Attachment 1.

Date 7.1.90.

ETHICAL REVIEW COMMITTEE, ICDDR,B.

22

Principal Investigator Dr N.S. Shahid

Application No. 90-001

Title of Study Studies on neonatal rotavirus infection in Bangladesh

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) _____

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 Stool Sample, — (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:
   ___ 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
   ___ 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.

(PTO)

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

Nigar S Shahid
Principal Investigator

Trainee

A-033772

90-001
10/1/90

1. Investigator : Dr. Nigar Shahid

2. Co-Investigators : Dr. Fu Bingnan
Dr. G. Podder
Ms. Leanne Unicomb
Prof. Khaleda Banu
Prof. N. Nahar
Dr. B. Elahi

3. Title of Project : **Studies on neonatal rotavirus infection in Bangladesh.**

4. Starting date : February 1990

5. Date of completion : July 1990

6. Total budget requested : US$ 7,500

7. Funding Source :

8. Programme Co-ordinator : Dr. S. Tzipori S. Tzipori 7/1/90

9. Aims of Project :

a) General aim:

To identify whether rotavirus infected neonates in different epidemiological settings occur in Bangladesh as they do in other countries.

b) Specific aims:

1. To determine the presence (if any) of rotavirus in the stools of neonates.
2. To determine whether strains adapted to nursery environment are found as asymptomatic infection in neonates.
3. To characterise these nursery strains by serotyping (using ELISA and/or RNA hybridization) and electropherotyping.
4. To compare the nursery strains with those circulating in the community and with nursery strains in other countries.
5. To determine whether these nursery strains are endemic in the hospital nurseries over a period of time .

c) Significance:

Rotavirus nursery strains have been found to be both similar and dissimilar to those circulating in the community of both developed and developing countries. These strains are thought to be avirulent and have been shown to persist for many years in some hospital nurseries. Since these strains have been shown in some cases to confer protection against subsequent severe rotavirus diarrhoea in infancy they are currently being considered as potential vaccine strains.

The identification and characterization of such strains from Bangladeshi neonates may help add to the understanding of epidemiology of rotavirus infection in neonates in developing countries and may contribute to future development of vaccines.

10. Ethical implications:

Sequential stool or rectal swab samples will be required for this investigation. A written consent will be obtained both from patients and attending physicians (see page 22).

11. Background:

Rotavirus has been identified in many parts of the world as the most common agent of acute gastroenteritis in children aged less then 5 years both in developed[1] and developing countries[2]. As part of a series of papers speculating possible strategies for diarrhoeal disease control, it is estimated that an efficient rotavirus vaccine administered

to infants in lesser developed ccountries (LDCS) under 6 months of age could reduce diarrhoeal episodes by over 50 million and deaths by 400,000-800,000/year[3].

Rotavirus has also been detected from stools of symptom-free newborn babies in neonatal nurseries in several parts of the world[4,5,6] and could be isolated from about 50% of babies in those nurseries. If neonates are colonized, these nursery strains are shed as early as the first day of life[7] often continuing for up to 7 days[8]. Both specific local and systemic immune responses are evoked - mucosal response being earlier than systemic[9].

A direct relation among early infection with nursery strains and subsequent infection, severity of clinical symptoms and the amount of virus excreted in faeces has been observed[10]. In some nurseries infection has been reported to be endemic with neonates excreting the endemic virus within a few days of introduction to the nursery.

Rotaviruses possess a double-stranded RNA genome consisting of 11 segments. Segment 4 is known to be responsible for restriction of growth in cell culture, protease-enhanced plaque reduction and possibly virulence. Sequence analysis of this genome segment from nursery strains and community strains revealed differences indicating that this could account for the attenuated nature of nursery strains[11]. The electrophoretic patterns of the strains identific

over several months or years from the same nursery has revealed remarkable genetic stability[12]. Electrophoresis indicates that the nursery strains in Melbourne hospitals during 1975-9 belonged to only one serotype and that these strains were electrophoretically different than those in the community causing severe diarrhoea in young children[12]. A similar persistance of serotype 1,2 and 4 have been noted in newborn nurseries in Venezuela, Sweden and England respectively[13]. These viruses have been adapted to culture and found to be electrophoretically similar to one another[13]. Bishop *et al* have noted that neonatal rotavirus infection does not confer immunity against reinfection but does protect against development of clinically severe disease with other community strains[10].

