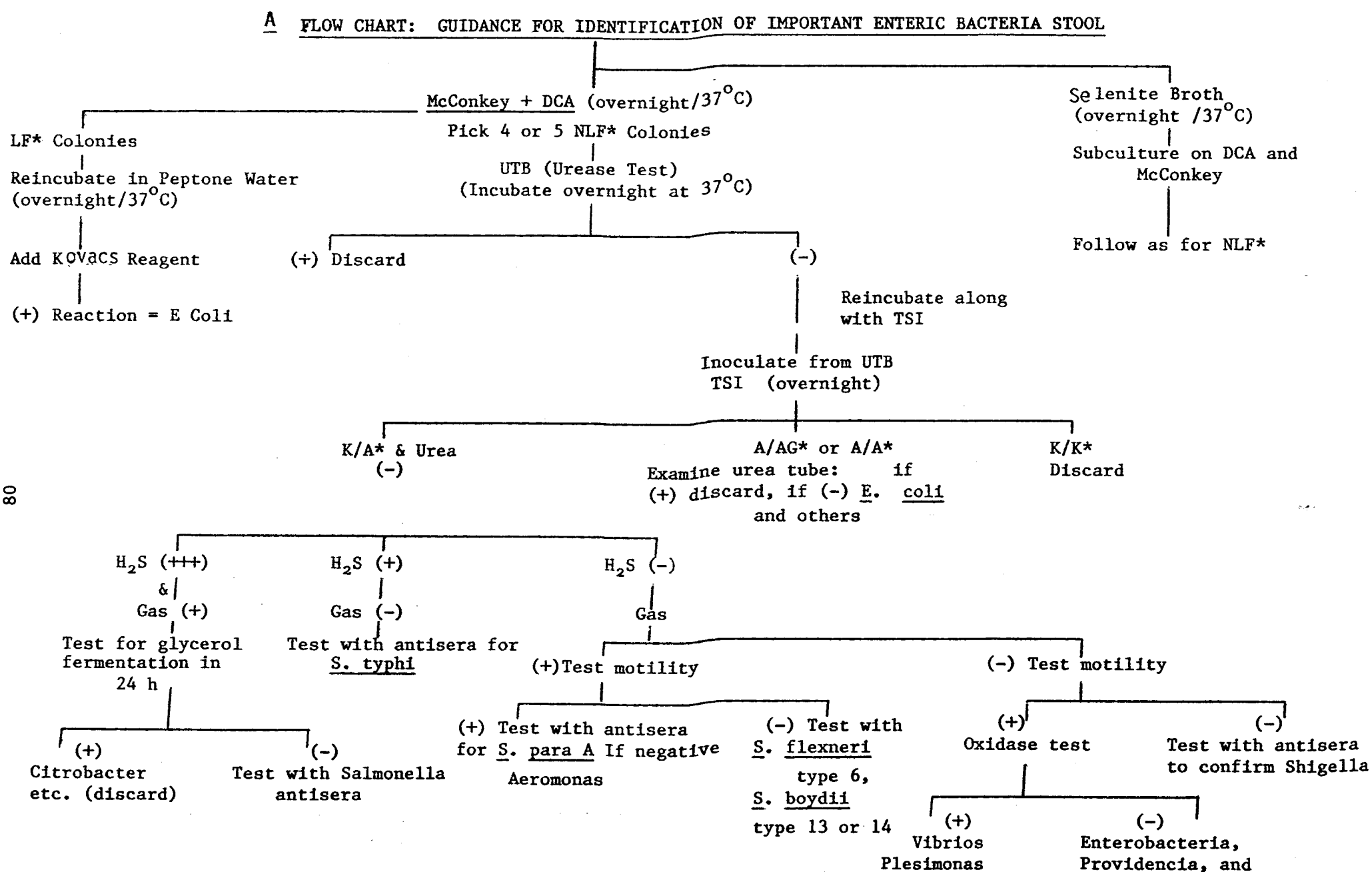
This method fixes cysts in formol saline. The fatty debris is separated by flotation into an ether layer and the cysts are left in the sediment at the bottom of the tube.

