Attachment 1.

Date 18-8-1981

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. G.H. Rabbani

Application No. 81-034

Title of Study STUDIES ON ROTAVIRUS SEROTYPES IN BANGLADESH AND KENYA.

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) WHO/University of Nairobi, Kenya.

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects (Yes) No
   (b) Non-ill subjects Yes No
   (c) Minors or persons under guardianship (Yes) No
2. Does the study involve:
   (a) Physical risks to the subjects Yes (No)
   (b) Social Risks Yes (No)
   (c) Psychological risks to subjects Yes (No)
   (d) Discomfort to subjects Yes (No)
   (e) Invasion of privacy Yes (No)
   (f) Disclosure of information damaging to subject or others Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) Yes (No)
   (b) Use of fetal tissue or abortus Yes (No)
   (c) Use of organs or body fluids (Yes) No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study (Yes) No
   (b) Procedures to be followed including alternatives used (Yes) No
   (c) Physical risks (Yes) No
   (d) Sensitive questions (Yes) No
   (e) Benefits to be derived (Yes) No
   (f) Right to refuse to participate or to withdraw from study (Yes) No
   (g) Confidential handling of data (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure (Yes) No
5. Will signed consent form be required:
   (a) From subjects Yes (No)
   (b) From parent or guardian (if subjects are minors) (Yes) No
6. Will precautions be taken to protect anonymity of subjects (Yes) No
7. Check documents being submitted herewith to Committee:
   NA Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ✓ Informed consent form for subjects
   ✓ Informed consent form for parent or guardian
   ✓ Procedure for maintaining confidentiality
   NA Questionnaire or interview schedule *

   * If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
   1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
   2. Examples of the type of specific questions to be asked in the sensitive areas.
   3. An indication as to when the questionnaire will be presented to the Cttee. for review.

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

G. H. Rabbani
Principal Investigator

Trainee

81-034
Recd 19/8/81

SECTION I. - RESEARCH PROTOCOL

1. Title : STUDIES ON ROTAVIRUS SEROTYPES IN BANGLADESH AND KENYA

2. Principal Investigators : Dr. G.H. Rabbani, (ICDDR,B, Dacca)
Dr. L.N. Mutanda, (Nairobi, Kenya)
Dr. S.N. Kinoti, (Kenyatta Hospital, Nairobi, Kenya)

Collaborating Investigators : Prof. G. Ziszis, (Brussels, Belgium)
Dr. Flewett, (Birmingham, U.K.)
Betty Kirkwood, (London, U.K.)
Dr. A.R. Samadi, (ICDDR,B, Dacca)

Advisor : Dr. W.B. Greenough (Director, ICDDR,B, Dacca)

Co-investigators : Drs. Shafiq, Prodip

3. Starting Date : 1st September 1981

4. Completion Date : 30th August 1982

5. Total Direct Cost : US $ 40,845 (Staff comitment $ 12,628, Operation $ 28,217).

6. Scientific Program Head : This protocol has been approved by the Pathogenesis and Therapy/Disease Transmission Working Groups.

7. W.B. Greenough
Signature of Scientific Program Head Date: 19/8/81

7. Abstract Summary:

Serotype specific clinical and epidemiologic studies of rotavirus gastroenteritis in Bangladeshi and Kenyan children are planned. At the ICDDR,B, 500 children aged 0-5 years will be studied over one year period specifically examining the clinical presentation and severity of illness associated with each serotype and electrophorotype of rotavirus. Similar studies will be undertaken at Nairobi in collaboration with the Kenyan Scientists. Standardised methods have been developed to compare the results of the African and Asian Studies. These data would be helpfull in the understanding of the relative role of different serotypes of rotavirus in these two different geographic populations. Serotype specific information are also crucial for the development of an effective rotavirus vaccine at the moment.

9. (a) Research involving human subjects: _____

(b) Research Review Committee: _____

(c) BMRC: _____

(d) Director: _____

(e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective:

(a) To determine the number of different serotypes of human rotavirus prevalent in Bangladesh and Kenyan children.

(b) To characterize and compare the clinical and epidemiological features of the circulating Rotavirus Serotypes in Asian and African children.

2. BACKGROUND

Establishment of collaboration:

This collaborative project for studying the Rotavirus diarrhoea of children in Bangladesh and Kenya originated because of the following backgrounds:

(a) At the ICDDR,B research activity in the field of Rotavirus diarrhoea began in 1978 when Dr. David Sack undertook a number of clinical and field trials and also set up the laboratory procedure for the isolation of the virus at the centre. Subsequently the research area is further expanded by Dr. Robert E. Black who also carried out some field studies in the Matlab area. Results of these investigations showed that i) the virus is highly prevalent in the community (second in incidence to E. coli) ii) has been associated with severe dehydrating diarrhoea in children and (iii) can be effectively managed using oral electrolyte solution. Both these investigators completed their tenure of work and left the country in the later part of 1980 and no further Rotavirus work has been undertaken at the centre since then. During this lag period initiative effort to study this important virus amongst the centre's scientists were mostly lacking. Since rotavirus diarrhoea is coming out as an important public health problem of high priority, activity to study this virus would be essential for the centre.

(b) Secondly, it is being increasingly realised that rotavirus diarrhoea is a global problem for todays children. Evaluation and characterization of the clinico-epidemiological features associated with the different strains of the virus prevalent at different geographic regions of the world would be extreamly important to understand the natural course of the disease. It will also bring out the biological and socio-cultural variables in relation to the distribution of the disease in two geographically distinct communities.

(c) Thirdly, since Dr. Mutanda has already gained considerable experience in rotavirus diarrhoea in the East African region and also conducted some serotyping studies in Bangladesh, this is a unique opportunity for utilizing his experience to run further comparative studies in this areas.

(d) Fourthly, at the moment ICDDR,B laboratory is not competent to carry out serotyping studies of Rotavirus due to technological difficulty. Therefore it would be logical to collaborate with expert personnel of the world who have more technological advancement to have our strains serotyped.

(e) Fifthly, form the very inception of ICDDR,B in early 60's as the Pakistan-SEATO Cholera Research Laboratory, its activities were entirely limited within the regions of Bangladesh, which is quiet understandable considsidering the limited nature of the centre. Today the scopes and objectives of the centre is an international one and extension of its activities outside Bangladesh is a logical outcome of its international collaboration.

(f) Lastly, Dr. Mutanda and the Kenya Institute of Medical Research has expressed an interest to orginize a collaborative effort to study the rotavirus diarrhoea in children in these two countries. The objectives of

this study will be determined by and funds shared between the collaborating participants.

In order to give an effect to the above mentioned facts and to initiate the collaborative work with the Kenyan counterpart the Director of ICDDR,B promulgated a draft proposal on 15.4.1981 to establish a body called INTERNATIONAL SCIENTIFIC AND TECHNOLOGICAL COOPERATION PROGRAM at ICDDR,B to negotiate all kinds of overseas activities of the centre.

The program reads as follows:

INTERNATIONAL SCIENTIFIC AND TECHNOLOGICAL COOPERATION PROGRAM

1. *INTRODUCTION*

Reference to Resolution 17.12.1980 of ICDDR,B Board of Trustees which states: " THE BOARD REQUESTS THE DIRECTOR TO PREPARE A WORKING PAPER ON STRATEGIES FOR COLLABORATION WITH COUNTRIES AND AGENCIES AND TO DEVELOP CRITERIA FOR ESTABLISHING PROGRAMMES OUTSIDE BANGLADESH".

ICDDR,B should initiate assisting other countries and agencies in providing technical scientific collaboration for control of diarrhoeal diseases.

In this context ICDDR,B is already assisting the Government of Saudi Arabia by development of a central laboratory in Riyadh and an animal house in Jeddah. Also ICDDR,B has started a joint research protocol with Kenyatta Hospital in Kenya in regard to rotavirus studies. Recently the Government of Kuwait requested that ICDDR,B to provide technical assistance in assessment and advice on diarrhoeal diseases in that country.

In this point ICDDR,B must develop a system by which a more efficient and high quality technical and scientific service can be delivered to requesting countries and agencies. In providing a sound technical and scientific collaboration, it is be necessary to establish in sub-committee of the Management Committee and an office for the International Scientific and Technical Co-operation.

II. OBJECTIVES

1. The underlying objectives of this committee should be the creation or the strengthening of a process that will make it easier for the centre to be able to respond to the requests from collaborating countries or in actively generating proposals for collaboration. The Committee will be assigning Program Heads or Associate Directors the responsibility of preparing project proposals.

2. The Committee will be responsible to review all proposals for collaboration in projects and help the Director to decide on the projects to be undertaken by the centre.

3. The Committee will advise the Director in taking decisions on actions related to International Co-operation and collaboration.

4. The creation of this Committee would in no way reduce the responsibilities of the existing offices within the current organizational structure of ICDDR,B.

This Committee will be the central policy making body for all scientific and technical collaborating proposals and proposals for project funding.

III. ORGANIZATION OF THE ISTC

1. The ISTC Committee has been formed as a Sub-committee of the Management Committee.

2. The Chairman of the ISTC Committee will be the Director, ICDDR,B.

3. The ISTC office will be headed by the Secretary of ISTC Committee who shall be a person of Associate Director level.

4. The members of the ISTC Committee will be the Deputy Director and the Associate Directors for Resources Development and Training and Extension.