Some investigators have found rotavirus strains infecting neonates to be the same as those circulating in the community[5]. Therefore we wish to investigate the presence and nature of any rotavirus strains infecting newborn babies in Bangladesh and compare them with the strains infecting children and causing rotavirus diarrhoea within the same community and with neonatal strains found in other countries, where possible. If any evidence of neonatal infection is found we shall plan another study to follow neonates with rotavirus infection to see whether protection occurs against post-neonatal RV-diarrhoeas as has been found in the Melbourne study[10].

## Materials and Methods

### 1. Recruitment of Infants

Fifty infants will be selected from those delivered at Holy Family Hospital and Dhaka Medical College Hospital from the neonatal cases at Dhaka Shishu Hospital and housed in nurseries or "baby rooms", living in Dhaka city. If frequency of neonatal infection is very low, an additional 50 babies will be recruited. Consent will be obtained from parents and attending physicians. Stool/rectal swabs will be requested for the first 7 days of life both at the hospital and home and will be collected in specimen bottles supplied by LSD. Demographic and clinical information will be obtained and recorded on forms. It is hoped that recruitment will commence at the beginning of the "rotavirus season" in Dhaka in order to maximize the potential rates of rotavirus detection in the event that neonatal rotaviruses also show seasonality.

### 2. Laboratory methods

In the laboratory an attempt will be made to detect the presence of any group A rotavirus and to characterize them following the scheme outlined below:

1. Detection of group A rotavirus in stools by ELISA (using Dakopatt Rotavirus test).
2. Extraction of dsRNA from stools that are RV positive for electropherotyping. (Appendix A).

3. Serotyping of ELISA positive specimens using commercially available monoclonal antibodies (Serotype 1-4)(Appendix B)

4. Serotyping of ELISA positive specimen by dot hybridization using serotype specific, radioactively labelled synthetic oligonucleotide probes (available from the Armed Forces Research Institute and Medical Sciences - AFRIMS -Bangkok. (Appendix C).

5. Adaptation to growth in cell culture of rotavirus strains that show unusual electrophoretic or serotype patterns and suspected true nursery strains for further characterization (Appendix D).

6. Production of hyperimmune sera for extensive antigenic analysis (Appendix E and F).

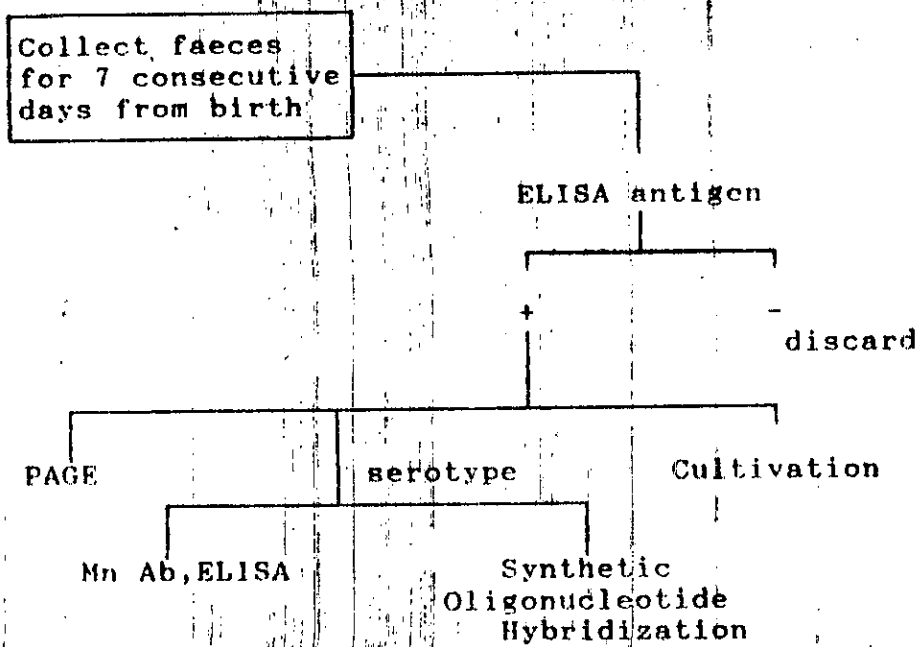
7. Collaboration with a laboratory that could compare $VP_4$ of any suspected nursery strains with that of standard nursery strains from other countries.

