Date 7-5-91

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Leanne Unicomb / Christine Moe

Application No. 91/006

Title of Study : Importance of astroviruses as agents of acute diarrhoea in Bangladeshi children

Trainee Investigator (if any) S

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).

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 — stool (Yes) No

Are subjects clearly informed about: N/A
(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.

Specimens used were collected as part of the protocol number 86/021 "Giardia and persistent diarrhoea...." P.I. Dr Andrew Hall.

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

Leanne Unicomb
Principal Investigator

Trainee

A-032075

91-005
7/5/91

APPLICATION FOR PROJECT GRANT

1.	PRINCIPAL INVESTIGATORS	:	Christine Moe (CDC, Atlanta) Leanne Unicomb
2.	CO-INVESTIGATORS	:	Jim Allen (CDC, Atlanta) N. Nahar Banu Roger Glass (CDC, Atlanta) Andrew Hall
3.	TITLE OF PROJECT	:	Importance of astroviruses as agents of acute diarrhoea in Bangladeshi children
4.	STARTING DATE	:	May, 1991
5.	COMPLETION DATE	:	August, 1991
6.	TOTAL BUDGET REQUESTED	:	US$ 5,133
7.	FUNDING SOURCE	:	Core funds
8.	HEAD OF PROGRAMME	:	Dr. M. Moyenul Islam Acting Head Laboratory Sciences Division

9. AIMS OF PROJECT

The aim of the project is to determine whether astroviruses are an important cause of diarrhoea in children by comparing the incidence of astrovirus in stool specimens from children with diarrhoea with the incidence in stools from matched control children without diarrhoea.

Significance

This study will provide an indication of whether astroviruses are a cause of diarrhoea in Bangladeshi children. It may also provide a preliminary information about clinical features of astrovirus diarrhoea. As part of the study, an ELISA assay will be set up in Bangladesh and will develop the

capability to pursue further studies of this pathogen at ICDDR,B. If astrovirus is found to be reasonably important as a cause of diarrhoea, further studies will be considered to examine the disease burden of astrovirus and to look more carefully at issues of immunity and transmission.

10. ETHICAL IMPLICATIONS

This study will use stool specimens collected for an ELISA for *Giardia* by Dr. Andrew Hall as part of the protocol entitled "*Giardia* and persistent diarrhoea ..." (Protocol No. 86/021), and therefore, will not directly involve human subjects.

11. BACKGROUND

Astroviruses were discovered in 1975 by Madeley and Cosgrove who used electronmicroscopy to examine faecal specimens obtained from children with gastroenteritis. The virus is about 28 nm in size, has a smooth surface and 5-10% of particles have 6-pointed star-like internal form that is a distinguishing morphologic feature. Astroviruses are known to cause diarrhoea in a variety of animals but their importance as a cause of diarrhoea in man is unclear.

Since their discovery astroviruses have been continually identified in studies where the electronmicroscope has been included in the repertoire of tests used to determine the cause of diarrhoea. In general, astrovirus has not been a major contributor to diarrhoeal disease and have accounted for 3-5% of diarrhoeal illness leading to hospital admission. The rare detection of astroviruses in faecal specimens stands in contrast to the observation of

Cubitt and Kurtz that most children acquire antibodies to astrovirus by their fifth year of life. The high prevalence of antibodies despite infrequent detection of virus could be explained if astrovirus caused infection but not disease or if electronmicroscopy (EM) was an insensitive diagnostic method.

An answer to this problem came with the recent development of an immunoassay for astrovirus developed by Hermann and its further development by Moe and Allen at CDC. These assays employ a monoclonal antibody for capture and rabbit antisera for detection in different formats of classic enzyme immunoassays. The assays are significantly more sensitive than EM and lead to estimates of prevalence much higher than that determined by EM alone. At the same time, ELISA-positive specimens have been confirmed as true positive with either solid-phase immune electronmicroscopy (IEM) using astrovirus-coated grids or riboprobes developed at CDC by Monroe. The increased sensitivity of the ELISA assay has led to a higher prevalence of astrovirus in field studies of causes of diarrhoea. For example, a recent survey of children with gastroenteritis in Thailand identified astrovirus in 8.6% of more than 1000 children with diarrhoea but in only 2% of children without diarrhoea.