1. Emulsify about 2 grams of faeces in 10ml of 10% formol saline.
2. Strain through 2 layers of gauze into a centrifuge tube.
3. Add 3 ml of ether and shake vigorously.
4. Centrifuge so that 2000 rpm is reached in 2 minutes and switch off.
5. Loosen the fatty middle layer at the interface of the fluids and decant the whole of the supernatant so that only the small deposit remains.
6. Resuspend the deposit in the small drop of fluid remaining in the tube and remove in a Pasteur pipette.
7. A drop of double-strength Lugol's iodine solution is added to the deposit. Cover with a cover slip and examine microscopically.

APPENDIX IV

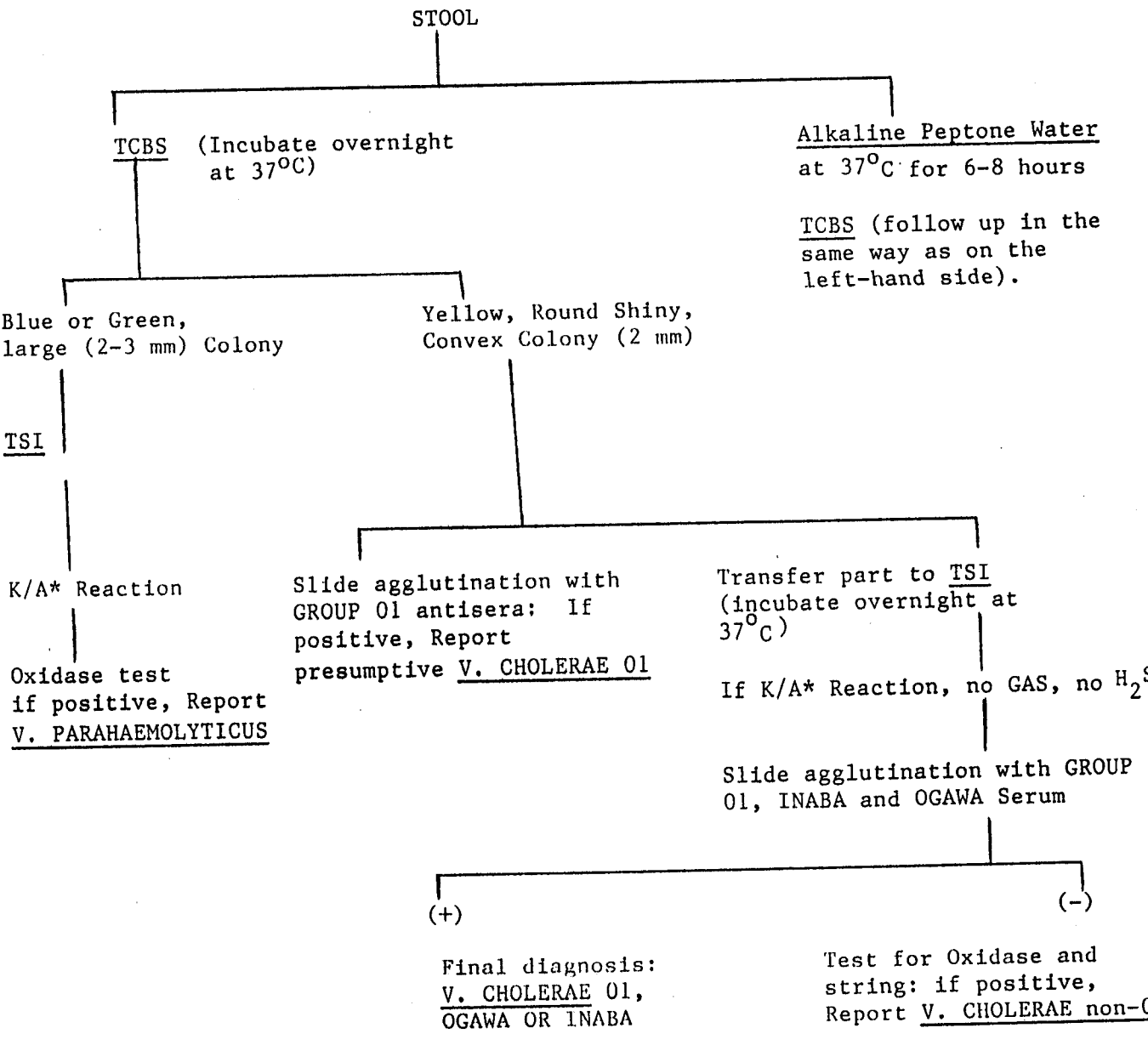
THE MODIFIED ZIEHL-NIELSON METHOD FOR CRYPTOSPORIDIUM (74)