## BUDGET

**Personnel**

| | | | 6 months |
|---|---|---|---|
| a. Dr. N. Shahid | 30% Salary NOC Step 6 | - | 2,600 |
| SHA | 100% Salary GS3 Step II | - | 1,600 |
| SHA | 50% Salary GS3 Step II | - | 800 |
| Helper | 100% @ US$ 40/month | | 240 |
| b. Operating cost | | | |
| Travel | | | 800 |
| c. Reagents and disposibles : | | | |
| ELISA | | | 300 |
| PAGE | | | 300 |
| Hybridization | | | 560 |
| Cultivation | | | 300 |
| | TOTAL DIRECT COST US$ | = | 7,500 |

## FLOW CHART


Collect faeces for 7 consecutive days from birth
ELISA antigen
+
-
discard
PAGE
serotype
Cultivation
Mn Ab, ELISA
Synthetic Oligonucleotide Hybridization

**Abstract Summary**

Rotavirus "nursery" strains have been found to be dissimilar to those circulating in the community mostly in developed countries. These avirulent strains have also been shown to persist for many years in hospital nurseries and have been shown to confer some protection against subsquent severe RV disease in infancy. These strains are being considered as potential Vaccine strains. The identification of such strains in Bangladeshi nurseries may also help formulate a future vaccine.

In order to address this issue, fifty newborn will be enrolled for the study. Stool and R/s will be examined for the presence of rotavirus for 7 consecutive days starting from birth. Nothing is known about the epidemiology of neonatal rotavirus infection in Bangladesh.

Specific task for each investigator

Dr. Nigar Shahid

- Collaborate with DMCH staff for collection of complete clinical information and stool sample.
- Collaborate with the laboratory in getting the sample processed.
- Maintain all clinical, epidemiological and laboratory information up-to-date.
- Performance of hybridization of RV RNA with serotypes oligonucleotide probes.
- Collate and write-up results.

Dr. Fu Bingnan

- Provide advice on the performance of hybridization of RV RNA with serotypes oligonucleotide probes.

Dr. G. Podder

- Help performance of ELISA tests and RNA gel electrophoresis.

Ms. Leanne Unicomb

- Collaborate with Dr. Shahid in matters relating to setting up of the assays and analyis of results.

Prof. N. Nahar, Prof.K. Banu, Dr. B. Elahi

- Collaborators at Dhaka Medical College Hospital, Dhaka Shishu Hospital and Holy Family Hospital.
- Enrollment of neonates at the above hospital.

## References


1. Brandt CD, Kim HW, Rodriquez WJ, *et al.*. Pediatric Viral gastroenteritis during eight years of study. J. Clin. Micro. 1983; 18:71-78.

2. Stoll BJ, Glass RI, Huq MI, *et al.* Surveillance of patients attending a diarrhoeal diseases hospital in Bangladesh. Br. Med. J. 1982;285:1185-8.

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## APPENDIX A

**RNA Electropherotyping** : Double stranded RNA segments are extracted directly from faecal samples or from tissue culture supernatant and electrophoresed according to the method Herring *et al* (1982)[22].

1. Extraction of ds RNA

(a) Adjust pH to 5.6

i.e. if faecal homogenate is made in PBS or $H_2O$ or if TC-SNT is used, add one-tenth of the vol. used of 1M sodium acetate pH 5.6 (i.e. if extracting 200 ul).