5. The Committee will have powers to co-operate with other members as and when necessary.

6. The Committee may request anyone to attend one or more meetings of ISTC relevant to the particular project proposals concerned.

7. ISTC Sub-committee will initiate and request senior members of the staff to undertake specific project proposals.

8. The office of ISTC will be responsible for periodic reports for projects or will ensure that the project head submits the reports by expected deadlines.

9. The Secretary of the ISTC will convene meetings, keep minutes and have responsibility for follow-up of the resolutions adopted in the meetings.

Kenya - Bangladesh technical collaboration:

This joint rotavirus project has been organised by the ICDDR,B in collaboration with the following institutes of different countries:

Dr. L.N. Mutanda (ICDDR,B)
(Virologist)
Virus Research Centre
Kenya Institute of Medical Research
P.O. Box 20750
Nairobi
Kenya

Dr. S.N. Kinoti
Paediatrician/Nutritionist
Department of Paediatrics
Kenyatta National Hospital
University of Nairobi
P.O. Box 30588
Nairobi
Kenya

Professor T.H. Flewett
(Virologist)
Regional Virus Laboratory
East Birmingham Hospital
Bordesley Green East
Birmingham B9 5ST
United Kingdom

Professor George Zissis (Virologist)
Hospital Saint Pierre
Free University of Brussels
1000 Brussels
Belgium

J.P. Lambert
Department of Virology
Hospital Saint Pierre
1000 Brussels
Belgium

Dr. P.M. Tukei (Virologist, WHO)
Director
Virus Research Centre
Nairobi
Kenya

Professor Kauro Hayashi
(Virologist)
C/O. Dr. J.N. Kaviti
NPHLS
P.O. Box 20750
Nairobi
Kenya

H. Ensering (Miss)
Department of Virology
Medical Research Centre
Department of Royal Tropical
Institute
P.O. Box 20750
Nairobi
Kenya

Prof. Nimrod O. Bwibo
Professor and Chairman
Department of Paediatrics
P.O. Box 30588
Kenyatta Hospital
Nairobi
Kenya

Dr. W.H. Mosley M.D.
Population Studies and
Research Institute
P.O. Box 60190
University of Nairobi
Kenya

Ms. Betty Kirokwood (Statistician)
Tropical Epidemiology Unit
London School of Hygiene and
Tropical Medicine
Gower Street (Keppel St.)
London WC1F 7HT
United Kingdom

Professor Dr. Tatsuro Naito
Department of Bacteriology
Institute of Tropical Medicine
Nagasaki University
Japan

(Presently stationed Nairobi, Kenya).

Dr. I.A. Wamola
Department of Microbiology
Chairman
Diarrhoeal Disease Control Program
Ministry of Health
Nairobi
Kenya

Dr. Graham Beards (Virologist)
East Birmingham Hospital
Bordesley Green East
Birmingham B9 5ST
England
United Kingdom

Dr. Roy Saunders (Virologist)
East Birmingham Hospital
Bordesley Green East
Birmingham B9 5ST
England
United Kingdom

Dr. Gekonyo
Director
Kenya Institute of Medical Research
Ministry of Health
Nairobi
Kenya

Dr. H. Ramba (Parasitologist)
Department of Parasitology
University of Nairobi
Kenya

Dr. (Mrs.) N. Mirza
Department of Bacteriology
University of Nairobi
Kenyatta Hospital
Nairobi
Kenya

Dr. T.A. Siongok
Director
Divn of Communicable Disease Control
Ministry of Health
Nairobi
KENYA

Professor D.J. Bradley
Director
Ross Institute
London School of Hygiene and Trop.
London, U.K.

Joint meeting of ICDDR,B and Kenyan Scientists:

To work out the details of Scientific collaboration with Kenya a two member team (Drs. Rabbani and Samadi) from ICDDR,B paid a visit to Nairobi in late April 1981 and had discussions with the respective investigators in Nairobi, Kenya. At Nairobi, the ICDDR,B team met the following investigators: (a) Director of the Kenya Institute of Medical Research at the Ministry of Health, Government of Kenya, (b) Scientific investigators of the departments concerned at the University of Nairobi, Kenya, (c) the Paediatricians at the Kenyatta National Hospital and (d) many other investigators in the related field including the Japanese virology research team from the Nagasaki Universi presently stationed at Nairobi and the Dutch Research team from the Royal

Tropical Institute, Amsterdam. In two lengthy sessions the ICDDR,B team introduced their research objective in the field of Rotavirus diarrhoea to the Kenyan Health and Medical Research Authority. From the Kenyan side, the collaborative proposal for the study of Rotavirus diarrhoea was heartily welcomed. In the meeting it was decided that the clinical study will be conducted at the Diarrhoea observation ward of the Kenyatta National Hospital and laboratory work will be carried out at the Virus Research Centre in conjunction with the Royal Tropical Institute of Amsterdam in Nairobi and also at the departments of bacteriology and parasitology of the University of Nairobi, Kenya.

The preliminary protocol on Rotavirus submitted by Dr. G.H. Rabbani was discussed and the general principles were approved. However, some suggestion and alteration were made in the methodology for the protocol and these were later worked out at separate individual sessions. The methods for clinical observations were discussed and standardised between Drs. Rabbani and Kinoti by direct observation and demonstration on patients. The laboratory part of the study were also discussed among the investigators concerned and the methodology were standareised. The official resolutions of different meeting were enclosed at the end. (see Annex V, VI).

Funding of the Kenya Project:

The funding for Kenyan part of the study is expected to come from the WHO research grant for the CDD program, while Dr. Mutanda will be supported by the ICDDR,B. It was also decided that Dr. S.N. Kinoti who will be responsible for the clinical study at the Kenyatta Hospital would also pay a visit to Bangladesh and will be sponsored by ICDDR,B.

Time schedule:

It was anticipated that by the time the WHO fund is available for the Kenyan part of the study, the final protocol would be ready and work would eventually begin both at Dacca and Nairobi by the end September 1981.

Collaboration for virology work:

Since, the laboratory facilities for Serotyping the virus does not exist either at Dacca or Nairobi collaborative arrangements have been made with expert virologists in England and Belgium for the characterization of the virus. It is proposed that two types of characterization will be done on the virus isolate from Bangladesh and Kenya.

Characterization by Serotype:

It will be done by Professor Zissis at the Free University of Brussels in Belgium. Professor Zissis has already visited ICDDR,B research facilities at Dacca, Matlab and Teknaf in Bangladesh and has been in collaboration with ICDDR,B for Serotyping studies since 1979. He also visited the facilities at Nairobi, Kenya and is well cognigant about the background of this collaborative study. Technical details of how the faecal samples will be collected, preserved and shipped to Brussels for Serotyping has already been worked out with him.

Electropherotyping of the virus:

It will be undertaken by Professor Flewett at the WHO reference laboratory in Birmingham. It has been shown that viral RNA migration pattern on Polyacrylamide gel electrophoresis can be used to destinguish types, subtypes or strains of human rotaviruses. Since this method is extremely sensitive and reproduccible it may provide an important epidemiological tool for characterization of human rotaviruses strains.

It has been decided that Professor Zissis would exchange samples between his and Professor Flewett's laboratory to cross-check and correlate the virology results.

Dr. Mutanda will be responsible for the general supervision of the Kenyan project and he will also do the virology work at the Virus Research Centre as well.

Dr. W.H. Mosley who is presently working in Nairobi for the Population Council was an additional consultant and voluntary adviser to this Rotavirus Project and will be consulted in future for further progress of the work.

2.2 SCIENTIFIC BACKGROUND:

Epidemiologic and clinical studies of rotavirus by serotypes are crucial for the development of an effective rotavirus vaccine. Previously the prospects of a rotavirus vaccine was looked upon as a rare possibility because the virus was difficult to grow in conventional tissue culture systems to yield enough antigenic material. They also grew inefficiently in tissue culture of bovine intestinal cells (Albrey, 1976) and human embryonic kidney cell (Wyatt et al, 1976). Recently rotavirus were grown more effeciently in continuous line of African green monkey kidney cells (Bryden et al, 1977). Hopefully this might lead to further progress in the techniques of virus propagation and vaccine development.

After a brief review of existing literature it was observed that the knowledge about the clinical and epidemiological features associated with different serotypes of human rotavirus is truely non-existent. However such findings would be extremely important in the understanding of the relative role of the different serotypes in the natural history of the disease. Most

of the morbidity and mortality reports on rotavirus diarrhoea (not by serotype) came from hospital or nursury bas-d studies in the temperate countries like UK, USA, Canada and Australia and none has been reported from this part of the globe.

In this study, clinical features of rotavirus by serotype will be established in Bangladesh and Kenyan children over a period of one year. This will bring out the relative severity associated with each serotype of rotavirus in these two populations of different geographic locations. These informations are crucial for the understanding of relative virulence of each serotype and their pattern of circulation at different times of the year. Also the development of a prospective rotavirus vaccine depend to a great extent on the identification of number of serotypes existent in the community.