12. RESEARCH PLAN

Stool specimens collected during a 22-month study of *Giardia* as a cause of persistent diarrhoea will be tested for astrovirus using an ELISA technique. A total of up to 333 diarrhoeal and 666 non-diarhoeal specimens will be tested, where quantity of stool is sufficient. The specimens were collected from children aged 2 to 30 months who were suffering from at least 48 hrs of diarrhoea. All specimens were examined microscopically and cultured for *V. cholerae*, *Salmonella* spp., *Shigella* spp. and enterotoxigenic *E. coli*. No

bacterial pathogens were detected and neither was rotavirus detected by an ELISA. Each diarrhoeal case will be matched with two non-diarrhoeal control specimens, controlled with respect to age (±30 days), date of specimen collection (±30 days) and gender.

Laboratory assay

The assay for astrovirus is based on the principles of immunoassays used commonly to detect rotavirus or cholera toxin. 96-well microtitre plates are coated with a monoclonal antibody, faecal suspensions are added to each well, and after washing and blocking, a biotinylated rabbit antiserum to astrovirus is added and reacted with streptavidin peroxidase. The assay has a level of sensitivity of 95% and specificity of 96% using clinical specimens. The CDC-prepared reagents have been lyophilized for uniformity and easier handling. A manuscript describing the full development and characterization of the assay is in preparation.

Analysis

Patients with astrovirus detected in their specimens will be compared with controls to identify the features of astrovirus-associated gastroenteritis. Specifically, we will attempt to examine the frequency of astrovirus diarrhoea among children aged 2-30 months, the prevalence of astrovirus among cases of diarrhoea and controls without diarrhoea and correlates of the severity of symptoms.

13. REFERENCES

1. Hermann JE, Nowak NA, Blacklow NR *et al.* 1990. Astrovirus etiology of pediatric gastroenteritis (abstract 1221). Program and abstracts of the 13th Interscience Conference on Antimicrobial Agents and Chemotherapy, p.289.

2. Hermann JE, Nowalk NA, Perron-Henry DM, Hudson RW, Cubitt WD, Blacklow NR. 1990. Diagnosis of astrovirus gastroenteritis by antigen detection with monoclonal antibodies. J Infect Dis, 161:226-229.

3. Kurtz J, Lee T. 1978. Astrovirus gastroenteritis age distribution of antibody. Med Microbiol Immunol (Berl), 166:227-230.

4. Kurtz JB, Lee TW. 1984. Human astrovirus serotypes. Lancet, 2:1405.

5. Lew JF, Glass RI, Petric M *et al.* 1990. Six-year retrospective surveillance of gastroenteritis viruses identified at ten electron microscopy centers in the United States and Canada. Pediatr Infect Dis J, 9:709-714.

6. Madeley CR, Cosgrove BP. 1975. 28 nm particles in faeces in infantile gastroenteritis. Lancet, 2:451-452.

14. TASK OF EACH INVESTIGATOR

a) Ms. Leanne Unicomb

- Supervision of laboratory work

b) Dr. Christine Moe

- Set up ELISA test at ICDDR,B
- Train Ms. N. Nahar Banu to help perform ELISA
- Test stool specimens for astrovirus
- Analyse results