OR Make up faecal homogenate in 0.1M sodium acetate pH 5.6

(b) add an equal volume of the following extraction mixture to faecal homogenate/TC-SNT etc. (use a tube that fits in microfuge/centrifuge).

e.g. 200 ul faecal homogenate + 200 ul of the following extraction mixture.

3:2*(phenol-m-cresol-8 hydroxyquinoline - phenol mixture)

(chloroform-isoamyl alcohol 24:1)

For 10 ml

160 ul isoamyl alcohol + 3.9 ml chloroform + 6 ml phenol mixture.

recipe for phenol mixture:500g crystalline phenol + 70g m-cresol + 0.5g 8.OH quinoline + 200g $H_2O$.

- this can be aliquotted in 20 ml lots and stored at -20°C.

(c) Vortex sample and extraction mixture for 1 min. and spin in a microfuge for 2 mins.10,000 rpm).

(d) retain top layer (this is the nucleic acid fraction) and discard the rest.

(e) ds RNA can be concentrated if required by adding 2 volumes of ethanol and freezing at -20°C for a few hours. After freezing, centrifuge in a microfuge and resuspend the pellet in an appropriate volume of distilled water or can be frozen, neat, at -20°C.

2. Electrophoresis. RNA samples are mixed with sample buffer containing bromophenol blue, and applied to 7.5mm thick, 10% polyacrylamide gels using a discontinuous buffer system, according to Laemmli[24], in the absence of SDS. Samples are electrophoresed for 16-18 hours at 15 mA set current.

3. Silver Staining of Polyacrylamide Gels : Gels are fixed and stained with silver according to the method of Dyall-Smith and Holmes[23].

(a) Cut stacking gel from the top of the gel and soak gel for a minimum of 30 mins. in fixer (10% ethanol, 0.5% acetic acid) - gels can be kept overnight in fixer if required.

N.B. : Make sure that there is an appropriate marker on the gel (such as a cut off corner) so that first and last samples etc. can be distinguished.

(b) Aspirate fixer and soak gel in 0.011M silver nitrate for a minimum of 30 minutes.

(c) Aspirate silver nitrate, rinse with distilled water and add a reducing solution of -

0.75M NaOH, 0.1M formaldehyde and

(d) aspirate reducing solution after the bands have been visible for 10 minutes, and store gels in 5% acetic acid until photographed.

N.B.: use 200 ml volumes.

## APPENDIX B

ENZYME IMMUNOASSAY (EIA) FOR SEROTYPING GROUP A HUMAN ROTAVIRUSES USING MONOCLONAL ANTIBODIES - B (according to the method of Coulson *et al* 1987)[25].

1. Coat 96F NUNC tray with 100 ul/well of rabbit antisera to rotavirus serotype 1-4 diluted in phosphate buffered saline (PBS) pH 7.2 as follows:

| | | | |
|---|---|---|---|
| Row | 1 | Anti-RV4 (serotype 1) | 1:8,000 |
| | 2 | Anti-RV5 (serotype 2) | 1:6,000 |
| | 3 | Anti-RV-3 (serotype 3) | 1:6,000 |
| | 4 | Anti-ST-3 (serotype 4) | 1:8,000 |

2. Incubate tray at 37° for 2 hours in a moist environment.

3. Wash x 3 with phosphate buffered saline pH 7.2 containing 0.05% Tween 20 (PBST).

4. Add 75 ul of skim milk powder solution (SMP) to each well, followed by 25 ul of 10% (W/V) faecal supernatant or 100 ul of control sample. Add each sample to all 4 wells comprising a column of the plate, e.g. sample No. 1 in column 1, sample No. 2 in column 2.