Review of current knowledge:

The rotaviruses have emerged during the past several years as a major public health problem of world-wide importance for which there are as yet no adequate control measures. The disease predominantly affects the children under 5 years of age with peak incidence in 6-24 month old children. Of the children hospitalized for gastroenteritis in various parts of the world, rotavirus infections have accounted for 42% to 55% (see Table).

PROPORTION OF CHILDREN HOSPITALIZED WITH ACUTE GASTROENTERITIS ASSOCIATED WITH ROTAVIRUS INFECTION

Geographic Location	Reference	Year	No. of cases	Percent with Rotavirus
Washington D.C.	Kapikian et al (1)	1976	143	42
Atlanta, Ga	Gomez-Barreto et al (2)	1976	29	55
Birmingham, UK	Flewett et al (3)	1974	73	54
Oslo, Norway	Orstavick et al (4)	1974	35	54
Melbourne, Australia	Davidson et al (5)	1975	378	52

Adapted from Gomez-Barreto et al (2).

The disease is observed frequently in children upto 4 years of age and occasionally in older children. Rotavirus are not often associated with diarrhoea in adults. Although in one study 35% of parents of infected children showed serological evidence of recent infection, illness occurred in only 3 to 14 parents (Kapikian et al, 1976). Most of the studies were conducted in hospitalized children and were likely to emphasize the severe forms of the disease. In late 70's studies from Bangladesh indicate that rotavirus and enterotoxigenic E. coli were associated with 70% of the dehydrating diarrhoea and 77% of the total potential deaths in Matlab population (6). In a prospective sero-survey in rural Bangladesh 93% of the children 0-4 years old was found to have antibodies against rotavirus and rises in the antibodies against rotavirus and rises in the antibody tire occured in 67% of these children (7).

Rotavirus serotypes:

None of the these studies have undertaken any effort to examine the serologic variants of the virus. In 1978 Flewett et al reported from Birmingham the existence of two serotypes of human rotavirus (Flewett et al 1978). Zissis and Lambert (Zissis et al, 1978) and Thouless et al 1978 have detected different serotypes using complement fixation and serum neutralization test respectively. Initially two, possibly three serotypes were reported. Later Flewett et al, 1978 produced evidence for the existence of a fourth using ELISA and Radio-immunoassay technique. Very recently Beards et al reported the confirmation of 3 and indicated that a fifth serotype probably exists (Beards et al 1980).

Electrophorotyping of rotavirus:

Rotaviruses are difficult to grow in conventional tissue culture and this has hampered the development of serologic tests by which the species identification and strain differences of the virus can be made. Because of this limitation of growth in-vitro, a different approach for distinguishing rotaviruses was undertaken on the basis of electrophoretic migration of viral nucleic acid. This method has also proven a useful tool for distinguishing many other viruses such as influenza virus, cytomegalovirus, herpes virus etc. The method consists in extraction and purification of viral RNA material from cell-culture grown human rotaviruses using centrifugation in cesium chloride gradient. A solution of RNA materials was then made in polyacrylamide gel and subjected to electrophoresis at $0^{o}C$ at a constant 200 volts for 5 hours. The gels were stained and photographed using a polaroid type 105 film and a short wave ultraviolet transilluminator. On the basis of differences in RNA migration pattern in polyacrylamide gel human rotaviruses can be seperated into 8-11 distinct bands which represent the viral genome structure (Newman et al 1975, Rodger et al 1975, Kalica et al 1976, Verly and Cohen 1977). It is important to note that a difference in RNA migration pattern does not necessarily reflect an antigenic difference. However, genetic studies with Bluetongue virus and reoviruses have now produced evidence that viral RNA segments which code for particular polypeptide on the viron is responsible for immunologic specificity (Huisman 1973, Weiner et al 1977). Determination of which segment is responsible for serotype specificity among the rotaviruses needs further research and development in immunologic techniques.

If the differences and similarities of RNA patterns for the human rotaviruses ultimately correlate with distinct serotypes among the human

rotaviruses, then the current analysis with human rotaviruses may have implications for understanding the epidemiology of rotavirus infection.

Epidemiologic study of Transmission:

Subsequent to the clinical study an epidemiological study will be undertaken in children in 3-4 villages in Matlab field area or Dacca urban area under a diarrhoea surveillance program where age and sex specific distribution of rotavirus serotypes and the incidence of clinical disease produced by each of them will be determined over a period of one year. This study would provide information about the relative prevelance of the different existing serotypes of rotavirus in this community at different times of the year.

This epidemiological field study will be undertaken after the clinical part of the project has efficiently progressed to a considerable extent and the serotyping art has been functioning fairely efficiently. Family studies will be undertaken and contacts of index cases will be followed, specifically examining the possible vehicle of transmission.

Rationale of the study:

Delineation of the clinical and epidemiologic spectrum of the disease caused by different serotypes of human rotavirus is expected to through adequate light in the understanding of the disease process both in the individual patient and in the community as a whole.

The difference in the relative prevelance of the various serotypes in different seasons of the year may have important implications in the development of control strategies particularly vaccines. The clinical disease that may be associated with different serotypes of rotavirus producing varying degrees of severity may indicate different lines of management. The main

objectives of this study are to define the total number of human rotavirus serotypes in Bangladesh and Kenya to determine their type specific clinical and epidemiological features.

B. SPECIFIC AIMS:

This study will be designed to answer the following questions as specific aims:

1. How many serotypes of rotavirus are associated with acute gastro-enteritis in children of two geographically distinct population of Asia and Africa ?
2. What is the relative importance of different serotype in clinical severity of the disease in these two population ?
3. Whether or not the clinical expression of the type-specific disease are different in population of different time, place and person ?
4. Whether infection by one serotype protects the host from subsequent attacks ?

C. METHODS AND PROCEDURES:

The methods and procedures as described in the previous protocol submitted by Dr. G.H. Rabbani were reviewed and discussed in detail between the clinicians from ICDDR,B and Kenyatta National Hospital. The following methods have been developed after complete review and will be followed for clinical evaluation of the patients both in Kenya and Bangladesh Research Centre.

Size of the sample: It was decided that 500 patients under five years of both sexes will be studied over one year period both in Kenya and Bangladesh.

Selection of patients: All patients reporting at the Kenyatta National Hospital, Nairobi and the Treatment Centre at ICDDR,B, Dacca will be eligible

for the study. Patients should have a hisotry of acute diarrhoea with 3 or more watery or loose motions within the last 48 hours without receiving a treatment with antibiotics, before coming to the hospital. All patients with a severe and chronic infection(s) and those with 3rd degree malnutrition will be excluded from the study. (For criteria of severe infection and malnutrition - see Annexture I,II).

Randomization: At each centre, 2 children will be selected randomly for admission into the study each day. Randomization will be made by taking every 3rd child who would meet the study criteria. If the 3rd child does not satisfy the admission criteria then the next will be selected and so on. Each child admitted into the study will be identified by a study number and will be written in the admission chart.

Clinical Assessment: History: Immediately on admission a detailed clinical history of present illness will be obtained from the attendant of the child and will be recorded in prescribed forms. This will include the personal memorandam of the patients, duration and type of diarrhoea, treatment received, frequency of vomiting and general food habit. (for clinical history see Annexture III).

Assessment of dehydration: A complete physical examination will be made by the attending physician and height and weight will be recorded. Degree of dehydration will be classified into mild, moderate and severe types as judged by the clinical signs and symptoms. These observations will include examination of skin turgor, anterior fontanelle, eyes, tongue, mucus membrance, radial pulse etc. (for sign/symptoms of dehydration - see Annexture IV). Status of dehydration will be repeatedly examined at 4 hours and 8 hours after

admission and then every 24 hours till the patient is discharged.

Standardization of clinical observations: Clinical observation generally varies to a considerable extent in most cases between physician and non-physician observers. To minimize such interobserver variations the clinical methods to be followed in this study have been standardized by practical demonstration between the investigators at the paediatric observation ward at the Kenyatta National Hospital. On the same patients, blind observations were exchange for the determination of degree of dehydrations, fluid deficit, etc. and were statistically evaluated to examine their extent of correlation. After such trial, the standardized methodology has been developed. Dr. S.N. Kinoti, who will be conducting the clinical aspects of the study in Kenya is expected to visit ICDDR,B to see Bengali diarrhoea patients for himself and to cross-standardize the methodology at Dacca following a similar procedure as was done in Kenya. After such procedure, the final flow-sheet for recording clinical observations will be developed and will be used both in Kenya and Bangladesh. Patients will be clinically evaluated following a list of clinical variables (see Annexture V).

Treatment and Management: Rehydration: Rehydration will be started with intravenous fluid only in cases of severe degree of dehydration and then switched over to oral fluid for subsequent maintenance provided the child is able to tolerate the oral rehydration. In mild and less severely dehydrated patients only oral fluid will be used both for initial replacement and subsequent maintenance. No antibiotics will be given to patients throughout the course of illness unless there is indication(s) for giving antibiotics, such as isolation of Vibrio Cholerae or pathogenic shigellae from the stool. However, treatment with multivitamin, aspirin or topical skin lotion may be prescribed

when indicated. For patients with pyrexia, antipyretic drug such as pracetamol may be given in regular does.

