Date 4/1/80

REVIEW BOARD ON THE USE OF HUMAN SUBJECTS, ICDDR,B.

VC

Principal Investigator Dr. L.N. Mutanda

Trainee Investigator (if any) _____

Application No. 80-015

Supporting Agency (if Non-ICDDR,B) _____

Title of Study SEROTYPING OF ROTAVIRUS

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).

Source of Population:
(a) Ill subjects (Yes) No
(b) Non-ill subjects Yes (No)
(c) Minors or persons under guardianship Yes (No)

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)

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)

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 Board:
___ Umbrella proposal - Initially submit overview (all other requirements will be submitted with individual studies)
___ Protocol (Required)
___ Abstract Summary (Required)
___ Statement given or read to subjects - 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
___ 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 Board for review.

I agree to obtain approval of the Review Board on the Use of Human Subjects for any changes involving the rights and welfare of subjects before making such change.

L.N. Mutanda A-033758

Principal Investigator Trainee

80-015
Rec'd 4/3/80

SECTION 1 - RESEARCH PROTOCOL

1) Title: SEROTYPING OF ROTAVIRUS

2) Principal Investigators: Drs L.N. Mutanda and G. Zissis

3) Starting Date: 1st April 1980

4) Completion Date: End of March, 1981

5) Total Direct Cost:

6) Availability of Funds:

(a) Scientific Director's Remarks:-

(b) Controller's Remarks:

7) Abstract Summary:

Serotyping of rotavirus is planned. Stool specimens will be screened for the presence of rotaviruses, and the viruses will be typed by use of ELISA technique. Also blood previously collected from children 0-5 years old from Dacca and Nairobi, East Africa will be tested for rotavirus antibodies. Those found positive will be typed, employing already prepared rotavirus antigens.

8) Reviews:

(a) Research Involving Human Subjects: _____

(b) Research Review Committee: _____

(c) BMRC: _____

(d) Director: _____

(e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objectives:

The goals of the proposed research are to work out a collaborative arrangement with Dr. Zissis on rotavirus detection and serotyping to be established at ICDDR,B; undertake serotyping of rotaviruses isolated from Dacca, Matlab, Teknaf and Kenya.

2. Background

Although our knowledge that enteropathogenic or toxigenic E. coli could cause outbreaks of serious diarrhoea in infants and were often found in endemic infantile and adult diarrhoea as well dates back 30 years, three quarters of the cases of childhood acute gastroenteritis could not be classified aetiologically. The scene has just changed in 1973 when a new viral contender - rotavirus - was detected by Bishop et. al (1973) in the duodenal epithelium of six infants with acute non-bacterial gastorntèritis. Today rotavirus diarrhoea has become as well established as that due to toxigenic strains of E. coli. Rotavirus infections have also been found in calves antelope, rabbits, piglets, apes, monkeys, lambs, deer, mice and foal (Flewett, 1976). Antibodies to the virus have been found in some of the above listed animals and also in wild rats (Mutanda, 1979; Tokahashi et. al, 1979). The human virus has been transmitted to piglets, calves and colostrum - deprived baby monkeys; the calf virus also has been transmitted to piglets, calves and colostrum - deprived baby monkeys (Flewett, 1976; Flewett and Woode, 1978). The rotaviruses from the different species have been shown to cross-react in complement fixation, immunofluorescence and gel diffusion tests (Kapikian et al, 1976 and Woode et al, 1976), indicating the presence of a group rotavirus antigen. Subsequently

the group antigen has been found to be in the inner capsid layer and the specific antigen in the outer layer (Spence et al, 1978).

In humans and animals antibody to rotavirus was found in 50 - 100 % of young adults. This observation suggested subclinical infections, and reports of a high percentage of subclinical infections in neonates further indicated that there might be a second serotype. In one study of rotavirus infection in children, Orstavick et al (1978) observed that gastroenteritis was often recorded among contacts, including parents. The antibody response of two patients in their study group differed from the responses observed in infants and children. This difference was deemed to be either due to a secondary immune response in adult patients or due to reinfections by antigenic variants of rotavirus. In another study, when Zissis and Lambert (1978), prepared guinea pig immune sera to two purified rotaviruses of human origin, interestingly, the antisera clumped only the homologus antigen in enormous aggregates. This finding, together with the results of Thouless et al (1978) convincingly revealed the existence of at least two human rotavirus types. Subsequently, additional serotypes of human rotavirus have been reported by Flewett et al (1978), making now a total of four.

Following these observations enteric illnesses associated with different rotavirus serotypes have been reported by Fonteyne et al (1978), Rodriguez et al (1978) and Wyatt et al (1979). Yolken et al (1979) have shown that type 2 was the most predominant serotype

associated with symptomatic disease - an indication that human rotaviruses like the pig rotaviruses (Woode, 1976), may vary in their virulence. In their sero-epidemiology of rotavirus diarrhoea in Matlab, Bangladesh, Sack et al (1979) observed multiple rises in titer in 9 of the 85 subjects. The high incidence of titer rises in the older children (Greater than 2 years) seemed to indicate that older children continue to be exposed to and become infected with rotavirus. These repeated infections occurring in older children, according to the authors, point to an important development of immunity to the pathogenic effect of the virus. However, studies aimed at establishing the total number of rotavirus serotypes in any of the developing countries, and the importance of these serotypes in the pathogenesis and induction of natural immunity against rotavirus infection have not been done.

3. Rationale:

The main objectives of this study is to establish the total number of rotavirus serotypes in Bangladesh and probably in another country which is geographically different in respect to people and climate. Information on the total number of serotypes of human rotavirus is crucial for the production of a vaccine and understanding the existence of serotypes of varied virulence.