1. Make a dry thick smear on a microscope slide from the formol-ether concentration technique deposit.
2. Fix in methanol for 5 minutes.
3. Stain in cold carbol fuchsin for 5 minutes.
4. Differentiate in 3% concentrated Hydrochloric acid in alcohol for 30 to 60 seconds.
5. Rinse in tap water.
6. Counter-stain in 0.25% malachite green for 30 seconds.
7. Rinse in tap water.
8. Blot dry.
9. Examine with x 100 objective (oil immersion). Cryptosporidium oocysts stain bright red on a green background.


A FLOW CHART: GUIDANCE FOR IDENTIFICATION OF IMPORTANT ENTERIC BACTERIA STOOL
McConkey + DCA (overnight/37°C)
Pick 4 or 5 NLF* Colonies
Selenite Broth (overnight /37°C)
Subculture on DCA and McConkey
Follow as for NLF*
LF* Colonies
Reincubate in Peptone Water (overnight/37°C)
Add KOVACS Reagent
(+) Reaction = E Coli
UTB (Urease Test) (Incubate overnight at 37°C)
(+) Discard
(-)
Reincubate along with TSI
Inoculate from UTB TSI (overnight)
K/A* & Urea (-)
A/AG* or A/A*
Examine urea tube: if (+) discard, if (-) E. coli and others
K/K* Discard
H2S (+++) & Gas (+)
H2S (+) Gas (-)
H2S (-) Gas
Test for glycerol fermentation in 24 h
Test with antisera for S. typhi
(+)Test motility
(-) Test motility
(+) Citrobacter etc. (discard)
(-) Test with Salmonella antisera
(+) Test with antisera for S. para A If negative Aeromonas
(-) Test with S. flexneri type 6, S. boydii type 13 or 14
(+) Oxidase test
(-) Test with antisera to confirm Shigella
(+) Vibrios Plesimonas
(-) Enterobacteria, Providencia, and

OW CHART FOR ISOLATION AND IDENTIFICATION OF V. CHOLERAE AND

PARAHAEMOLYTICUS (73)



LF = Lactose Fermenter
NFL = Non-Lactose fermenter
/AG = Butt and slant acid + GAS (yellow)
/A = Butt and slant acid (yellow)
/A = But acid and slant alkaline (yellow + red)
/K = Butt and slant alkaline (red)

APPENDIX VI

TEST FOR OCCULT BLOOD IN FAECES

1. **Reagent**

HEMATEST REAGENT TABLETS (AMES COMPANY, ENGLAND) containing O-Tolidine, Strontium Peroxide, Tartaric Acid and Calcium Acetate.

2. **Method**

1) Make a thin smear of faeces on the filter paper square provided.

2) Place the hematest tablet across the edge of the smear.

3) Flow one drop of water on top of the tablet, wait 5 to 10 seconds and flow a second drop of water on th tablet so that it runs down the sides onto the filter paper.