Patients will be kept in the hospital ward so long as the diarrhoea continues (average 3 days of hospitalization) and will be discharged when the stool has become formed.

Type of IV-Fluid: In Nairobi at present Half strength Darrow's solution is being used for intravenous rehydration. This solution has similar electrolyte composition with "Dacca Solution" (Acetate) being used in ICDDR,B. In addition Darrow's solution has added glucose in it whereas Acetate has not.

Half Strength Darrow's	Half Strength Acetate (Dacca Solution)
Sodium 62 MEQ/L	67 MEQ/L
Potassium 18 MEQ/L	7 MEQ/L
Bicarbonate 28 MEQ/L	24 MEQ/L
Chloride 52 MEQ/L	48 MEQ/L
Glucose 120 MMOL/L	NIL

However, after Dr. Kinotis visit to ICDDR,B attempts will be made to prepare IV-solution with identical electrolyte composition to treat patients at both places.

Oral fluid: For use as oral rehydration solution WHO formula will be used both in Dacca and Nairobi.

WHO oral fluid composition

Na	90 MEQ/L
K	20 MEQ/L
C	80 MEQ/L
HCO_3	30 MEQ/L

Amount of fluid to be given: In dehydrated patients, initial deficit will be replaced within 6 hours of admission depending upon the percent of body weight lost. The volume will be replaced according to the following principles.

1. In severly dehydrated patients 10% of body weight loss will be replaced within the first 6 hours.
2. In moderate cases 6-9% will be replaced and
3. In mild cases 5% will be replaced.

Maintenance: If diarrhoea continues, hydration will be maintained by replacing fluid to match the loss measured at 8 hourly intervals. The requirement will be calculated according to the following principle.

0-1 month	=	125ml/Kg/24h
1-12 month	=	150ml/Kg/24h
12m-5 yrs.	=	100ml/Kg/24h

However, in every cases the amount of fluid to be given will be assessed on the clinical grounds by the physician on the merit of individual patients.

Intake - Output measurement: At every 8 hour, total volume of intake and output of fluid will be recorded.

The intake will include :
1. IV - fluid
2. Oral fluid
3. Plain water
4. Breast milk
5. Bottle milk

The output will include
1. Stool volume
2. Urine volume
3. Vomitus

Output recording: At the ICDDR,B stool will be collected and measured using specially designed cholera cots for children (see Figure below). Urine will be collected for quantitation using special plastic urine collecting bags. Vomitus will be collected in small containers.

At the Nairobi Hospital, similar facilities for excreta collection do not exist at the moment. It has been suggested that after Dr. Kinoti's visit to ICDDR,B some kind of collection method will be developed for use at the Kenyatta Hospital for the study. The following suggestion were made:

1 Weighting the child at frequent intervals
2 Using special plastic pants
3 Devising Cholera cots.

Input recording: The attending nurses would record the volume of IV and oral fluid given at definite time intervals. For the breastfed babies cases will be grouped as follows:

1 Breastfed plus ORS
2 Non Breastfed plus ORS

For the quantification of breast milk ingested by the baby, lactating mothers may be randomly sampled to estimate the average volume of milk at each feed.

Rotavirus in the stool: Collection, preservation and mailing of samples from Dacca - Kenya.

1. On admission stool samples will be collected using rectal cathaters from children with rotavirus diarrhoea in Dacca and Nairobi.
2. Within one hour of collection - the stool samples will be divided into 4 aliquots of 1 dram vials, labell and freez at less than - 20°C using Revco Freezers.
3. All these samples will be kept frozen at - 20°C until tested.

4. When shipped to England or Belgium for serotyping they will be packed in insulated box with dry ice to prevent thawing. Shipment of specimens will be within 3 to 4 months or even earlier than that depending upon the size of collection.

5. Data sheet will be prepared and each sample will be numbered with patient's identification and date.

6. How the results of serotyping-test would be expressed has to be developed after discussion with laboratory personnels.

Exchange of samples between virology labs:

It has been agreed after discussion among W.B. Greenough, Flewett, Zissis, Rabbani, Merson and Barua (WHO) that all stool specimen from Dacca and Nairobi will go to Dr. Zissis in Brussels first as per the original protocol of Dr. Mutanda. He (Zissis) and Dr. Flewett will later exchange standard sera and samples for Rotavirus assay.

Two categories of typing studies will be done on the isolated samples of Rotaviruses. Dr. Flewett is expected to carry out electrophoresis of viral RNA to determine the electropherotypes of the virus. This will be done at the WHO reference laboratory at Birmingham in association with Drs. Sanders and Graham Beards. On the other hand conventional serotyping of the Rotavirus will be done by Dr. Zissis at Brussels, Belgium in association with Dr. Lambert.

Clinical investigation:

Baseline laboratory investigations will be done immediately after clinical examination, before starting any rehydration. 3cc venous blood will be drawn in all cases for initial electrolytes, specific gravity and osmolity determination. Electrolytes would include the following: Sodium, Potassium, Bicarbonate and Chloride. Others will include: Glucose, Creatinine and Blood Urea Nitrogen (BUN). Finger prick blood for specific gravity will be repeated

at 8 hours and 24 hours after admission. Blood haemotocrit and complete blood count will be performed on admission and when clinically indicated. Differential white count will be done and malaria parasite will be looked for in blood smears.

Other pathogens in the stool:

Besides rotavirus, other diarrhoeal pathogens will also be looked at for clinical evaluation of mixed infection. Rectal swabs from all patients will be plated on Telurite-Taurocholate-Gelatin-Agar (TTGA), Mansoor's Agar, Salmonella Shigella Agar (SS media) and Mac Conkey's Agar and the Plates will be incubated for 18-24 hours. These plates will be looked for Vibrio cholerae, shigella and salmonella. From the Mac Conkey's plate 5 lactose positive colonies will be picked those are typical of E. coli and will be stored in blood agar slant for testing Heat-Labile (LT) toxin using either Chinese Hamster Ovary cell assay (CHO) or Y_1 Adrenal cell assay. Two of the colonies will be tested for Heat-Stable toxin (ST) by infant mouse assay.

Intestinal Ova and Parasites:

For intestinal ova and parasites (Giardia, E. histolytica), stool samples will be examined using MIF (Merthiolate-Iodine-Formaline) concentration technique on the day of admission and on 4th and 7th post admission days.

Statistical analysis:

Data will be collected in precoded forms and be directly transfered to IBM Cards or diskette without intermediate coding. Analysis will be mainly carried out using IBM-34 at ICDDR,B Computer Centre. Data will mainly consist of clinical descriptive variables such as frequency of vomiting, degree of dehydration, electrolyte concentrations, volume of fluid required etc in each

type specific groups. These frequencies will be compared using appropriate statistical principles between the Bangladeshi and Kenyan children. The following statistical significance tests may be used - Chi Square test, Students T-Test, Analysis of variance (Anova), correlation and regression analysis etc. It is to be mentioned here that if required overseas collaboration will be made for statistical consultancy with Betty Kirkwood, London. Data from Nairobi and Dacca study will be analysed together to examine the comparibility of the clinical variables. Hypothetical tables for analysing data have been shown in Table 1-4.

D. SIGNIFICANCE

(See Rationale)

E. FACILITIES REQUIRED

Office space for Dr. Rabbani: The present study room will be utilized. Some new furniture like desk, file cabinet may be needed.

Laboratory space: Existing ICDDR,B Laboratory (Immunology, Biochemistry and Microbiology) will be utilized.

Hospital resources: Study physician (one) and full time ward nurse (two) will be required.

Animal resources: 300 suckling mice for ST assay will be required.

Logistic support: Data processing and computer support from the Statistical Branch of ICDDR,B.

Major items of equipments: None.

Others: None

Transport: Transport will be supported by the existing transport facilities.

F. COLLATORATING ARRANGEMENTS:

For the Serotyping work of rotavirus collaborative arrangements have been provisionally made with Professor G. Zissis, at St. Piere Hospital, Free University, Brussels, Belgium. Faecal specimen from the study patients will be sent to him for serotyping analysis in batch of samples. Similar collaboration will also be made from Dr. Flewett in Birmingham, England for serotyping. This will ensure a cross-check between different laboratories.

REFERENCES

1. Kapikian AZ, Kim HW, Wyatt RG, Cline WL, Arrobio JO, Brandt DC, Rodriguez WJ, Sack DA, Chanock RM, Parrot RH: Human Reovirus like agent as the major pathogen associated with 'winter' gastroenteritis in hospitalized infants and young children. *N Eng J Med* 294:964-972, 1976.

2. Comez-Barreto J, Palmer EL, Nahmias AJ, Bat h MH: Acute enteritis associated with reovirius-like agents. *JAMA* 235:1857-1869, 1976.

3. Flewett TH, Davies H, Bryden AS, Robertson MJ: Acute gastroenteritis associated with reovirus-like particles. *J Clin Pathol* 27:608-614, 1974.

4. Orstavick I, Figenschaw KJ, Ulstrup JC: Rotavirus in stool specimen of faecal extracts. *Lancet* 2:1083, 1974.

5. Davidson GP, Bishop RF, Townley RRW, Holmes IH, Ruck BJ: Importance of a new virus in acute sporadic enteritis in children. *Lancet* 1:242-246, 1975.