B. Objectives:

1) To type rotaviruses isolated in Dacca, Teknaf and those from Kenya, East Africa.

2) To use the available human sera in stock to study the epidemiology of the individual rotavirus serotypes

3) To study the clinical features of infants and children infected with individual rotavirus serotypes and determine the importance of the different serotypes in the pathogenesis and induction of antibodies against rotavirus.

C. Methods and Procedure:

Collection of faecal samples: Faecal material will be collected from children and adults to be admitted to ICDDR,B for acute diarrhoea. Also faeces will be collected from diarrhoea patients in Teknaf. In the two places, specimens will be collected 4 times over a one year period during the months of April, July, October, 1980 and January. 1981. Also faecal specimens will be transported from Nairobi, Kenya to Dacca where they will be screened for the presence of rotaviruses and these viruses be typed. The specimens from Nairobi were collected in 1977 from different parts of Kenya and are being kept in a freezer at -20°C.

Collection of Blood samples:

In Dacca, use will be made of the sera already separated from blood samples and kept at -70°C. Also the sera of infants and children now being kept in Nairobi will be employed to study the epidemiology of the individual serotypes of rotavirus inoone of the African countries.

Isolation and Typing of Rotaviruses:

Rotaviruses will be isolated and typed according to Dr. Zissis' techniques.

Serology:

Sera will be screened for rotavirus antibodies by the complement fixation technique, and those found positive will be further tested for neutralizing antibodies against prepared rotavirus antigens.

Clinical features of rotavirus - infected patients:

Dr. A.M. Molla will be the in-charge of seeing all the patients with diarrhoea attending ICDDR, Dacca clinic. The selected patients will each provide a stool specimen and Dr. Molla will examine and treat the patients. Dr. M.M. Rahman will be the in-charge of seeing and getting stool specimens from patients with diarrhoea in Teknaf. Already he has an active programme on dysentery. Use will thus be made of the stool specimens being collected. These specimens will be transported by air, to Dacca during the months specified above.

D. SIGNIFICANCE

The knowledge of the total number of serotypes of human rotavirus is crucial for the production of a vaccine and understanding the existence of serotypes of varied virulence. The results of serology will also set the age at which vaccine against rotavirus can be given.

E. FACILITIES REQUIRED:

A fluorescent microscope is required.

F. COLLABORATIVE ARRANGEMENTS:

Collaborative arrangements with Drs. Zygraich and Zissis from Belgium are underway.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICE

Names	Position	% Effort	Annual Salary US Dollars	Project Requirement US Dollars
Dr. L.N. Mutanda	Investigator	100%	18,069	18,069
Dr. C. Zissis	Co-Investigator	20%		
Dr. A.M. Molla	Paediatrician	15%	36,200	7,200
Dr. M.M. Rahman	Deputy Director	15%	48,200	3,215
Ms M.N. Mansur	Research Assistant	100%	3,246	3,246
	Technician	100%	1,370	1,370
	Sub-Total:			33,100

2. SUPPLIES:

Items	
Reagents	15,000

3. EQUIPMENT:

Fluorescent Microscope	6,000

4. PATIENT HOSPITALIZATION: None

5. OUT-PATIENT CARE: None

6. ICDDR,B TRANSPORT:

1000 miles @ Tk.3.00: Mileage	200
Sub-Total:	21,200

7. TRAVEL AND TRANSPORTATION OF PERSONS

Travel for Dr. Zygraich or Dr. Zissis to Dacca (2 trips)	7,500
Travel for Dr. Mutanda to Kenya and Hotel expenses for 1 month	7,500
Travel for Dr. Mutanda to Belgium (1 trip)	3,750

		US Dollars
8.	TRANSPORTATION OF THINGS:	
	Transport of specimens from Kenya	750
9.	RENT, COMMUNICATION AND UTILITIES : None	
0.	PRINTING AND REPRODUCTION:	200
11.	OTHER CONTRACTUAL SERVICES : None	
12.	CONSTRUCTION, RENOVATION, ALTERATIONS : None	
	SUB-TOTAL:	19,750

B. BUDGET SUMMARY

Category		Dollars
1.	Personnel	33,100
2.	Supplies	21,200
3.	Equipment	6,000
4.	Hospitalization	
5.	Outpatients	
6.	ICDDRB Transport	200
7.	Travel Persons	18,750
8.	Transportation Things	750
9.	Rent/Communication	
10.	Printing/Reproduction	200
	GRAND TOTAL:	741,050

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ABSTRACT SUMMARY

A research project aimed at serotyping rotavirus is proposed. Stool specimens already being collected from diarrhoea patients attending our clinics, in both Dacca and Teknaf will be used for this study. In the two places, specimens will be collected 4 times over a one year period during the months of April, July, October, 1980 and January 1981. Also faecal specimens already collected from infants and children, will be transported from Nairobi, Kenya to Dacca. Stools will be screened for the presence of rotavirus, employing either complement fixation (CF) or ELISA techniques. Those found positive will be typed, and the clinical features of infants and children infected with individual rotavirus serotypes will be determined from hospital records. Sera, already separated from the blood previously collected from infants and children in Bangladesh and Kenya will also be tested for rotavirus C.F. antibodies. Those found positive will be typed by ELISA technique.

The main objective of this study is to establish the total number of rotavirus serotypes in Bangladesh and in another country geographically different in respect to people and climate. Knowledge of the total number of serotypes of human rotavirus is crucial for the production of a vaccine and understanding the existence of serotypes of varied virulence.

This project involves no interviews, physical, psychological, social, legal or any other risks, and a signed consent is therefore not required.