c) Dr. Jim Allen

- Provide feedback regarding test performance and results

d) Ms. N. Nahar Banu

- Perform astrovirus ELISA test
- Provide technical back-up

e) Dr. Roger Glass

- Overall supervision of study and study plan

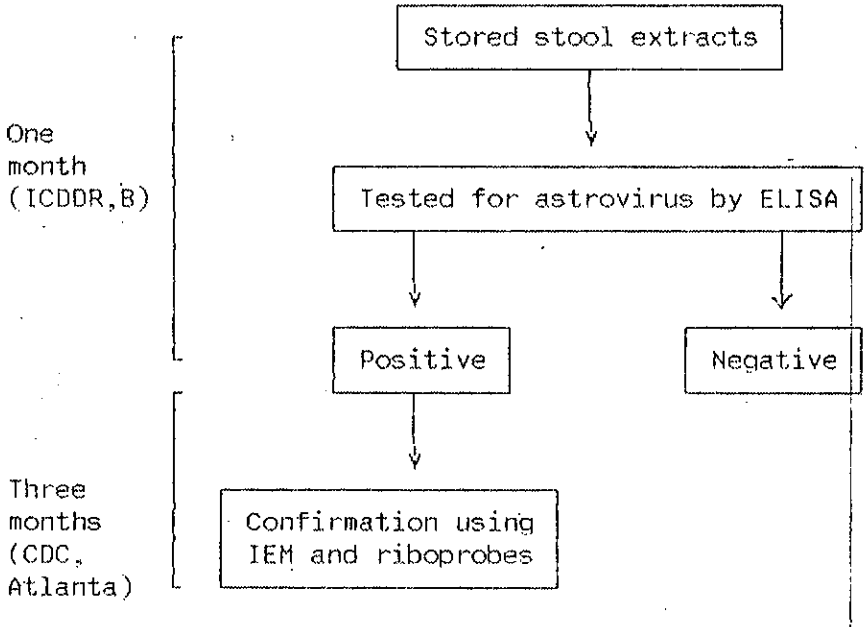
f) Dr. Andrew Hall

- Providing specimens and appropriate data for analysis
- Providing assistance with data analysis

15. COLLABORATION

To initiate this pilot study, Christine Moe from CDC will come to Dhaka to set up the test, train and work with N. Nahar Banu and assist in the analysis of data. Leanne Unicomb and Andrew Hall from ICDDR,B will be designated to work with this project as coinvestigators and facilitate work with specimens and related data. Specimens positive for astrovirus will be sent to CDC for confirmation by IEM or riboprobe. CDC will provide further reagents according to protocols developed after the original screening project is complete.

16. FLOW DIAGRAM



17. PUBLICATIONS

L. E. Unicomb

1) Bishop AF, Tzipori SR, Coulson BS, Unicomb LE, Albert MJ and Barnes GL. (1986). Heterologous protection against rotavirus-induced disease in gnotobiotic piglets. J Clin Microbiol. 24: 1023-1028

2) Albert MJ, Unicomb LE and Bishop RF. (1987). Cultivation and characterization of human rotaviruses with super-short RNA patterns. J Clin Microbiol. 25:183-185.

3) Coulson BS, Unicomb LE, Pitson GA and Bishop RF. (1987). Simple and specific enzyme immunoassay using monoclonal antibodies for serotyping human rotavirus. J Clin Microbiol. 25:509-515.

4) Albert MJ, Unicomb LE, Tzipori SR and Bishop RF. (1987). Isolation and serotyping of animal rotaviruses and antigenic comparison with human rotaviruses. Arch Virol. 93:123-130.

5) Albert MJ, Unicomb LE, Barnes GL and Bishop RF. (1987). Cultivation and characterization of rotavirus strains infecting newborn babies in Melbourne, Australia (1975-1979). J Clin Microbiol. 25:183-185.

6) Unicomb LE, Coulson BS and Bishop RF. (1989). Experience with an enzyme immunoassay for serotyping human group A rotaviruses. J Clin Microbiol. 27:586-588.

7) Unicomb LE and Bishop RF. (1989). Epidemiology of rotavirus strains infecting children throughout Australia during 1986-1987. A study of serotype and RNA electropherotype. Arch Virol. 106:23-34.

8) Genera G, Passarani N, Unicomb LE, Parea M, Sarasini A, Battaglia M and Bishop RF. (1989). Solid-phase immune electonmicroscopy and enzyme-linked immunosorbant and monoclonal antibodies: A comparative study. J Infect Dis. 159:335-339.