6. Black RE, Merson MH, Huq I, Alim ARMA, Yunus M: Incidence and severity of diarrhoea associated with Rotavirus and Enterotoxigenic Escherichia coli in rural Bangladesh (in press). 1980.

7. Sack DA, Gilman RH, Kapikian AZ, Aziz KMS: Seroepidemiology of Rotavirus infection in rural Bangladesh. *J Clin Microbiol* 11(5):530-532, 1980.

8. Flewett TH, Thouless ME, Pilfold JN, Bryden AS, Candeias J: More serotypes of human rotavirus. *Lancet* 2:632, 1978.

9. Zissis GP, Lambert JP: Different serotypes of human rotavirus. *Lancet* 1: 38, 1978.

10. Thouless ME, Bryden AS, Flewett TH: Serotypes of human rotAvirus. *Lancet* 1: 39, 1978.

11. Bryden AS, Davies HA, Thouless ME, Flewett TH: Diagnosis of rotavirus infection by cell culture. *J Gen Microbiol* 10:121-125, 1977.

12. Beards MG, Pilfold JN, Thouless ME, Flewett TH: Rotavirus serotypes by serum neutralization. *J Med Virol* 5:231-237, 1980.

13. Albrey MB, Murphy AM: *Letter:* Rotavirus growth in bovine monolayers. *Lancet* 1: 753, 1976.

14. Wyatt RG, Gill VW, Serero MM, Kalika AR, Vankirk DH, Chanock RM, Kapikian AZ: *Letter:* Probable invitro cultivation of human reovirus like agent of infantile diarrhoea. *Lancet* 1: 98-99, 1976.

15. Jelliffe DY: Diarrhoea in childhood; *In* Medical care in Developing countries, Oxford University Press, Nairobi, Chap. 15.

16. Kalica AR, Sereno MM, et al: Comparison of human and animal Rotavirus strains by get eleotrophoresis of viral RNA: *Virology* 87(2):247-255, 1978.

17. Newman JFE, Brown F, Bridger JC, Woode GN: Characterization of Rotavirus. *Nature* (London) 258:631-633, 1975.

18. Rodger SM, Schnagi RD, Golmes IH: Biochemical and biophysical characterization of diarrhoea viruses of human and calf origen. *J Virology* 16: 1229-1235, 1975.

19. Kalica AR, Garon CF, Wyatt RG, Mebus CA, Vankirk DH, Chanock RM, Kapikian AZ: Differentiation of human and calf reovirus like agents associated with diarrhoea using polyacrylamide gel electrophoresis of viral RNA. *Virology* 74: 86-92, 1976.

20. Verely E, Cohen J: Demonstration of size variation of RNA Segments between different isolates of calf rotavirus. *J Gen Virology* 35: 583-586, 1977.

21. Huismans H, Howell PG: Molecular hybridization studies on the relationships between different serotypes of blue tongue virus and on the difference between virulent and attenuated Strains of the same Serotype. *Onderstepoort J Vet Res* 40: 93-103, 1973.

22. Weiner HL, Fields BN: Nutralization of reovirus; The gene responsible for the nutralization antigen. *J Exp Med* 146: 1305-1310.

SECTION III - BUDGET

A. DETAILED BUDGET

PERSONNEL SERVICES:

NAME	Position	% effort	Annual salary US $	Project requirement US $
Dr. G.H. Rabbain	Principal Investigator	60%	4479	2688
Dr. S.N. Kinoti	Investigator, Nairobi	ICDDR,B Honorarium		
Dr. L.N. Mutanda	Co-Investigator, Nairobi	5%	58280	2914
Dr. A.R. Samadi	Co-Investigator	5%	67320	3366
Dr. Zissis	Collaborator (Virologist)	5%	-	-
Dr. Flewett	Collaborator(Virologist,WHO)	5%	-	-
Betty Kirkwood	Consulting Statistician	3%	-	-
Dr. Shafiq Alam	Co-Investigator	100%	2400	2400
Dr. P.K. Bardhan	Research Physician	20%	2400	480
Nilóofar	Microbiology Technician	30%	1600	480
Dr. W.B. Greenough	Advisor	1%	-	-
Mr. Huda (ELISA Analyst)		10%	1500	150
Animal Technician (for E. coli Toxin)		10%	1500	150
SUB TOTAL				US $ 12,628

SUPPLIES:

ITEMS	Unit Cost Taka	Amount required	Project requirement Taka	Dollars
Culture for V. cholerae	3.28	300		65
Culture for Shigellae/Sal	2.50	300		50
Culture for E. coli	2.00	300		40
ST Assay	3.00	300		60
ELISA Assay (Rotavirus)	1.50	300		30
SUB TOTAL				US $245

EQUIPMENTS:

Calculators	1	US $ 80	Tk.4,800.00	US $ 320
	SUB TOTAL		Tk.4,800.00	US $ 320

PATIENTS HOSPITALIZATION:

Number of patients days @Tk.150.00/day:		Tk.2,25,000.00	US $ 15000
500 patients (3 days stay) @$10.00/day	SUB TOTAL		US $ 15000

OUT PATIENTS CARE: None.

CRL TRANSPORT:

2 trips to Matlab and back per week		Tk.4,800	US $ 320
48 (Total trips, 2880 milage)	SUB TOTAL	Tk.4,800	US $ 320

TRAVEL AND TRANSPORTATION PERSONS:

Exchange of visits for investigators		US $ 10,000
between Bangladesh - Kenya	SUB TOTAL	US $ 10,000

TRANSPORTATION OF THINGS:

Import : None.

RENT, COMMUNICATION AND UTILITIES: None

PRINTING AND REPRODUCTION:

Forms and record sheet		Tk. 4,000	US $ 226
Publication Cost		Tk. 3,000	US $ 200
Reprint puchase		Tk. 3,000	US $ 200
	SUB TOTAL	Tk.10,000	US $ 666

CONTRACTUAL SERVICES:

Computer time @Tk.800/hour:		Tk. 25,000	US $ 1,666
	SUB TOTAL	Tk. 25,000	US $ 1,666

B. BUDGET SUMMARY

	Category	US Dollars
1	Personnel Services	12,628
2	Supplies	245
3	Equipments	320
4	Patients Hospitalization	15,000
5	Out patient care	None
6	Transport	320
7	Travel of Persons	10,000
8	Transport of Things	None
9	Rent/Communication	None
10	Printing and Reproduction	666
11	Contractual Services	1,666
SUB TOTAL		US $ 40,845

Staff comitment	US $ 12,628
Operational cost	US $ 28,217
GRANT TOTAL	US $ 40,845

ABSTRACT SUMMARY

A collaborative study to determine the serotype specific clinical and epidemiological features of gastroenteritis associated with rotavirus in Bangladeshi and Kenyan children is planned. 500 hospitalized children aged 0-5 years will be studied at the ICDDR,B Hospital, Dacca over a period of 12 months, starting from September 1981. Clinical study would include duration of illness, course of the disease, severity of dehydration, volume of stool lost, history of vomiting, physical description of stool and subjective and objective clinical symptoms. Similar study will be undertaken in Kenya in collaboration with the Kenyan scientists in Nairobi for which standardized techniques have been developed to measure these characteristics between Kenyan and Bangladeshi children. This collaborative study is expected to provide basic clinical characteristics which would help a clinician to diagnose rotavirus diarrhoea among gastroenteritis patients. Clinical expression of mixed infection with shigellae, salmonellae, E. histolytica, V. cholerae and Enterotoxigenic E. coli will also be examined.

As an extension of this study, an epidemiologic study, would start at Matlab Field area to investigate the epidemiologic features of Rotavirus diarrhoea by serotypes. This will include the determination of the most prevalent serotype in the community, the effect of seasonal variation, case to infection ratio, age sex distribution etc.

Characterization of the virus strains by serotyping will be done in collaboration with Prof. G. Zissis at the Free University of Brussels, Belgium. Also electrophorotyping of the virus will be done by Professor Flewett at the WHO viral reference laboratory in Birmingham, U.K.

ANNEXTURE I

CRITERIA FOR SEVERE INFECTION

Those children with the following criteria will be excluded from the study:

1 Meningitis

2 Pneumonia

3 Schistosomiasis

4 Evidence of Renal disease

5 Cardiovascular disease

6 Convulsion

7 Kala-azar with hepato spleeno megally

8 Asymptomatic parasitemia for malaria will be included.

9 Chronic infection such as Tuberculosis, filariasis, amoebic hepatitis will be excluded.

ANNEXTURE II

CRITERIA FOR DIAGNOSING MALNUTRITION

In Bangladesh, standard of reference for classifying degrees of malnutrition on the basis of anthropometric data is not yet available. Use of western standard for nutrition (Harvard, NCHS, Gomes etc.) for Bangladesh children may not be satisfactionally applicable. However, we prefer to use the data available from Mehran growth study in Matlab as a standard of reference of nutritional status for local children in Bangladesh. (Khan et al. 1979, Bangladesh Medical Journal 7:74-90).