4) Observe the colour of the filter paper around the tablet exactly 2 minutes later.

POSITIVE: The filter paper around the tablet turns blue within 2 minutes.

NEGATIVE: The filter paper around tablet remains unchanged at 2 minutes.

Ignore any colour on the tablet or smear, and colour appearing on the filter paper after 2 minutes. The concentration of blood is roughly proportional to the intensity of the blue colour and the speed with which it develops.

APPENDIX VII

THE WELLCOME ROTAVIRUS LATEX TEST (72)

1. **Intended Use**:

Wellcome Rotavirus is a rapid latex agglutination test for use in the qualitative detection of rotavirus antigen in human faecal specimens.

2. **Principle of the Test**

The Wellcome Rotavirus Latex test is a rapid slide test in which latex particles coated with specific antibody react with rotavirus and rotavirus antigen present in a faecal specimen to give agglutination. Some faecal specimens cause non-specific agglutination of coated latex particles and a Control Latex preparation is provided to identify these samples. Agglutination of the Test but not the Control Latex indicates the presence of rotavirus in the sample.

3. **Reagents**

1) **Test Latex**: contains a suspension of polystyrene latex particles sensitised with rabbit antibody raised against Nebraska Calf Diarrhoea rotavirus in buffer with protein stabilisers. It contains 0.1 per cent sodium azide as preservative.

2) **Control Latex**: contains polystyrene latex particles sensitised with non-immune rabbit serum in buffer containing the same preservatives and stabilisers as the Test Latex.

3) Positive Control Antigen: contains inactivated Nebraska Colt Diarrhoea rotavirus with added stabilisers and 0.1 percent sodium azide as preservative.

4) Extraction Buffer: contains 0.1 M phosphate buffered in 0.85 percent saline, pH 7.3, with 0.1 percent sodium azide as preservative.

4. TEST PROCEDURE:

1. Bring all reagents to room temperature, preferably 18 to 25^{0}C while the sample is being processed. Prepare an approximately 10 percent suspension of the specimen in extraction buffer by transferring 1ml of extraction buffer (two 0.5ml volumes from the calibrated dropper in the bottle) into a centrifuge tube. Using a spatula or sampling stick, add approximately one tenth of the volume of the extraction buffer, cap the tube and vortex to homogenise the pellet. Allow to stand for at least 5 minutes at room temperature (preferably 18 to 25^{0}C) and then centrifuge at 1000g for 10 minutes. The supernatant may be stored at 2 to 8^{0}C for up to 3 days or at -20^{0}C for longer periods. Do not thaw and re-freeze the extract as this may reduce the sensitivity of the test.

If a swab specimen is to be used, put the swab into a small tube containing 1ml of extraction buffer and mix thoroughly. Remove swab and centrifuge as above. It is essential that a clear supernatant is obtained if non-specific reactions are to be avoided. Repeat the centrifugation if necessary then process the sample as described below.

3. Using one of the disposable droppers provided, dispense one drop of sample supernatant onto each of two circles on the card. Take care to correctly identify the samples.

4. Shake the latex reagents and dispense one volume (40ul) of Test Latex next to one of the drops of supernatant, and one volume (40 ul) of Control Latex next to the other.

5. Mix the contents of each circle using a separate mixing stick for each sample, spreading the mixture over as much of the circle as possible. Keep each sample within its circle or cross contamination could lead to false results. Discard each mixing stick after use.

6. Gently rock the card and observe for agglutination for up to two minutes, holding the card at normal reading distance (25 to 35 cm) from the eyes. Do not

use a magnifying lens. The patterns obtained are clear cut and can be recognised under any normal lighting conditions.