5. Incubate at 4° for 16-20 hours.

6. Wash as in step 3.

7. Add 100 ul of mouse monoclonal antibodies to each well, diluted in SMP as follows:

| | | | | |
|---|---|---|---|---|
| Row | 1 | Mab RV-4.2 | Protein A fraction | 1:200 |
| | 2 | Mab RV-5.3 | Protein A fraction | 1:2,000 |
| | 3 | Mab RV-3.1 | Protein A fraction | 1:2,000 |
| | 4 | Mab ST-3.1 | Protein A fraction | 1:1,000 |
| | | or kit abs as above but diluted 1/200 | | |

8. Incubate at 37°C for 2.5 hours in a moist environment.

9. Wash as in step 3.

10. Add 100 ul/well anti-mouse immunoglobulins conjugated to horseradish peroxidase at 1/800 (DAKO) diluted in SMP, or SILENUS brand (1/2,000).

11. Incubated at 37°C for 1.5 hours in a moist environment.

12. Wash as in step 3.

13. Add 100 ul TMB substrate to each well. Incubate at room temperature for 10 minutes and then stop the reaction with 50ul 2M $H_2SO_4$.

14. Read plates by eye or at 450 nm in a spectrophotometer.

## APPENDIX C

**DNA Hybridization for serotyping of group A rotaviruses**

Rotavirus RNA Extraction

1. Suspend 50-100 mg faecal sample in 0.5 ml of 0.1M sodium acetate, 1% SDS pH5.
2. Extract with phenol-chloroform mixture and keep the supernatant (dsRNA).

Blotting of RNA onto Nitrocellulose

1. Mix the RNA solution with one volume of 6.15M formaldehyde, 10X SSC.
2. Incubate at 65°C for 15 mins.
3. Spot on the nitrocellulose using Bio-dot apparatus.
4. Rinse with 10X SSC.
5. Air dried and bake at 80°C for 2 hrs.

Oligonucleotide probes labeling

1. 5-end labelled 8 pmol of probe with r-32p-ATP (3000 Ci/mmol).
2. Separate the labelled probe from unincorporated ATP by passing through Sephadex G-25 column.

Probe Hybridization

Buffer : 3X SSC, 0.5% BSA, 0.5% PVP, 1% SDS

Probe concentration : $10^6$ cpm/mL hybridization buffer

Hybridization Temperature :

38°C for HuG2Ac and HuG3Ac

42°C for HuG1Ac, HuG4Ac, BoG6Ac and PoG5Ac

45°C for Hug8Ac

Washing condition :

1. 3 X SSC at Room temperature for 30 mins.
2. 3 X SSC at the hybridization temperature for 10 mins. twice.
3. 2 X SSC at room temperature for 1 hr.

## APPENDIX - D

**PROCEDURE FOR CULTIVATION OF HUMAN ROTAVIRUS FROM STOOL**
(According to the method of Albert & Bishop 1984)[11]

Selection of Stool Specimens :

Use preferably fresh stool specimens, or specimens stored at -70°C. These should be positive for rotavirus by ELISA using commercial kit.

Preparation of Sample for Inoculation :

Prepare a 20% homogenate of faeces in phosphate buffered saline (PBS, pH 7.0). Remove bacteria by passing though a 0.45 um membrane filter after prewetting filter with neat foetal calf serum (FCS). Activate with 10 ug/ml of trypsin (sigma type IX trypsin) for 30 minutes at 37°C.

Preparation of MA-104 Cells :

Cells are grown in Dulbecco's modified medium (DMM) with 12.5 ug/ml each of polymyxin B and neomycin sulphate to form confluent monolayers of cells in culture flask (75 $cm^2$ area, 200 ml capacity containing 20 ml of medium). Culture fluid is drained off and cells are removed with trypsin - EDTA (2 ml for each flask). When cells are stripped, 150 ml of DMM with 10% foetal calf serum is added (1:5 split ratio). Two ml each of diluted cells are seeded into screw-capped tubes and incubated at 37°C on a stationary rack until a confluent monolayer is obtained (3-4 days usually). These tubes are used for inoculation of activated faecal samples. Rinse the monolayer twice with PBS (containing phenol red indicator) just before inoculating the stool filtrate.