Weight for age is considered reasonably satisfactory upto the age of 2 years. However, weight for height is considered more useful indicator in older children or in cases where age is not accurately known.

3rd degree malnutrition: (Using local standard):

1 Children with 60% and below weight for age.
2 Children with 70% and below weight for ht.
3 All cases of nutritional oedema
4 Children with mid-arm circumstances less than 12 cm.

McLaren's classification:

MacLaren's classification of the severe forms are the following:

1 Marasmus
2 Marasmic kwashiorkor
3 Kwashiorkor

This classification is based on simple scoring system as follows:

Scoring system for PEM (after MacLaren:

Signs present	Points
WT/HT - 80% with Oedema	5
WT/HT - 70-80% with Oedema	4
WT/HT - Below 70% with Oedema+	3
WT/HT - 70-80% without Oedema	2
WT/HT - Below 70% without Oedema	1
Oedema + Dermatosis (WT/HT not counted)	6
Hepatomegaly	1
Hair changes	1
Dermatosis	2

Total protein

Less than 3.25	7
3.25 - 3.99	6
4.00 - 4.74	5
4.75 - 5.49	4
5.50 - 6.24	3
6.25 - 6.99	2
7.00 - 7.74	1

Score = Sum of points

0 - 3 = Marasums (67.5% or less)

4 - 8 = Marasmic Kwashiorkor

9 - 15 = Kwashiorkor

ANNEXTURE III

CLINICAL HISTORY

1. Patients information:

Name: _____ Age: / / / (7-8) Sex: / / (9) (M=1, F=2)

Patient No. / / / / / / (10-14)

2. History of patient:

a) Duration of diarrhoea (h): / / / (15-16)

b) Nos. of stool/day: / / / (17-18)

c) Stool consistency: / / (19) (Liq=1,Soft=2,Formed=3)

d) Quality of stool: / / (20) (Normal=1,Small=2,Large=3)

e) Colour or stool: / / (21) (Normal=1,Green=2,Clay=3)

f) Blood or mucus: / / (22) (Present=1, Absent=2)

g) When last urinated? (h): / / / (23-24)

h) Colour of Urine: / / (25) (Normal=1,Red=2,Yellow=3)

i) Urine quantity: / / (26) (Normal=1, Less=2)

j) Fever: / / (27) (Yes=1, No=2)

k) Is child breast-fed? / / (28) (Yes=1, No=2)

l) What was given to child since diarrhoea started? / / (29) (Rice water=1, Barley=2, Cow's milk=3,Bottle milk=4, 1+2=5, 1+3=6)

m) Food taken during the last 24 h. Specify _____

n) Medicine taken: / / (30) (Antibiotic=1,ORS=2, Homeop=3)

o) Source of drinking water: / / (31) (Tap=1,Well=2,Tank=3, Tube=4)

p) Vomiting frequency: / / (32) (1-3=1,3-5=2, >5=3)

q) Diarrhoea or vomiting appeared first? / / (33) (Diarrhoea=1, Vomiting=2)

ANNEXTURE IV

ASSESSMENT OF DEHYDRATION

MILD DEHYDRATION:

Mental State	Alert or Restless
Sense of Thirst:	Thirsty
Body wt. loss:	4%- 5%
Estimated fluid deficit:	40 - 50ml/Kg

MODERATE DEHYDRATION*:

Mental State:	Alert, Restless, Lathergic
Radial Pusse:	Rapid and weak
Ant. Fontanelle:	Sunken or depressed
Blood Pressure:	Normal - Low
Skin Elasticity**:	Pinch Retract slowly
Mucus Membrane:	Dry
Eyes:	Sunken
Tears:	Absent
Urine Flow:	Reduced and Dark
Body wt. loss:	6%- 9%
Estimated fluid deficit:	60 - 90ml/Kg

* As a guide if two or more signs present indicate moderate dehydration

** Skin Turgor is of no value in malnourished children

SEVERE DEHYDRATION:

Mental sign:	Conscious, Drowsy, Comatose
Extrimities:	Cold, Sweaty, Cyanotic, wrinkled skin of fingers and toes.
Radial Pulse:	Very Rapid, Feble, Impalpable
Respiration:	Deep and very rapid
Ant. Fontanel:	Very depressed
Skin Elasticity**:	Pinch Retracts very slowly more than 2 secs
Mucus membrane:	Very dry
Tears:	Absent with straing eyes
Urine flow:	None passed more than 8 hours
Body weight loss:	10% or more
Estimated fluid deficit:	100 - 110ml/Kg

*As a guide 2 or more signs present indicate severe dehydration.

** Skin elasticity is of no value in malnourished children.

TABLE 1

FREQUENCY DISTRIBUTION OF DIFFERENT SEROTYPES OF ROTAVIRUS BY AGE GROUPS

Age Group	Type 1		Type 2		Type 3		Type 4		Type 5	
	M	F	M	F	M	F	M	F	M	F
0 - 6m	No (%)									
7m - 1yr										
2 - 3 yrs										
4 - 5 yrs										
5 yrs										
TOTAL										

(Format for Data Analysis)

TABLE 2

PLAN FOR DATA TABULATION

CLINICAL FINDINGS OF CHILDREN INFECTED WITH DIFFERENT SEROTYPES OF ROTAVIRUS

	Type 1	Type 2	Type 3	Type 4	Type 5
Vomiting					
Fever					
Dehydration					
Mild					
Moderate					
Severe					
Adp Pain					
Rash					
Cramping					
Tenesums					
Stool					
Blood					
Mucus					
Watery					
Soft					
Formed					

(Format for Data Analysis)

TABLE 3

SEROTYPE-SPECIFIC CLINICAL FEATURES OF ROTAVIRUS
IN BANGLADESHI AND KENYAN CHILDREN

	BANGLADESH					KENYA				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Duration of diarrh(Pre-Adm)										
Duration of diarrh(Post-Adm										
Volume of Stool(Litres)										
Volume of Urine (Litres)										
Volume of IV given										
Serum Electrolytes										
Sodium (m.mols/L										
Potassium (")										
Bicarbonate (")										
Chloride (")										

(Format for Data Analysis)

TABLE 4

MIXED INFECTION IN ASSOCIATION WITH DIFFERENT SEROTYPES OF ROTAVIRUS

	BANGLADESH			KENYA		
	Shigella T1 T2 T3 T4 T5	Cholera	E. coli	Shigella	Cholera	E. coli
0 - 6m						
7m - 1 yr						
2 - 3 yrs						
4 - 5 yrs						
5 yrs						

(Format for Data Analysis)

FLOW SHEET 1

Patient's Name:_____ Age:_____ Sex:_____

Date of Adm._____ Adm. No._____

	Blood sp.gr.	Hct	Eyeball	Pulse	Mucus memb	Skin Turgn	Fontanell	Thirst	Stool vol (litres)
Adm	(34-39)	(40-41)	(42)	(43-45)	(46)	(47)	(48)	(49)	(50-52)
4h after Adm		(53-54)	(55)	(56-58)	(59)	(60)	(61)	(62)	(63-65)
8h after Adm	(66-71)	(72)	(73)	(74-76)	(77)	(78)	(79)	(80)	(81-83)
24h after Adm	(84-89)	(90)	(91)	(92-94)	(95)	(96)	(97)	(98)	(99-101)
48h after Adm	(102-106)	(107)	(108)	(109-111)	(112)	(113)	(114)	(115)	(116-117)
72h after Adm	(118-123)	(124)	(125)	(126-128)	(129)	(130)	(131)	(132)	(133-135)
			1=Normal 2=Sunken 3=Dry	1=Normal 2=Week 3=Absent	1=Normal 2=Dry 3=Moist	1=Normal 2=Mod 3=Sev 4=Mild	1=Normal 2=Depressed	1=Present 2=Absent	

FLOW SHEET 1 (contd.)

Patient's Name: _____ **Age:** _____ **Sex :** _____

Date of Adm.: _____ **Adm. No.** _____

	Stool Quality	Mucus or Blood in stool	Total vol I.V. given (lits)	Total ORS (ml)	Urine Volume (ml)	Milk intake (ml)	Vomiting (Nos)	Temp (oC)
Adm	136	137	138-139	140-141	142-143	144-146	147-148	149-150
4 h	151	152	153-154	155-156	157-159	160-162	163-164	165-166
8 h	167	168	169-170	171-172	173-174	175-177	177-178	179-180
24 h	181	182	183-184	185-186	187-189	190-192	193-194	195-196
48 h	197	198	199-200	201-202	203-205	206-208	209-210	211-212
72 h	213	214	215-216	217-218	219-221	222-224	225-226	227-228

1= Formed
2= Soft
3= Liquid

1=Mucus
2=Blood
3=Both
4=None

1=Breast milk
2=Cow's milk
3=Formula

FLOW SHEET 2

Patient's Name:_____ Age:_____ Sex:_____

Date of Adm:_____ Adm. No.:_____

Adm	Abd Pain	Abd Tenesums	Skin Rash	Bowel Sound	Lymph Gland Enlargement	Bleeding Spots	Convulsions	Coma
24 h	☐ 213	☐ 214	☐ 215	☐ 216	☐ 217	☐ 218	☐ 219	☐ 220
48 h	☐ 221	☐ 222	☐ 223	☐ 224	☐ 225	☐ 226	☐ 227	☐ 228
72 h	☐ 229	☐ 230	☐ 231	☐ 232	☐ 233	☐ 234	☐ 235	☐ 236
	1=None 2=Mild 3=Mod Pain 4=Sev Pain	1=None 2=Present	1=Present 2=Absent	1=Present 2=Absent	1=Enlarged 2=Normal	1=Present 2=Absent	1=Present 2=Absent	1=Present 2=Absent