7. Discard the card for safe disposal. Do not re-use.

5. INTERPRETATION OF RESULTS (Figure 6):

a) Positive Result: clear agglutination of the Test Latex accompanied by a lack of agglutination of the Control Latex indicates the presence of rotavirus in the faecal sample.

b) Negative Result: Lack of agglutination in both Latex reagents means that rotavirus is not detectable in the faecal extract. This result does not eliminate the possibility of infection as insufficient antigen may be present due to inadequate or improper sampling. It is desirable to perform the test on a subsequent faecal specimen taken as soon as possible.

c) Non-interpretation Result: Visible agglutination of the Control Latex, whether stronger or weaker than the Test Latex, indicates a non-specific reaction and the unsuitability of the specimen for this test procedure.

d) Trace Reaction: Trace (±) reactions should be recorded as negative.

Reading of Results

A **positive** result is indicated by the development of an agglutinated pattern showing clearly visible clumping of the latex particles within two minutes of mixing the latex with the faecal extract. The speed of appearance and quality of agglutination depends on the strength of the antigen, varying from large clumps which appear within a few seconds of mixing to small clumps which develop more slowly. (See Figure 1).

In a **negative** result, the latex does not agglutinate, and the milky appearance remains substantially unchanged throughout the two minute test. Note, however, that faint traces of granularity may be detected in negative patterns, depending on the visual acuity of the operator. (See Figure 2).

Figure 1

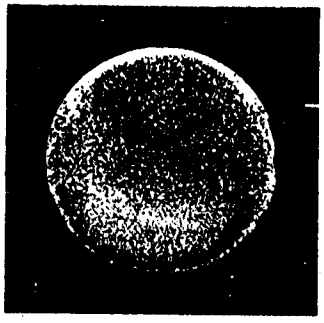


Figure 2

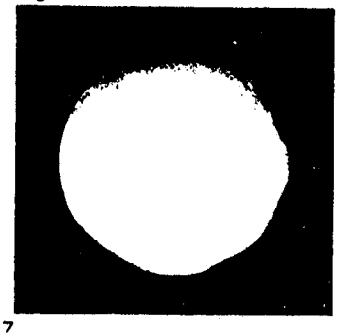


Figure 6:

The Wellcome rotavirus latex test. Reading of results.

6. PERFORMANCE CHARACTERISTICS:

The Wellcome Rotavirus Latex test has been evaluated in two laboratories, one in the United Kingdom and one in Finland. In both laboratories, electron microscopy was used as the reference method but one laboratory used an enzyme immuno-assay procedure incorporating antibody neutralization to confirm results found positive by the latex test but negative by electron microscopy. Of the total of 408 specimens tested which had been stored frozen, 10 gave non-specific reactions and were excluded from the totals. The majority of the specimens were taken from children ranging in age from neonates up to the age of 24 months but some samples tested were from adults. All patients had symptoms of gastroenteritis. The sensitivity of the latex reagent was found to be 90 percent (138/153) and specificity 99 percent (244/245) (72).

7. EVALUATION OF SPECIFICITY:

The Latex reagent has been tested and found to give a positive reaction with representative rotaviruses of Serotypes 1 to 4, (WA7729, SA-11, and VA70 respectively) as well as the Nebraska Calf Diarrhoea virus.

APPENDIX VIII

ROTAVIRUS ELISA TEST (Cambridge Bioscience U.S.A)

1. Mix the captive antibody (0.05 ul) and the specimen (0.05 ul). Incubate for 1 hour at room temperature.
2. Wash three times with diluent solution.
3. Add 0.05 ul of buffer solution and then 0.05 ul of enzyme substrate. Incubate at room temperature for approximately 10 minutes.
4. Add a reaction stopper.
5. Interpretation of the test

   a) Without the reaction stopper

      Production of a Blue colour = + Reaction.

   b) With the reaction stopper

      Production of a yellow colour = + Reaction.

      Use a spectrometer for a better interpretation of results.

APPENDIX IX

DETERMINATION OF UREA BY DI-ACETYL-MONOXINE (DAM) METHOD (75).