9) Bishop RF, Unicomb LE, Soenarto Y, Suwardji H, Risanto and Barnes GL. (1989). Rotavirus serotypes causing acute diarrhoea in hositalized children in Jogjakarta, Indonesia. Arch Virol. 107:207-213.

10) Sethabutr O, Unicomb LE, Holmes IH, Taylor DH, Bishop RF and Echeverria P. (1990). Serotyping of human group A rotavirus with oligonucleotide probes. J Infect Dis. 162:368-372.

11) Bishop R, Lund J, Cipnani E, Unicomb LE and Barnes G. Clinical, serological and intestinal immune responses to rotavirus infection of humans. In: Medical Virology 9. Ed. L.M. de la Maza and E.M. Peterson. Plenum Publishing Corporation, New York.

12) Tzipori S, Unicomb L, Bishop R, Montenaro J and Vaelioja LM. (1989). Studies on attenuation of rotavirus: A comparison in piglets between virulent virus and its attenuated derivative. Arch Virol. 109:197-205.

13) Bishop RF, Unicomb LE and Barnes GL. Epidemiology of rotavirus serotypes in Melbourne, Australia, 1973-1989. J Clin Microbiol. (in press)

14. Bingnan Fu, Unicomb L, Rahim Z, Banu NN, Podder G, Clemens J, van Loon FPL, Rao MR, Malek A and Tzipori SR. Rotavirus associated diarrhoea in rural Bangladesh: A two-year study of incidence and serotype distribution. J Clin Microbiol. (in press)

15. Barnes GL, Unicomb LE and Bishop RF. Severity of rotavirus infection in relation to serotype, monotype and electropherotype. J Paed Child Health. (in press).

16. Shahid NS, Banu NN, Bingnan F, Tzipori SR and Unicomb LE. Rotavirus infection detected in neonates from hospitals in urban Bangladesh. Arch Virol. (in press).

BUDGET

a)	Personnel		
	Salaries of all investigators paid by CDC, Atlanta/ICDDR,B and British ODA		
b)	Operating costs		
	Lab costs at ICDDR,B (remainder borne by CDC)	...	US$ 500
c)	Capital equipment	not applicable	
d)	Travel		
	Return airfare - Christine Moe		3,218
	Per diem for one month	...	775
	In-transit per diem	...	540
	Insurance	...	70
	Transport	...	30
e)	Computing (borne by CDC)		
Total cost			US$ 5,133

LU:mh/L3:ASTROVI.PRT

ABSTRACT SUMMARY

Astrovirus was first identified as a cause of acute gastroenteritis in children in 1976. The discovery was made using electronmicroscopy of faecal specimens which has, until recently, been the principle diagnostic technique. While early studies indicated that most children acquired antibodies to astrovirus by five years of age, detection of astrovirus was relatively uncommon, so the true importance of this agent as a cause of diarrhoea was difficult to assess.

We have recently developed a biotin-avidin assay to detect astrovirus in stool specimens that is easy to use and appears to be much more sensitive for detecting astrovirus than EM. Initial screening studies using ELISA indicates that astrovirus may be an important agent of gastroenteritis in children in their first years of life.

We propose to examine whether astrovirus is an important cause of gastroenteritis in Bangladeshi children. The astrovirus assay developed at CDC will be set up in the virology laboratory at ICDDR,B so that the test can be done locally. A collection of faecal specimens from a recent study of the aetiology of childhood diarrhoea will be screened to determine the prevalence of astrovirus in these specimens, the association of astrovirus with disease (comparison with non-diarrhoea controls), the age distribution of infection, and the relative severity of clinical presentation. Positive specimens will be confirmed at CDC using solid-phase IEM and riboprobes.

If astrovirus is identified to be an interesting or important cause of childhood diarrhoea, this pilot study will allow us to initiate planning for a larger project to examine further the epidemiology of disease caused by this agent.