LABORATORY INVESTIGATION

Pt. No. _____

Serum Electrolytes	Urine analysis	Stool	Blood
NA 237-239	PH 268-269 (Acid-1,Alk=2)	Colour 288 (1=Nor. 2=Red, 3=Black)	Hct 299-300
K 240-242	Sp. gr. 270-275	Smell 289 (1=Nor. 2=Foil)	TWBC 301-302
Cl 243-245	Albumin 276-278 (1=Presnt,2=Abs. 3=Trace)	(Consistency) Quality 290 (Liq-1 Formed =2)	Poly 303-306
HCO3 246-249	Sugar 279-280 (1=Present,2=Abs.)	Puscell 291-293	Lympho 305-306
Sp. gr. 250-255	Puscell 281-283 (Nos)	RBC 294-296	Bands 307-308
Bun 256-259	Cast 284-285 (Present=1,Abs=2)	Ova/Parasites	Eosino 309-310
Creatinine 260-263	Epth. cell 286-287 (1=Present,2=Abs.)	Macrophage 297-298	Platelets 311-312
Urea 264-267	Creatinine 313-316		

CONSENT FORM

ICDDR,B is carrying out research on rotavirus diarrhoea to characterize the clinical severity of acute Gastroenteritis associated with different serotypes of the virus. This study will provide knowledge about the clinical characteristics of serotype specific disease and help diagnosis of rotavirus gastroenteritis: These information will also help the development of Rotavirus vaccine for long term control.

If you want to have your child participate in this study then you can expect the following:

(a) Your child with diarrhoea will be hospitalized in the study ward for 3-4 days. Foods will be provided for the attendants of the child.

(b) Proper treatment will be given using oral fluid, IV fluid, antibiotics and other drugs and all stool, urine and vomitees will be collected and measured. These specimens will also be tested to detect disease.

(c) Blood (2 C.C.) will be drawn from arm vein to determine electrolytes. Finger prick blood will also be taken twice for testing.

(d) You will enjoy full right to withdraw from the study at any point in time after admission.

(e) You will also have the right to refuse admission into the study.

In either case, your child will get proper treatment and would not be penalised in any way.

(f) Results of the study will be published without reference to the identity of the individuals.

(g) All records will be kept confidential.

If you want to participate, please sign your name below:

Signature_____

Admn. No._____

Date_____

MEDICAL RESEARCH CENTRE
DEPARTMENT OF THE ROYAL TROPICAL INSTITUTE, AMSTERDAM (NETHERLANDS)

P. O. Box 19762
NAIROBI (KENYA)
PHONE 28727/-28/-29
CABLE ADDRESS "KETROPELTH"

NAIROBI, 2nd May, 1981

Ref. No.....

ANNEXTURE V

COLLABORATIVE STUDIES OF ROTAVIRUS

Minutes of the Joint Meeting of University on Nairobi Kenya Institute of Medical Research & International Centre for Diarrhoeal Diseases Research, Bangladesh (28/4/1981)

Participants:

Dr. Wamola (Chairman)
Miss Ensering
Dr. Kinoti
Dr. Mutanda
Dr. Pamba
Dr. Rabbani
Dr. Samadi (Rapporteur)
DR. R. Nsenze (absent)
Dr. Tukei

The meeting opened at 11.30a.m. 28/4/1981 in the conference room of Virus Research Centre, Kenya. Dr. Tukei introduced Dr. Wamola as the chairman of Diarrhoeal Disease Control Program of Kenya and asked upon him to take the chair.

Dr. Wamola opened the meeting by explaining that in June 1980 the Government of Kenya established a Diarrhoeal Disease Control Program. A WHO team came and assisted in devising a program for control of diarrhoeal diseases control. It was planned that the problem of diarrhoeal diseases to be incorporated into framework of health services. He pointed out to previous studies of Dr. Mutanda in Kenya that revealed over 40% of diarrhoeas are due to rotavirus. The other etiologies for diarrhoeal diseases are shigellosis, salmonellosis, ETEC and EPEC. He also referred to some preliminary work that showed high incidence of campylobacter in children (over 10%).

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Dr. Wamola added that protozoa and helminths are also common. He referred to significance of research on etiology of diarrhoeal diseases, community studies and ORS studies to see which modification of ORS may suit Kenya.

Dr. Tukei commented that they are happy to have Dr. Mutanda whose previous work is appreciated. He pointed out that they have submitted the rotavirus project to WHO for funding and it seems that in August 1981 decisions will be taken by WHO and the fund would be available in November 1981. He also referred to Japanese input and mentioned that Dr. Makino, the Japanese virologist will work together with Dr. Mutanda in the same laboratory.

Dr. Samadi briefed the group on ICDDR,B as an international centre for diarrhoeal disease research. He explained the objectives of ICDDR,B as follows: To undertake and promote research on diarrhoeal disease and related subjects with a view: (1) to developing improved methods and standards for control of diarrhoeal diseases to be utilized by developing countries within the frame of public health programs, (2) to disseminate the knowledge gained through research on diarrhoeal diseases and related subjects, and, (3) to provide facilities for training of local and international staff in the field of diarrhoeal diseases control programs.

Dr. Samadi referred to Diarrhoeal Diseases Control Program of Kenya and commented that it is a very nice comprehensive program. He pointed out that ICDDR,B is interested to collaborate on rotavirus studies and already Dr. Mutanda has been seconded to Virus Research Centre for this purpose. He explained that a collaborative research project on serotyping of rotavirus was prepared by Dr. Mutanda last year. This was the first stage of study which stool samples were collected from children in both Dacca & Kenya & were sent to Dr. Zissis Laboratory in Belgium. The second and third stages of rotavirus studies are clinical description of rotavirus diarrhoea in relation to specific serotypes and epidemiological studies of rotavirus in both urban and rural comunities respectively. In this connection Dr. Rabbani has developed a protocal on clinical features of rotavirus and this protocal will be submitted to Kenyan counterparts for comments. This protocal has to be standardized for both documentation and the interobservers variation; hence, we have come for this standardization. Dr. Samadi suggested that when

Wamola

the clinical study is started, a protocal on epidemiology of rotavirus in urban area based on index-case contacts can be developed, since the exposed contacts would be available for study. In a later stage rural studies will be carried out.

Dr. Samadi pointed out the significance of rotavirus study with particular reference to its specific serotyping for further development of a rotavirus vaccine which could be used in Asia and Africa.

Dr. Pamba commented that it seems the rotavirus study in Dacca is ready to be implemented while we are not in a position to start it mainly due to lack of equipment, media and technicians. He also mentioned that he does not see any arrangements for microbiological and parasitological study. Dr. Samadi pointed out that however, the ICDDR,B is collaborating in rotavirus aspect of diarrhoeal diseases, the clinical protocal which has been prepared includes the parasitological and microbiological investigations as well.

Dr. Wamola commented that however, at present the existing research facilities in Nairobi is limited and there are budgetary constrains, I hope that we can make a start with clinical aspects of rotavirus and then WHO will be approached for funding to extend further studies.

Dr. Samadi referred to his suggestion to Dr. J. Gekonyo, the Director of Kenya Medical Research Institute, that WHO normally provide fund upto $25,000.- for each research project, it may be rational to split the whole study of diarrhoeal diseases into 3-4 separate studies inorder to get sufficient funds. He mentioned that his suggested approach was accepted by the Director.

Regarding the ICDDR,B contribution, Dr. Samadi mentioned that Dr. Mutanda already has been seconded to Virus Research Centre, Kenya. Also the pediatrician responsible for clinical study in Nairobi will be sponsored by ICDDR,B to pay a visit for further standardization of inter-observar variation.

Dr. Mutanda pointed out that the Director, ICDDR,B has authorized him to recruit a technician as well.

Dr. Kinoti mentioned that he needs a place for research study and two nurses.
Dr. Wamola commented that though there are certain problems, there is a general concensus that these problems can be over-comed.

Dr. Tukei pointed out that the virological aspects can be studied right now. We have to see what is available and what can we supplement. The Japanese Team also assists us in this regard. Then Dr. Tukei commented on the first draft of clinical protocal that the age of children for study was suggested upto 15 years and he thinks that this age to be limited upto 5 years. Dr. Rabbani replied that in the second draft the age has been limited upto 5 years only.

Then the persons were assigned to discuss the different aspects of the project:

Clinical Study:	Dr. Kinoti
	Dr. Njai
	Dr. Rabbani
	Dr. Samadi
Laboratory Methods:	Dr. Tukei
	Dr. Nasanzi
	Dr. Pamba
	Dr. Mutanda
Epidemiology:	Dr. Islam
	Dr. Kenya
	Dr. Samadi

It was decided the final reports on clinical laboratory and epidemiological aspects to be submitted inaa meeting which will be held on Monday 4/5/1981 at 12.15p.m.. The meeting was closed at 4.30 p.m.