Inoculation of Trypsin Activated Filtrate :

0.2 to 0.5 ml of filtrate (or 1.0 ml whenever available) is inoculated into each of two tubes of monolayers and rolled in the drum for 1 hour at 37°C for virus adsorption. Excess fluid is drained from the tubes. Cells are rinsed once with PBS and inoculated with 2 ml of DMM containing 1 ug/ml of trypsin. The tubes are then incubated in the roller drum at 37°C for up to 5 days, checking for evidence of CPE everyday.

Detection of Rotavirus in Inoculated Tubes :

After 3 freeze thaw cycles, supernatants can be tested in ELISA test.

Further Passages :

The duplicate tube is frozen and thawed three times, and the supernatant is treated with trypsin (as above). 1.0 ml of trypsin treated supernatant is passaged into each of two tubes containing fresh monolayers of cells as before. Tubes are not rinsed with PBS from passage 2 onwards after adsorption of virus. Cellular damage due to trypsin can occur. This does not seem to interfere with viral multiplication.

## APPENDIX E

**Concentration of rotavirus from tissue culture supernatant (ELISA antigen)**

1. After one freeze-thaw cycle, cell debris is removed from pooled tissue culture supernatant by centrifugation at 5,000 g at 4°C for 10 mins.
2. Supernatant is pooled and ultracentrifuged at 100,000g for 1 hour at 4°C.
3. The pellet is resuspended in 0.01M Tris-Hcl pH 7.5 with 10mM $CaCl_2$ and 15mM NaCl to give a 100 fold concentration of the original supernatant.

## APPENDIX F

**Production of hyperimmune antisera**

1) Seronegative animals are chosen (see appendix J)

2) Rabbits are immunized with 0.5ml of purified virus preparation (see appendix G) at a concentration of approximately $10^6$ FFU/ml or greater - mixed with 0.5 ml of Freund's complete adjuvant and injected, subcutaneously, on 3 sites on the back.

3) Three weeks later rabbits are injected with 0.5 ml of virus preparation (as above) mixed with Freund's incomplete adjuvant and injected as above.

4) Three weeks later 0.5 ml of virus preparation only is injected as above.

5) Ten to 14 days later rabbits are kill bled, if ELISA antibody titre is greater than 1/20,000 (see appendix J) and/or neutralizing antibody titre is greater than 1/20,000.

Note 1 : Guinea pigs are immunized with volumes approximately half those given to rabbits.

Note 2 : Blood is taken at each time of immunization, if possible, to determine antibody levels.

Child Name : _____ Sex : _____

Child No. : _____

Father's name : _____

Mother's name : _____

Where delivered : _____

Residential address : _____

_____

_____

No. of siblings ☐☐

Full term baby Yes [ ] No [ ]

Type of delivery CS [ ] Normal [ ] Forceps [ ]

Diarrhoea : Yes [ ] No [ ]

No of stool in last 24 hours ☐☐

Type of stool normal [N], Watery [W], Uncertain [X]

Is your child Breast Fed.

| No BM | BM only | BM + WATER | unk |
|---|---|---|---|

Was colostrum given Yes [ ] No [ ]

For lab use only

Specimen No. _____
Date of specimen collection _____
Date specimen received _____
Received by _____

# CONSENT FORM

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is focussing its research efforts on ways of preventing diarrhoea. Rotavirus infection of newborn babies with a harmless virus has been observed in other countries. Its presence in stool has been found to protect against later severe diarrhoea.

We would like to see whether newborn Bangladeshi children have this harmless virus in their stools. The identification and characterization of these virus from Bangladeshi newborns may contribute to a future development of vaccines.

If you agree to let your child participate in this study please sign below. We will require stool or rectal swab specimens of your child everyday for 7 days after birth.