1. REAGENTS

1. Trichloracetic acid : 100g/L (TCA).
2. Di-acetyl-monoxime : 25g/L in water (DAM).
3. Thiosemicarbazide : 5g/L in water (TSC)
4. Acid Ferric Chloride solution.
5. Acid Reagent
6. Colour Reagent (DAM + TSC).
7. Working Urea Standard: 10 mmol/L

2. METHOD

REAGENTS	TEST	BLANK	STANDARD	CONTROL
SERUM OR PLASMA	0.2 ml	-	-	-
STANDARD	-	-	0.2 ml	-
DISTILLED WATER	1.0 ml	1.2 ml	1.0 ml	1.0 ml
TCA	1.0 ml	1.0 ml	1.0 ml	1.0 ml
QUALITY CONTROL	-	-	-	0.2 ml

Mix well and centrifuge at 1000 RPM for one minute.

REAGENTS	TEST	BLANK	STANDARD	CONTROL
SUPERNATANT	0.2 ml	0.2 ml	0.2 ml	0.2 ml
COLOUR REAGENT	3.0 ml	3.0 ml	3.0 ml	3.0 ml

Heat in a boiling water bath for 20 minutes. Cool to room temperature in cold water. Read test and standard against blank at 520 nm within 15 minutes in the colorimeter.

3. CALCULATION:

$$\text{Urea Concentration in mmol/L} = \frac{T \times C}{S}$$

T = absorbance reading of sample

S = absorbance reading of working Urea standard

C = Concentration of Urea working standard

Check the control results.

APPENDIX X:

POTASSIUM AND SODIUM ESTIMATION BY FLAME EMISSION SPECTROMETRY (75)

1. REAGENTS

1) Stock

Make a saturated solution of Lithium Chloride.

2) Working Solution

From the saturated solution, make a 1:400 dilution with deionised distilled water.

2. **PROCEDURE:**

Dilute sample (plasma or serum), standard and quality control one in a hundred with working lithium chloride solution.

REAGENTS	TEST	BLANK	STANDARD	CONTROL
SAMPLE	0.1 ml	-	-	-
STANDARD*	-		0.1 ml	-
DEIONISED DISTILLED WATER	-	0.1 ml		-
WORKING LITHIUM CHLORIDE SOLUTION	9.9 ml	9.9 ml	9.9 ml	9.9 ml
QUALITY CONTROL	-	-	-	0.1 ml

* Na (Sodium) Standard :140 mmol/L
* K (Potassium) Standard: 5.0 mmol/L

Mix well and measure the electrolytes on the flame photometer (spectrometer).

UNITS - mmol/L

Check the control results.

APPENDIX XI:

DETERMINATION OF BLOOD GLUCOSE BY O-TOLUIDINE METHOD (75)

1. REAGENTS:

1) O-Toluidine Reagent.

2) Working Glucose Standard: 10 mmol/L

2. METHOD

REAGENTS	TEST	BLANK	STANDARD	CONTROL
PLASMA	0.05 ml	-	-	-
STANDARD	-	-	0.05 ml	-
DISTILLED WATER	-	0.05 ml	-	-
O-TOLUIDINE	3.0 ml	3.0 ml	3.0 ml	3.0 ml
QUALITY CONTROL	-	-	-	0.05 ml

Mix well, cover and incubate all tubes at 100^{0}C for 12 minutes. Cool rapidly to room temperature and measure absorbances. colorimeter: Orange filter, ILFORD 607 (600 NM). Use a dry cuvette or rinse with glacial acetic acid and drain. Set the instrument to Zero with the reagent blank.

3. CALCULATION:

$$\text{Glucose Concentration in mmol/L} = \frac{T \times C}{S}$$

T = absorbance reading of sample

S = absorbance reading of working Glucose standard

C = concentration of working standard

Check the control results.

APPENDIX XII

HAEMOGLOBIN ESTIMATION: MODIFIED CYANMETHAEMOGLOBIN METHOD.