List of Members of the Joint Meeting with Particulars

University of Nairobi

1. I.A. Wamola, M.Sc., PhD.
   Senior Lecturer, Dept. of Microbiology and Chairman
   Diarrhoeal Diseases Control Program Ministry of
   Health, Government of Kenya.

2. R. Nsanze, M.R.C. Path.
   Professor, Dept of Microbiology

3. S.N. Kinoti, M.B.Ch.B, M.Med., M.P.S. - ID
   Lecturer, Dept. of Paediatrics.

4. H. Pamba, M.Sc. Ph.D.
   Senior Lecturer, Parasitology

5. W.H. Mosely M.D., M.P.H.
   Professor, Community Health and Population studies

6. N.B. Mirza
   Consultant Microbiologist

Kenya Institute of Medical Research

1. P.M. Tukei M.B. Ch.B., M.Sc., Cert. (Immunology)
   Director, Virus Research Centre (WHO Virologist).

2. H. Ensering
   Senior Technologist in Virology (Dutch Research Team)

International Centre for Diarrhoeal Diseases Research, Bangladesh

1. A.R. Samadi, M.D., D.P.H.
   Senior Scientist & Ag. Head, Disease Transmission
   Scientific Program.

2. L.N. Mutende, M.H.Sc., Ph.D Scientist

3. G.H. Rabbani M.B.B.S., M.Sc., D.P.H.,
   Assistant Scientist.

Rapporteur: ASamadi

Chairman: [illegible]

Extracts of the Minutes of the 2nd Joint Meeting of University of Nairobi, Kenya Institute of Medical Research and International Centre for Diarrhoeal Diseases, Bangladesh 4.5.1981

ANNEXTURE VI

Members Present:

DR. Wamola (Chairman)
Miss Enseering
DR. Kinoti
DR. Mirza
DR. Mosely
DR. Mutanda
DR. Nsanze
DR. Pamba
DR. Rabbani
DR. Samadi (Rapporteur)
DR. Tukei

The Meeting was opened by the Chairman, DR. Wamola at 2.30p.m. 4.5.1981.

Minutes:

The Minutes of the last meeting held on 28th April 1981 were reviewed by the Members. The errors regarding the names and titles of the participants and the timing of the meeting were corrected. The text of the Minutes were approved.

Matters arising out of the Minutes

Lengthy discussions again were made on the same subjects as title of collaborative study, ICDDR,B Contribution, study of all etiologies of diarrhoeas, development of research protocols in Nairobi, significance of a research protocol on clinical features of diarrhoeal diseases, present budgetary limitations and provision of funds for research by WHO. The Chairman pointed out that we in Nairobi are somehow late in development of research protocols on different aspects of diarrhoeal diseases.

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Agenda:

DR. Kinoti reported that the details of clinical standardization has been worked out with DR. Rabbani and DR. Samadi Again he showed his concern about budget for clinical studies in Nairobi. The group felt that parallel clinical protocol has not been prepared yet in Nairobi, otherwise could be submitted for funding to W.H.O.

Finally it was agreed that the development of a protocol on clinical aspects of diarrhoeal diseases to be studied in Kenya is of immediate paramount significance. Then DR. Kinoti was asked to prepare a clinical protocol based on already standardized protocol of ICDDR,B for Nairobi with full budgetary elements. The laboratory aspects of this protocol could be worked out with DR. Wamola, DR. Pamba and DR. Mutanda. Then this protocol to be sent to ICDDR,B to see what immediate contibution ICDDR,B can make in order to speed up the start of the Clinical studies and meanwhile this protocol will be submitted to WHO for funding.

It was felt that with secondment of DR. Mutanda and standardization of clinical protocol collaboration on rotavirus aspects of diarrhoeal diseases with ICDDR,B can be initiated and maintained.

The meeting was closed by the Chairman at about 4p.m.

Rapporteur:.....Samadi.....

Chairman:....Kinoti....

T. H. Flewett, M.D., F.R.C.P., F.R.C. Path.
Virologist

Tel.: 021-772 4311 - Ext.4075

Regional Virus Laboratory
East Birmingham Hospital
Bordesley Green East
Birmingham B9 5ST

3rd June 1981

Dr. W. B. Greenough,
Director,
International Centre for Diarrhoeal
Disease Research,
GPO Box 128,
Dacca 2,
BANGLADESH,
India.

Dear Dr. Greenough,

Thank you for your letter of the 27th May on which I would only comment that I think it very unlikely that there would be any qualitative difference between different rotaviruses caused by different serotypes. There might, however, be different quantitative differences in the degree of severity, but I do not think that such differences would be likely to be revealed by a hospital based study. However, hospital based observations certainly might be useful to determine the prevalence of serotypes and to gain practical experience in techniques for the wider investigation which we were discussing between ourselves and Rahbani. Electropherotyping is quite easy. The equipment is comparatively inexpensive and it is a good excuse for buying a good 35 mm camera, which of course can be used for other purposes. I will ask Ray Sanders or Graham Beards, who are doing it here, to send you the details of the equipment and method which we use. It is not original and it is virtually identical to the method we had from Ian Holmes and it is far less laborious than serotyping.

I hope that before too long we shall be able to provide some really good rotavirus diagnostic reagents.

Best wishes,

Yours sincerely,

Tom Flewett

T. H. Flewett.

London School of Hygiene and Tropical Medicine

Keppel Street (Gower Street) London WC1E 7HT

Telephone 01-636 8636
Telegrams Hygower London WC1

Department of Medical Statistics and Epidemiology

W. Brass MA
Professor of Medical Demography
and Departmental Head
M. J. R. Healy MA
Professor of Medical Statistics
G. A. Rose DM FRCP FFCM [redacted]
Professor of Epidemiology

28 May 1981

Dr. G.H. Rabbani,
International Centre for Diarrhoeal
Disease Research, Bangladesh,
G.P.O. Box 128,
Dacca-2,
Bangladesh.

12/6/81

Dear Rabbani,

I was very disappointed that you couldn't manage the meeting we planned for last week to discuss the rotavirus study. I hope that you didn't have too many problems with your travel arrangements.

The main points I wanted to raise with you concerning the proposed protocol are as follows.

(1) It is suggested that the Kenya and Bangladesh data be analysed separately. Such a procedure is unsatisfactory if a fully reliable comparison is to be made between the 2 parts of the study. I strongly recommend that the 2 sets of data be analysed together so that identical methods may be used. I am very willing to do this analysis myself, but I think it is very important that whoever does it should become familiar with the organisational setup in both Kenya and Bangladesh. Only in this way can proper account be taken of local factors which may affect the comparability of the 2 sets of data. Do you agree with this approach, and do you think money might be available for me to visit Kenya as well as Bangladesh?

(2) Another problem is that there seems to be no plan to study children without diarrhoea. Without this it will not be possible to evaluate if any serotypes are not associated with diarrhoeal disease.

(3) I agree with Professor Bradley's point on the problem of comparability of the hospital patients in Kenya and Bangladesh.

(4) I would like more details of the epidemiological study in the Matlab area. For example, what age range will be studied? Will specimens be taken regularly or only when a child is suffering from diarrhoea? Are there plans to do a similar epidemiological study in Kenya?

(5) How will the group of patients for the determination of the defect of carbohydrate digestion be chosen?

London School of Hygiene and Tropical Medicine

Keppel Street (Gower Street) London WC1E 7HT

Telephone 01-636 8636
Telegrams Hygower London WC1

Ross Institute of Tropical Hygiene
David J. Bradley
MA, DM, MRCPath, FIBiol, MFCM
Professor of Tropical Hygiene
and Director of Institute

7th May 1981

Dr. G.H. Rabbani,
International Centre for Diarrhoeal
Disease Research, Bangladesh.
G.P.O. Box 128,
Dacca-2,
Bangladesh.

Dear Dr. Rabbani,

Many thanks for your March letter and protocol, which Betty Kirkwood also showed to me.

I have only one major worry about what is otherwise a well-thought-out study. That concerns sampling problems. I don't really see how, when the criteria for hospital attendance must be mainly severity, you can get a really valid comparison of virulence between strains by comparing hospital cases. And the criteria for admission to the Kenyatta cannot really help being different in practice from those at the CRL. This is my worry, and it may be that it's not worth doing such a long series but rather to do say 200 (rather than 500) and then get into the community. At any rate you should think about how far the study objectives can be achieved in a hospital study.

I'm very pleased that you are going to be able to concentrate on the study and don't get too widely spread - the quality of work and of supervision are very important.

Hope to see you in June.

All good wishes,

Yours sincerely,

David Bradley

David Bradley

c. & Betty Kirkwood TEV.

(6) Please can you send me a copy of all the proposed reporting forms so that I can check their suitability for direct transfer to the computer and also that all data necessary for the analysis is being recorded in an appropriate way.

Hope to see you again soon. Please give my regards to Najma.

All best wishes, (And from Tom)

Betty

Betty Kirkwood

cc Professor D.J. Bradley