---

Signature of Parent

---

Signature of Investigator

# সম্মতিপত্র

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র উদরাময় প্রতিরোধের জন্য গবেষণা করে যাচ্ছে। অন্যান্য দেশে ক্ষতি হয়না এমন ধরনের রোটাভাইরাস দ্বারা আক্রান্ত নবজাতকদের পর্যবেক্ষণ করা হয়েছে। মনে এর উপস্থিতি পরবর্তীকালে মারাত্মক ডাইরিয়া থেকে রক্ষা করতে পারে বলে জানা গেছে।

আমরা ক্ষতিহীন ভাইরাস দ্বারা আক্রান্ত বাংলাদেশী নবজাতকদের দেখতে চাই। এ ধরনের ভাইরাসের উদ্ঘাটন এবং বৈশিষ্ট্যসমূহ খুঁজে বের করা ভবিষ্যতে এর প্রতিরোধে টিকা আবিস্কারে সহায়ক হতে পারে।

আপনি যদি এ গবেষণায় আপনার শিশুকে অংশগ্রহণ করাতে রাজী থাকেন তবে অনুগ্রহপূর্বক দস্তখত করুন। এ গবেষণার জন্যে আপনার শিশুর জন্মের দিন থেকে ৭ দিন পর্যন্ত পায়খানার নমুনা দরকার হবে।

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পিতা/মাতার স্বাক্ষর

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গবেষকের স্বাক্ষর

**Publication of Dr. Fu Bingnan:**

1. Comparison of passive immunohaemolysis assay with Y1 cell test for detection heat-labile enterotoxin for *E. coli*. Hygiene and Diseases Prevention 1985; 4:3-6.

2. Comparison of enterotoxin gene probe with single radial immune haemolysis test and other tests for detecting *Escherichia* [illegible]. Hygiene and Diseases Prevention 1986;2:1-1.

3. Etiological and epidemiological studies on acute infectious diarrhoea in central-south China. China Public Health 1986;5(1):21-6.

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## PUBLICATIONS : Leanne Unicomb

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2. Epidemiology of rotavirus strains infecting children throughout Australia during 1986-1987 : A study of serotype and RNA electropherotype.
Leanne E. Unicomb and Ruth F. Bishop
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3. Experience with an enzyme immunoassay for serotyping human group A rotaviruses (1989).
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J. Clin Microbiol **25**:509-515

6. Cultivation and characterization of rotavirus strains infecting newborn babies in Melbourne, Australia from 1975-1979 (1987).
M. John Albert, Leanne E. Unicomb, Graeme L. Barnes and Ruth F. Bishop J. Clin. Microbiol. **25**:1635-1640

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J. Clin. Microbiol. **25**:183-185

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Review of Protocol: "Studies of neonatal rotavirus infection in Bangladesh" - Dr. Nigar Shahid and others by Dr. Ruth Bishop, Royal Children's Hospital, Melbourne, Australia (given by discussion with Ms Leanne Unicomb).

1. Dr. Bishop suggested that the general aim be modified to include investigation of *any* rotavirus strain found in different epidemiological setting in order to broaden the aims and obtain as much information regarding the types of rotavirus strains that can infect infants in the neonatal age group.

   We have, therefore, included this suggested change.

2. In order to maximize the chances of detecting nursery rotavirus strains, special and intensive-care nurseries should also be used as recruitment sites.

   This suggestion has been incorporated into the protocol.

3. Under the heading of "Specific aims" nursery or neonatal strains should be altered to read "strains adapted to the nursery environment" in order to distinguish between any rotavirus that infects neonates and a group of strains that are found to only infect neonates. This suggestion has been incorporated into the protocol.

4. Under the heading of "laboratory methods" it should also be included that collaborative studies with another laboratory should be undertaken to investigate the nature of the VP4 protein of the gene 4 of any nursery strain in order to compare such strains with strains present in other countries. We have therefore proposed to set up collaborative links with Dr. A. Kapikian of NIH in Bethesda, USA for investigation of the sequence of gene 4 of any detected nursery strains.

   Final comment: A simple and worthwhile pilot-style project utilizing appropriate laboratory techniques.