1. REAGENTS:

   1) 0.9% Sodium Chloride (NaCl).

   2) Lysate: Lysing and haemoglobin reagent (COULTRONIC France).

2. METHOD:

   Add 10 ml of 0.9% NaCl to 0.02 ml of blood sample. Add 4 drops of lysate.

   Read haemoglobin estimation in a colorimeter (252) at 5 nm.

3. CALCULATON:

   Haemoglobin values of blood specimens are read from the calibration graph.

APPENDIX XIII:

SICKLING TEST: METABISULPHITE REDUCTION METHOD (75).

1. With a Pasteur pipette, add 2 drops of freshly prepared 2% Sodium Metabisulphite reagent to the middle of a microscope slide.
2. Dip an applicator stick into the blood and transfer a very small amount of blood to the metabisulphite reagent on the slide. Mix evenly.
3. Using an applicator stick, put a thin film of vaseline on the edges of a cover slip. Place the cover slip over the mixture on the slide.
4. With the high power lens of a microscope, examine immediately. Look for a thin area (one in which the cells are not touching each other). Examine at 15 and 60 minutes, and if negative, at 24 hours.
5. Avoid drying of preparation before examination.
6. Erythrocytes with haemoglobin S have a Sickle shape

APPENDIX XIV

FIELD'S METHOD FOR STAINING THICK SMEARS (76)

1. Make a thick blood film on a microscope slide.
2. Dip the dried slide into stain A (Polychromed Methylene blue) for 1 to 2 seconds.
3. Rinse in buffered water for 5 to 10 seconds.
4. Dip the slide into stain B (Eosin) for 1 to 2 seconds.
5. Rinse rapidly for 10 seconds in buffered water.
6. Set the slide upright to dry after the excess water has been shaken off.
7. Examine the slide for Malaria parasites with x100 objective (oil immersion).

APPENDIX XV

TOTAL LEUCOCYTE COUNT BY VISUAL METHOD (76)

1. Make a 1 in 20 dilution of blood by adding 0.02 ml of blood to 0.38 ml of diluting fluid (2% acetic acid coloured pale violet with 0.01% gentian Violet) into a test tube.
2. Mix the suspension by rotating in a cell suspension mixer for at least 1 minute.
3. Fill the improved Neubauer counting chamber by means of a capillary tube or a Pasteur pipette.
4. Allow 2 minutes for cells to settle.
5. View the preparation using a x 10 objective and subdued light. Count at least 100 cells in as many 1 mm^2 areas (0.1 uL in volume) as may be necessary.
6. CALCULATION:

$$\text{Count (/L)} = \frac{\text{Number of Cells Counted (N)}}{\text{volume counted (uL)}} \times \text{Dilution} \times 10^6$$

For S.I. units, convert count to 10^9/L

APPENDIX XV1

MAY-GRUNWALD-GIEMSA'S STAIN FOR DIFFERENTIAL LEUCOCYTE COUNT (76).

1. Make a thin blood film on a microscope slide.
2. Dry the film in the air.
3. Fix by immersing the slide in a jar of methanol for 10 to 20 minutes.
4. Transfer the slide into a jar containing May-Grunwald's stain diluted with an equal volume of buffered water for 15 minutes.
5. Transfer the slide without washing into a jar of Giemsa's stain freshly diluted with 9 volumes of buffered water for 10 to 15 minutes.
6. Transfer the slide into a jar containing buffered water and rapidly wash in 3 or 4 changes of water.
7. Allow to stand undisturbed in water for 2 to 5 minutes for differentiation to take place.
8. Stand the slide upright to dry.
9. Examine the slide with x 40 or x 100 objective (oil immersion lens).
10. Inspect the film from the head to the tail of the slide, and if less than 100 cells are encountered in a single narrow strip, examine one or more additional strips until at least 100 cells have been counted.

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