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# ALL INDIA INSTITUTE OF MEDICAL SCIENCES

DEPARTMENT OF PEDIATRICS
*Division of Gastroenterology*
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ANSARI NAGAR,
NEW DELHI - 110029
(INDIA)

*Dr. M. K. Bhan* MD
*Professor*

Date : 24/12

Dr. Saul Tzipori
Associate Director
International Centre for Diarrhoeal
Disease Research, Bangladesh,
Laboratory Sciences Division,
GPO Box 128, Dhaka 1000,
BANGLADESH

Dear Dr. Tzipori,

I regret the delay in sending comments on the proposal regarding Neonatal rotavirus infections.

I hope you will forgive me, just this time

Yours sincerely,

(MK BHAN)

## COMMENTS

The development of RV vaccine is an important area of research from a public health perspective.

Indepth characterisation of neonatal RV strains which may be naturally avirulent is one of the important approaches to development of RV Vaccines.

The preliminary proposal to determine if RV infection is endemic in the neonatal unit of the investigators, the electropherotype and serotype of the neonatal RV strains and its comparison with community strains is by itself of modest scientific interest. It must be undertaken only if the investigators intend to perform follow up studies which may include nucleotide sequencing of gene segments 4, 8 and 9 of neonatal strains and of reinfecting strains (in a longitudinally followed up cohort), synthesis of $VP_3$ and $VP_7$ based on gene sequencing results and production of monoclonal antibodies to both conserved and divergent regions of these major antigenic peptides. This would help to characterise serotype specific episodes of rotavirus, the nature of heterologous protection and escape mechanisms in reinfection.

In this regard, the reference under laboratory methods "production of hyperimmune serum for antigenic analysis", is inadequate and the whole approach would be best spelt out in greater detail in a second proposal after completion of the initial proposal.

Project title: ....Studies....on.....Neonatal....Rotavirus....Infection
.....in.....Bangladesh.....

Principal Investigator(s): .....Dr....Nigar....Shahid.....

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

| | Rank Score | | |
|---|---|---|---|
| | High | Medium | Low |
| Quality of Project | | ✓ | |
| Adequacy of Project Design | | ✓ | |
| Suitability of Methodology | ✓ | | |
| Feasibility within time period | ✓ | | |
| Appropriateness of Budget | ✓ | | |
| Potential value to field of knowledge | | ✓ | |

CONCLUSIONS

I support the application:

a) without qualification ☑

b) with qualification:
- on technical grounds ☐
- on level of financial support ☐

I do not support the application ☐

Name of Referee: Prof. M.K. Bhan

Position: Chief, Gastroenterology Unit, Christian Medical College, Ludhiana, Punjab

Institution: Department of Pediatrics, All India Institute of Medical Sciences, New Delhi.

Signature

7.12.89
Date

Protocol: Studies on Neonatal Rotavirus Infection in Bangladesh by Dr. N. Shahid et al.

Answers to comments by Prof. M.K. Bhan, AIMS, New Delhi.

A. Dr. Bhan has sugested in-depth charecterization of the neonatal strains in particular of segments 4, 8 & 9.

i. We do not forsee it be worthwhile to set up such an elaborate technique here at ICDDR,B at this stage and hope to collaborate with a laboratory abroad, where this is done routinely.

ii. We also feel that segment of gene 4 to be of highest priority to show relatedness of nursery strains (Gorziglia M. et al., Prof. Nath. Acad. Sci. USA 1986;83:7039-43).

iii. This is a six month proposal aimed at preliminary determination of the presence of any such (nursery) strains.

iv. If and when nursery strains are found a longitudinal study will be proposed --This is a pilot study.

B. Production of hyperimmune serum -- The purpose of production of such sera will be to use these reagents in cross-neutralization tests in order to demonstrate serotype relatedness. This is a standard and well established Virological Technique used to determine, unquivocally the serotype of a virus (Hoshino Y et al J. Infect. Dis. 1984; 149; 694-702).

## CONSENT FORMS

Since the DSS has been going on since 1966 in Matlab and since 1975 in Teknaf, the entire populations of the areas are well aware of the nature of the project. Every household in the two areas has a Family Visitation card; the fact that these cards are carefully maintained indicates an implied type of consent. As approved by the RRC and ERC in the past, no consent form will be used during the continuation of this protocol.